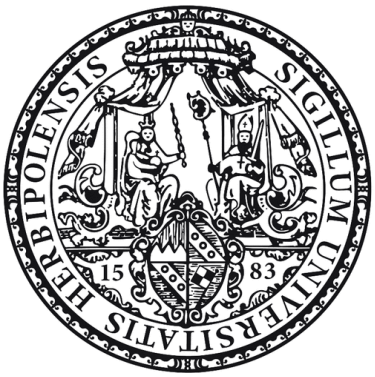
The stability of finished pharmaceutical products and drug substances beyond their labeled expiry dates

DISSERTATION

zur Erlangung des

naturwissenschaftlichen Doktorgrades

der Julius-Maximilians-Universität Würzburg



vorgelegt von

Markus Zilker

aus Würzburg

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A Introduction

1. General

The pharmacopoeias describe quality standards of drug substances and finished pharmaceutical products (FPP) to ensure the efficacy, safety, and quality of medicines. The three major pharmacopoeias are the European Pharmacopoeia (Ph. Eur.) [1], the United States Pharmacopeia (USP) [2], and the Japanese Pharmacopoeia [3]. The regulatory agencies ask for pharmacopoeial quality of the active pharmaceutical ingredients (API) and excipients used in the manufacturing process because of being responsible for the supervision and safety of remedies. In accordance with the International Conference on Harmonization (ICH) guidelines and Good Manufacturing Practices (GMP) regulations, appropriate FPP specifications must be established by the pharmaceutical manufacturer when applying for registration [4, 5]. In addition to clinical and non-clinical study reports, comprehensive data of stability tests have to be submitted to the regulatory agencies as well as a shelf-life has to be determined. In this regard, stability of the API and FPP is an integral part of the drug development process. The manufacturer is obliged to observe identity, content, and purity of the FPP over its entire market life [4]. The stability of APIs and FPPs beyond their expiry dates is largely unexplored and little-known issue, and therefore the subject of this doctoral thesis.

2. Stability testing and regulatory requirements

Drug stability defines the ability of the dosage form to comply with predefined chemical, physical, and microbial properties during the time of storage and usage by the patients [6]. Stability tests provide evidence on how the quality of a drug substance and drug product varies under controlled environmental conditions in the course of time. Typically, forced degradation, accelerated, and long-term stability tests are carried out in order to investigate the stability [7]. Moreover, potential interactions of all constituents of the FPP and the packaging material are scientifically analyzed. Based on evaluation of all data collected a retest period for the drug substance, a shelf-life for the drug product, as well as recommended storage conditions are established and will be applied to all future batches. In many jurisdictions throughout the world the maximum shelf-life which a regulatory agency will approve for a FPP is five years [8, 9]. In recent years, international regulatory agencies, such as the European Medicines Agency (EMA) and the American Food and Drug Administration (FDA), have stipulated harmonization of stability testing. They refer to GMP requirements and ICH guidelines [5]. The ICH guideline on stability testing of new drug substances and products Q1A(R2) describes specific conditions of stability testing which is sufficient for a registration application within the three regions of the European Community, the United States, and Japan [7]. In accordance with the guideline, at least 12-month long-term stability

testing on three primary batches has to be conducted at the time of submission. Moreover, uniform environmental conditions of temperature and humidity for long-term, intermediate, and accelerated testing are required (see Table 1). The applicant may decide to perform long-term studies at conditions depending on the climate conditions of the country where it is intended to become registered [6].

Table 1
Storage conditions for stability testing according to ICH Q1A(R2) guideline [7].

Study	Storage condition	Minimum time period covered by data at submission
General case		
Long term*	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate**	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months
Substances intended for storage in a refrigerator		
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months
Drug intended for storage in a freezer		
Long term	-20°C ± 5°C	12 months

* It is up to the applicant to decide whether long term stability studies are performed at 25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH
** If 30°C ± 2°C/65% RH ± 5% RH is the long-term condition, there is no intermediate condition

When conducting stability studies corresponding to the guideline, the occurrence of a “significant change” is defined as an important or remarkable failure to meet the specification in any physical or chemical condition. Usually, regulatory authorities do not accept failure in stability testing. Generally, the guideline states criteria for drug products as follows: (i) 5% change in content determination from its initial value, (ii) exceeded acceptance criteria of degradation products, (iii) any failure to meet acceptance criteria for predefined chemical and physical attributes and functionality tests, (iv) exceeding its pH limits, and (v) dissolution failure [7]. Special acceptance criteria regarding the content of the API and the impurities may be demanded for biologicals, herbal medicines, and some small molecules that are sensitive to instability, e.g. antibiotics. In such a case, individual justified specification limits are accepted by the regulatory authorities.

Furthermore, certain ICH guidelines play a vital role for drug development processes, ICH Q1B describes instructions for photostability tests, ICH Q1C gives requirements of testing new dosage forms, ICH Q1D provides bracketing and matrixing concepts, and ICH Q1E addresses issues of collection and presentation of stability data [10–13]. Aspects of

impurities which are formed during production processes and degradants contained in the drug substance and new drug products are stated in ICH Q3A(R2) and ICH Q3B(R2), respectively [14, 15]. Generally, impurities of the FPP must be reported, identified, or toxicologically qualified depending on the amount administered per day [15]. Specifications of chemical substances and biotechnological and biological products are addressed in ICH Q6A and Q6B [16, 17]. Furthermore, stability testing of biotechnological and biological products has to be conducted according to ICH Q5C [18]. After registration and marketing, ongoing studies are required in order to control the stability over the entire market life [19].

3. Instability

Instability of drug products can be caused by decrease of API content, loss of mass uniformity, variances in bioavailability, presence of pathological microorganisms, change in appearance, formation of toxic degradation products, loss of package integrity, and reduction of labeled quality [8]. All these items cause the therapeutic efficacy and safety to fail its original level. In general, the velocity of the degradation reaction, defined as rate coefficient (k), is affected by environmental conditions at storage, primary and secondary packaging systems, and microbiological contamination [20].

3.1. Rate of degradation

The degradation rate of API or FPP varies dramatically. For kinetic studies, it is mandatory to monitor the content of the parent drug. The most common degradation reactions undergo either a zero-order, first-order, pseudo first-order, or second-order kinetics (see Figure 1) [8, 21, 22]. For a zero-order reaction, the API concentration changes linearly with time, following the equation: $v = -\frac{dc}{dt} = k$ [8]. Enzymatic reactions commonly undergo such a kinetic type. The most common degradation reactions appear to be a first-order kinetic [23]. The reaction rate depends on the concentration of only one reactant, as shown in the equation: $v = -\frac{dc}{dt} = k \times c_1$ [8]. A hydrolysis reaction is properly speaking in terms of three reactants, the API, the solvent water, and the catalyst proton/hydroxide ions, e.g. hydrolysis of acetylsalicylic acid [24]. Eventually, the API concentration determines the velocity because of the great excess of water and proton/hydroxide ions in comparison to the API concentration being present in the system. This is stated as pseudo first-order reaction. Furthermore, the velocity of a second-order reaction depends on the concentration of two reactants: $v = -\frac{dc_1}{dt} = -\frac{dc_2}{dt} = k \times c_1 \times c_2$ [8]. Kinetic-orders higher than two do not play a role in describing degradation kinetics of pharmaceuticals.

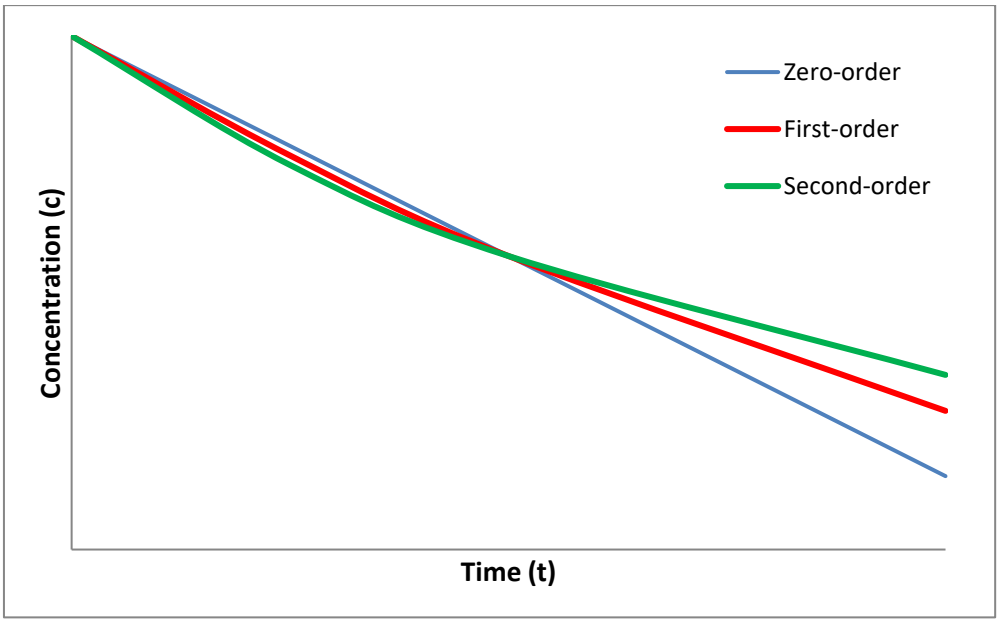


Fig. 1. Scheme of degradation kinetics

3.2. Environmental conditions

Temperature, oxygen, light, and moisture affect the quality of drug products during storage in a highly manner [20]. The Arrhenius equation describes the relationship between the kinetic rate of the degradation process and the storage temperature [8,19]. Based on results of accelerated stability studies, the degradation rates expected for ambient conditions can be calculated by using this equation. Typically, an increase of the temperature by 10 degrees redoubles the degradation rate [19, 21]. The next factor oxygen is abundantly contained in air, being critical to the stability of drug products. Oxygen permeates easily throughout the packaging, maybe interfering with the ingredients [25]. Furthermore, light exposure causes complex degradation processes of a drug. The spectral regions of ultra violet (UV) and visible radiation are in the ranges of 200–400 nm and 400–700 nm, respectively [26]. The shorter the wavelength of the light, the higher the energy [27]. Subsequently, UV radiation leads to massive drug decomposition. Exposure of moisture promotes preferably the occurrence of hydrolysis. The permeability of moisture throughout the container into a drug may causes interference of APIs and excipients.

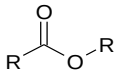
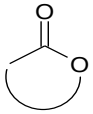
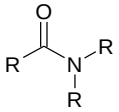
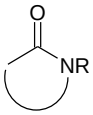
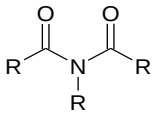
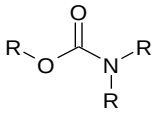
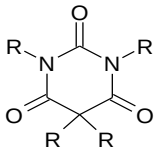
3.3. Chemical types of instability

The most common chemical degradation reactions are hydrolysis, oxidation/reduction, isomerization, racemization, etc. However, all types are associated with a decrease of API content over time [8].

3.3.1. Hydrolytic degradation

Hydrolysis describes the reaction of a molecule with water resulting in the cleavage of a chemical bond within the molecule [6]. It is the most important degradation reaction occurring in drug products because of the ubiquitous nature of water and the widespread of sensitive moieties, e.g. esters, amides, etc., in the majority of the therapeutically used compounds [22]. Functional groups of drugs, being prone to hydrolysis, are given in Table 2.

Table 2
Chemical moieties being sensitive to hydrolysis

Functional group	Chemical structure	API examples
Esters		Acetylsalicylic acid, atropine, procaine
Lactones		Erythromycine, pilocarpine, spironolactone, cardenolides
Amides		Chloramphenicol, lidocaine, indomethacin
Lactams		Penicillin and cephalosporine antibiotics
Imides		Mesuximide, glutethimide
Carbamates		Carbachol
Malonic ureans		Barbiturates (e.g. phenobarbital)

R could be an aliphatic, aromatic rest, or a hydrogen atom

The hydrolysis reaction of esters and amides starts with a nucleophilic attack of water on the carbonyl carbon, resulting in the formation of cleaved reaction products of a carboxylic acid and either an alcohol and an amine, respectively. The reaction runs more rapidly in esters than in amides because of the higher partial positive charge of the carbonyl carbon group in ester group [28]. Higher hydrolysis rates occur when being catalyzed by acidic and basic conditions. Likewise, some functional groups may react with water, resulting in splitting of

chemical bond, e.g. imines, sulfonic esters, and phosphate esters, found in diazepam, metamizole, and adenosine triphosphate, respectively [29].

3.3.2. Oxidative degradation

Oxidation is another pathway for drug degradation, in which an organic compound is restated as a loss of an electron and hydrogen, as well as a gain of oxygen [25]. Certain major drugs and excipients such as epinephrine, nifedipine, morphine, fatty acids, and ascorbic acid, are sensitive to undergo oxidations. In general, pathways are divided in (i) radical initiated auto-oxidation, (ii) electron transfer mediated oxidation, or (iii) peroxide mediated oxidation. Firstly, the free-radical process involves three stages, starting with the initiation phase in which a radical is formed by the presence of oxygen, being itself a biradical. In the following stage, the propagation phase converts the compound to a hydroperoxide and finally the reaction ends in a termination phase. Typically, the hydroperoxides are instable, subsequently forming ketone moieties. Secondly, direct and catalyzed electron transfer processes take place in pharmaceutical agents. Oxygen, peroxyl radical, or metal catalysts may gain of electron from the drug compound which is oxidized to an instable radical cation, readily reacting to final degradants. Generally, sulfide, sulfoxide, and phenol anion moieties are prone to be oxidized to sulfoxide, sulfone, and ketone, respectively, via the electron-transfer reaction. Primary and secondary amines may be oxidized to hydroxylamines or imines, while tertiary amines and pyridines can possibly form amine *N*-oxides. In the third place, since peroxide impurities are present in some excipients of a drug product, e.g. polysorbates and polyethylene glycol, they can trigger oxidations of pharmaceutical ingredients [25, 28].

The drug class of phenothiazines, e.g. chlorpromazine, promazine, perphenazine, and fluphenazine, is sensitive to oxidative degradation reactions [30]. Chlorpromazine as a prototype of phenothiazines is used to illustrate typical oxidative processes (Figure 2). The main degradation pathways of chlorpromazine include oxidative processes such as 3-hydroxylation, *N*-oxidation, and *S*-oxidation [31, 32]. The core structure is susceptible to become oxidized due to the high electron density at the sulfur atom of the heterocyclic compound. The oxidation process runs via the formation of a mesomerism-stabilized cationic radical, which is deeply red colored [33]. Final degradation reactions lead to sulfoxide, sulfone, hydroxyl, and *N*-oxide compounds. All degradation products have pharmacological profiles which are largely different from the parent agent, in spite of the minor structural differences [34]. Moreover, phenothiazines and closely related compounds of the class of thioxanthenes, e.g. chlorprothixene and zuclopenthixol, even undergo oxidation processes following the same reactions [30].

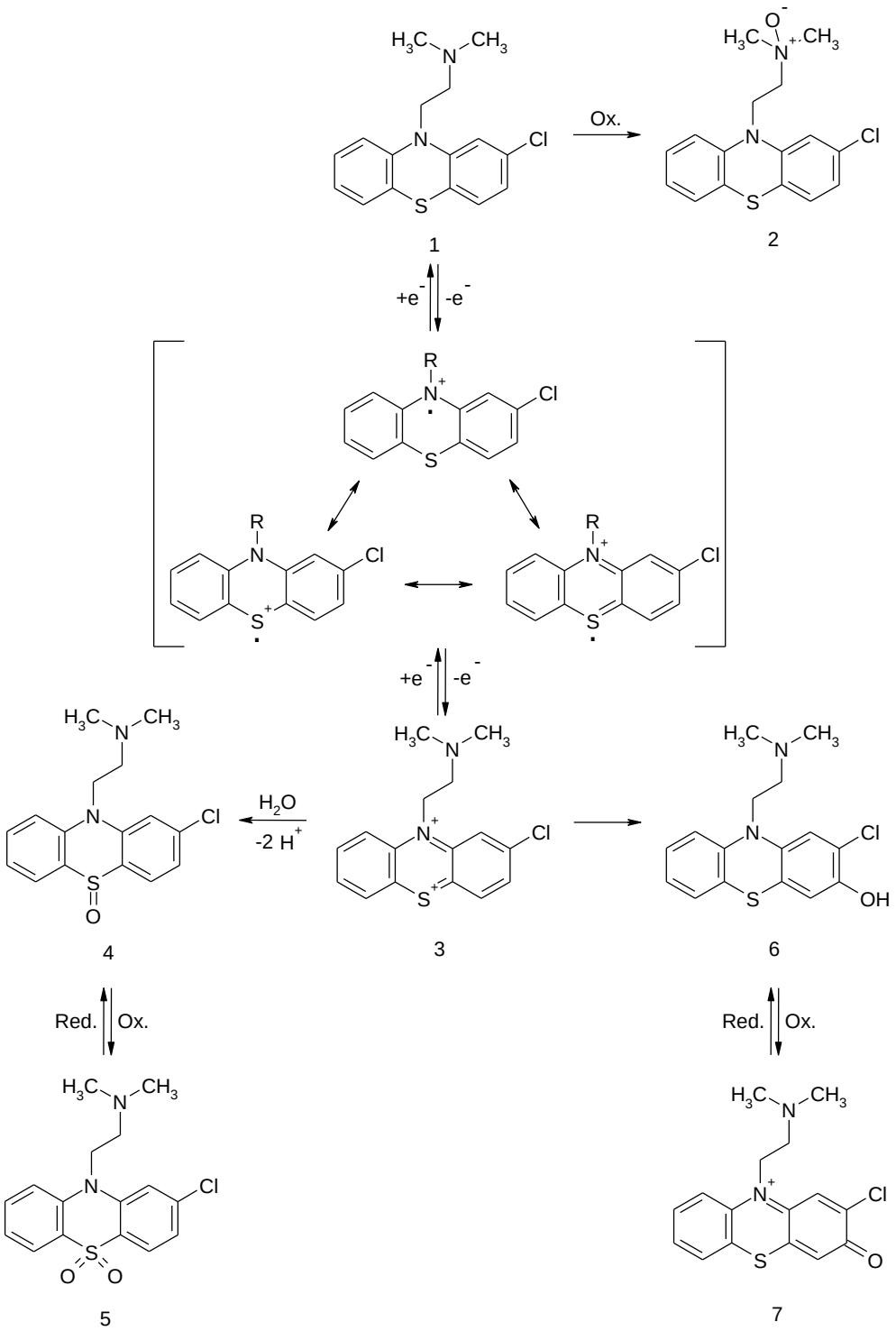


Fig. 2. Typical degradation products of chlorpromazine (1): Chlorpromazine-*N*-oxide (2), phenacetonium ion (3), chlorpromazine sulfoxide (4), chlorpromazine sulfone (5), 3-hydroxychlorpromazine (6), chlorpromazine-3-on (7) [30–32].

3.3.3. Isomeric degradation

Conversion of an API into another isomer can possibly make the substance less or even totally inactive regarding the pharmacological efficacy. Typical reactions are racemization and epimerization taking place in drug products [35]. Racemization is characterized by the

conversion of a pure optically active compound to a mixture of enantiomers, e.g. *D*- and *L*-epinephrine [36]. Usually racemization occurs via keto-enol tautomerism, in which the former chiral center becomes planar. The incoming group can approach from either side of the plane, resulting in either the *R*- or *S*-configuration [6]. Epimerization is the configurational change at one chiral center. A major drug undergoing epimerization is tetracycline, which is displayed in Figure 3.

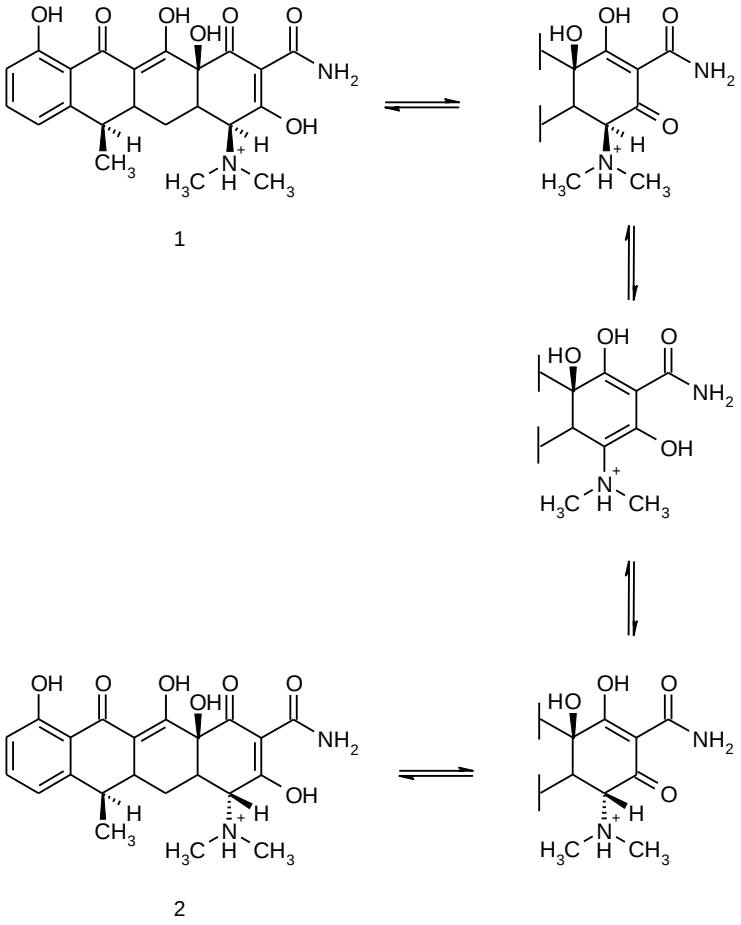


Fig. 3. Epimerization of (4*S*)-tetracycline (1) to (4*R*)-epitetracycline (2), under acidic condition [30, 37, 38].

Geometrical isomerization exists for compounds which can take a *cis*- or *trans*-configuration. However, this occurs rarely, e.g. in chlorprothixene. The separation of the diastereomers can be achieved by means of reversed phase chromatography, whereas techniques of chiral separation are necessary for the separation of enantiomers [39]. The Ph. Eur. requires respective limit criteria for all specified isomers [1].

3.3.4. Photolytic degradation

Photolysis is a light induced degradation process whereby any photon affects the chemical bonds of a molecule. Light sensitive drugs are chemically degraded by exposure to solar, UV, and visible light. Photolytic degradation reactions include miscellaneous types among

others oxidation, reduction, cyclization, dealkylation, decarboxylation, dehalogenation, dehydrogenation, dimerization, elimination, hydrolysis, isomerization, as well as ring cleavage [26].

3.4. Physical instability

The physical instability affects crucial properties of a drug product, e.g. its appearance, mass and content uniformity, and drug release. However, instability varies with the dosage form. Abrasion, impact, vibration, and fluctuation of temperature and humidity cause decomposition [8].

Tablets, capsules, and granules may change the friability, hardness, and dissolution rate. Hygroscopic powders preferably absorb water from external atmosphere, leading to formation of agglomerates. Moreover, solutions and gels are inherently sensitive to alteration of viscosity and homogeneity. Particularly, the formation of precipitations in parenteral dosage forms is critical in patient safety. Semisolid dosage forms, including suspensions, emulsions, ointments, and creams, may form several types of instability, like separation of phases, sedimentation, change of particle size and viscosity, creaming, cracking, and evaporation of water. Furthermore, adsorption of drug to the surface of a container is an example for the decrease of API content, possibly occurring in all dosage forms [22, 35].

The existence and formation of different polymorphic forms influence the physicochemical property of a drug, including its stability [40]. Typically, amorphous substances have a higher kinetic solubility and dissolution rate, and subsequently the bioavailability may be altered compared to crystal structures [41]. The polymorphic purity of drug samples can be characterized by means of techniques, such as x-ray powder diffraction, Raman, and infrared spectroscopy [4]. Spontaneous interconversion is noted for some APIs, e.g. ritonavir and fenofibrate, forming inactive forms, respectively [42].

3.5. Microbiological instability

The microbiological instability is based on the contamination with microorganism and viruses as well as with toxic and pyrogen substances of these. The sources of microbial contamination are water, raw materials, personnel, instruments, and apparatus [43]. Liquids are particularly at risk. Moreover, contamination may occur during storage caused by not tightly closed drug containers. The consequence of spoilage is often unpleasant smell, opacification, and discoloration [44].

3.6. Protection techniques

In order to ensure the stability of a drug product during the manufacturing process and storage, control of normal environmental conditions, utilization of stability promoting additives, and the usage of appropriate packaging material play vital roles [20].

Oxidative degradation can be sufficiently controlled by manufacturing and packaging under nitrogen or argon conditions to avoid oxygen. In addition, sensitive drugs are recommended to be stored under nitrogen or argon as well as in the absence of light [45]. The usage of a suitable primary and secondary packaging material is crucial. Typically, oxygen and moisture permeate faster through plastic material than through glass [22]. Furthermore, oxygen scavengers have found a wide application as part of the packaging to absorb oxygen. The metal oxidation with iron is the most commonly used scavenger, consequently degradation is effectively prevented [46].

Antioxidants are very commonly added to several pharmaceutical dosage forms. The manufacturer can choose from a large number of agents. Dibutylhydroxytoluene and propyl gallate are phenolic antioxidants which catch free radicals. Ascorbic acid and sulfites can terminate oxidation chain reaction due to their properties of becoming easily oxidized. Complexation of catalyzing metals can be reached by addition of citric acid, sodium ethylene diamine tetraacetic acid (EDTA), and tartric acid, respectively [35].

For prevention of hydrolysis, some parenteral agents are lyophilized or dry filled into ampoules because of their limited stability in aqueous solution [22]. Before dispensing, they are reconstituted in water. Furthermore, the pH control of the dosage form appears to have great leverage of reducing hydrolysis. Many agents are stable at about pH 4, but individual optimum conditions for each API have to be evaluated [35].

The light sensitivity of FPPs requires the use of an effective primary and secondary packaging system. Colored plastic and brown glass give suitable protection from light compared to clear flasks [22]. Additionally, glass is resistant to chemical and physical changes of the ingredients. The protection of solid dosage forms can be reached by using UV-opaque blisters. Aluminum foil wrappers and transparent foil consisting of colloidal titanium dioxide or iron oxide are widely employed [47]. It has been demonstrated that plastic films filled with ultrafine colloidal titanium dioxide particles lead to appropriate UV-absorption, too. Generally, the disadvantages of plastic container materials are adsorption of ingredients, permeation of gas and moisture, and leaching of container ingredients into the drug.

Antimicrobial preservatives are added to FPPs in order to prevent microbiological contamination. Typical agent classes are used in pharmaceutical fields, e.g. amino aryl acid

esters, alkyl/aryl alcohols, phenols, organo mercurials, and quarternary ammonium compounds [35, 48]. For sterile preparations there is either a terminal sterilization process or a closely controlled aseptic manufacturing procedure.

4. Stability indicating approaches

4.1. Organoleptic analysis

Organoleptic properties are important in the initial assessment of the quality of a drug product. The chemical instability of acetylsalicylic acid could be determined by smell of acetic acid and physical instabilities could be identified by organoleptic tests in most cases. Effects like alterations in smell, feel, taste, as well as visual appearance are typical for observation of degradation processes, which have been occurred [49]. In liquids, the occurrence of precipitation and in the case of emulsions, the separation in two phases leads to visual change with reference to the original one.

4.2. Titrimetric analysis

Usually, the pharmacopoeias provide titrimetric methods for assay of drug substances. Since most of the drugs are salts of weak bases, aqueous and nonaqueous acidimetric methods are widely employed. Generally, the determination of the equivalence point can be accomplished by either color change using an appropriate indicator or by a potentiometric indication. Likewise, the Ph. Eur. describes titrimetric approaches such as amperometric, conductrimetric, voltametric, and redox titration [50]. Titrations yield results with high accuracy and precision. Therefore, it is the first choice for quantification of bulk drug substances. Nevertheless, the assay of a FPP by means of titration is a difficult task to undertake. On the one hand excipients and degradation products of the API may interfere with the titrant, because of being a non-specific assay. On the other hand, drug products commonly contain small amounts of the API in a single unit dose. Regardless, a few hundred milligrams of the analyte are utilized per single titration. Hence, multiple single unit doses have to be unified, prior to assay.

4.3. Thin-layer chromatography

Thin-layer chromatography (TLC) enables the separation of mixture of chemical substances into its components. It has been established in pharmacopoeias for identification tests and sometimes for determination of related substances because of its simplicity, low costs, and unsophisticated devices used [51]. Commonly, dilutions of the main compound are utilized to quantify impurities being present in the sample solution. The lower quantitation limit of impurity determination is typically limited to a range of 0.1% to 0.5%, because of the weak detecting power of spots at low concentrations. In order to increase the ability of visual

detection of non-colored spots, several visualization techniques are possible such as fluorescent suppression and specific derivatization resulting in colored or fluorescent chromatographic zones. Spot intensity matching techniques require either extraction of the components from the sorbent followed by spectrophotometric measurements or densitometers for in situ quantification [52–54]. In recent years, the approach has been gradually replaced by high performance liquid chromatographic (HPLC) application. High separation power, more sensitive detectors, and HPLC software enable highly precise and accurate analysis based on area counts of the peaks.

4.4. High performance liquid chromatography

HPLC is the current state-of-the-art in the quality control of pharmaceutical products. A wide variety of sample mixtures can be qualitatively and quantitatively analyzed with very high selectivity. The approach is very quick, highly efficient, and delivers high chromatographic resolution. The reproducibility is enhanced compared to TLC because of the largely automated process. Most of the Ph. Eur. monographs provide liquid chromatography approaches for determination of related substances [55–57].

Several modes of the stationary phase are differentiated between normal phase, reversed phase (RP), ion exchange, ion-pair, size exchange, and chiral phase chromatography. RP-HPLC is the most employed chromatographic type in current pharmacopoeias. In RP-chromatography, the surface of the support particles is modified by more or less hydrophobic octadecyl-, octyl-, propyl-, cyanopropyl-, phenyl-, and amino- covalently bonded phases [58, 59]. The mobile phase in HPLC usually consists of a mixture of aqueous solution of a defined pH value and one or more organic solvents. Buffer salts, ion pairing reagents, and other additives may be present in the mobile phase to control chromatographic parameters, e.g. retention time and peak shape. A chromatographic method can be carried out either by isocratic or gradient elution.

The analytes are detected by means of a suitable detector device. Absorbance detectors, such as UV/VIS including photo-diode-array detectors, provide a linear signal over a wide range of analyte concentration. In addition, they are very robust, sensitive in the nanogram to picogram concentration range [59]. Certainly, in order to obtain a signal response, it requires the presence of a chromophore in a molecule.

Furthermore, refractive-index, electrochemical, fluorescence, charged aerosol, and light scattering detectors are described in the Ph. Eur. [1]. Mass spectrometry is one of the most powerful detection approaches available for HPLC, detecting analytes within a sample based on their mass-weights. It can assist identification and characterization of sample components in a wide type of applications [60].

5. Method validation

The objective of analytical method validation is to demonstrate that the approach is suitable for the intended application. The ICH guideline Q2(R1) “Validation of analytical procedures” comprises parameters which have to be considered during the validation of analytical procedures [61]. The methods of the pharmacopoeias are validated per definition. If using a new analytical method for determination of content and impurity profiling of a FPP, numerous parameters have to be verified (see Table 3) [61, 62]. Limit values or specifications are generally not mentioned in the guidelines, but information is provided in the technical guide for the elaboration of monographs of the European Pharmacopoeia Commission [63]. The effort of validation depends on the intended application of the scientist. In the subsequent sections all relevant parameters are elucidated being crucial for stability indicating HPLC-UV methods [6]. Generally, the validation comprises the sample preparation as well as the analysis of the sample.

Table 3

Required validation parameters of analytical methods for assay and impurity profiling of FPPs according to ICH Guideline Q2(R1) [61].

	Assay	Testing for impurities	
Parameter	Content	Quantitative test	Limit test
Accuracy	+	+	-
Precision			
Repeatability	+	+	-
Intermediate precision	+	+	-
Specificity	+	+	-
Detection Limit	-	-	+
Quantitation Limit	-	+	+
Linearity	+	+	-
Range	+	+	-

+ signifies that the parameter is evaluated
- signifies that the parameter is not evaluated

5.1. Accuracy

The accuracy is assessed by comparing the closeness of agreement of the value found and the true value. Typically, the percent recovery of both results is calculated and reported. For FPP assay by means of HPLC, the accuracy is verified by using quality control samples which are separately prepared, containing known quantities of reference standard at 80%, 100%, and 120% levels of the test concentration, respectively. Generally, a minimum of three replicates at three concentration levels each are recommended.

5.2. Precision

The precision is the variability in the data measured from replicate determinations of one homogeneous sample. Generally, three types of precision are divided in (i) repeatability, describing as a series of repeated measurements of the same sample, (ii) intermediate precision, verifying variations on different days, analysts, and equipment, and (iii) reproducibility, giving the variation between laboratories. A series of six measurements, each day, under the same operating conditions over a short time interval corresponds to the guideline requirements. Typically, the precision is expressed as coefficient of variation and a value not higher than 2% should be obtained.

5.3. Specificity

Specificity is the property to analyze unequivocally an analyte in the presence of related substances, excipients, or matrix that are expected to be contained in the sample. When carrying out HPLC investigations, overlapping of the target peak with any impurity peak is undesired. Therefore, specificity is demonstrated by the chromatographic resolution (R_s) between two compounds eluting close to each other. A R_s value of 1.5 or higher ensures sufficient separation.

5.4. Detection and quantitation limit

The limit of detection (LOD) of an individual analytical procedure is defined as the lowest amount of analyte in a sample which can be detected. The limit of quantitation (LOQ) is stated as the lowest concentration of analyte which can be qualified with safety. In HPLC, the Signal-to-Noise ratio is measured, being 3/1 for LOD and 10/1 for LOQ.

5.5. Linearity

The linearity of a method is the ability that signal values are directly proportional to the agent concentration of the sample. At the minimum five calibration solutions covering the defined range are recommended. By using a UV/VIS detector, the relationship between the analyte concentration and the absorbed light is described by the Lambert-Beer law, typically being valid over a wide concentration range. The coefficient of determination R^2 is typically given to assess the linear relationship.

5.6. Range

The range gives the concentrations of the analyte between the low and high limits of quantification. The linearity, accuracy, and precision must be acceptable within the specified range. For assay of an FPP, the aforementioned parameters should be validated in a range of 80–120% of the target API concentration. In the cases of planning long-term and

accelerated stability or controlled release studies, the range may be extended to an API content of 0–120%.

5.7. Robustness

The robustness of a method is the capacity to remain unaffected by small variations of external conditions. In the case of HPLC, the effect of variation in (i) pH value of the mobile phase, (ii) mobile phase composition, (iii) the column used, (iv) temperature, and (v) decrease or increase of flow rate may be investigated.

5.8. System suitability test

Conducting of a system suitability test (SST) is routinely done before starting with an analytical run in order to ensure the performance of the whole procedure. Typically, it must be applied when testing for related substances according to the Ph. Eur. The SST is used to verify resolution, column efficiency, and repeatability of the chromatographic system [53, 62].

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B Aim of the work

The pharmaceutical manufacturer is obliged to determine the shelf-life of a FPP and to submit stability studies to the regulatory authorities for approval. Generally, small molecules have to be in a range of 95–105% of the labeled content during the entire market life corresponding to the ICH guideline Q1A(R2). In recent years, a growing interest in stability of FPPs past their expiry dates has been observed. Physicians and patients have asked whether the application of outdated medications would entail harmful health effects. Only a few publications reported the actual shelf-lives to be markedly longer than the declared ones or even exceeding the maximum shelf-life of five years, but there is scattered or little information about the stability after extreme long storage. Nevertheless, in most cases, no or less decomposition has been occurred during the entire storage period and the impurity profile has not changed in a significant manner. These findings imply a doubt on setting of inaccurate expiry date ranges by the manufacturers. The objective of this thesis is to confirm or refute the general high stability of medicinal products beyond their expiry dates.

In a systematic review, the literature addressing the stability of expired drug products and old drug substances should be summarized and the actual shelf-lives with regard to the respective dosage form and the affiliation of the drug class were to be assessed.

In the experimental studies, the quality of old pharmaceuticals comprising 50 pure drug substances and 14 ampoules manufactured in the 20th century should be analyzed. The drug substances are part of a collection of old pharmaceuticals at the Institute of Pharmacy and Food Chemistry in Würzburg, kept for at least two decades. Drug classes with well established position on the pharmaceutical market are chosen, e.g. β-blockers, β-sympathomimetic drugs, anticholinergics, anti-infectives, non-steroidal anti-inflammatory drugs, antipsychotics, antihistaminic drugs, and one antiarrhythmic drug. The ampoules are preserved in the collection of long expired FPPs with an age of up to 83 years at the Institute for Biomedical and Pharmaceutical Research (IBMP) in Nürnberg-Heroldsberg.

The content and the degradation profile of the items shall be determined by means of appropriate instrumental analysis, mainly using liquid chromatography techniques based on pharmacopoeial approaches for impurity profiling covering all process and degradation related substances. The proposed methods have to be validated for the application of studying FPP stability. Hence, linearity, intra-assay precision, and accuracy must be verified in accordance with the ICH guideline Q2(R1).

C Results

1. A systematic review of the stability of finished pharmaceutical products and drug substances beyond their labeled expiry dates

Zilker, M., Sörgel, F., Holzgrabe, U.

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Abstract

In recent years, there has been a very active debate about the stability of drug products especially after exceeding the expiry dates. The regulatory authorities require comprehensive stability data for market approval. The shelf-life obtained determines the expiry date, which is typically between 1 and 5 years and commonly set in a conservative manner. Conducting stability studies is a resource- and time-consuming matter for the pharmaceutical manufacturer. Short shelf-lives of drug products are also a challenge for managers of hospitals, nursing homes, and strategic national stockpile agencies which have to dispose of large quantities of outdated medicines every year. This conflict raises the question whether shelf-lives are often longer than the labeled one. In the past years, the FDA has launched several programs for shelf-life extension in order to defer replacement costs and to prevent drug shortages due to supply disruption. The aim of this review was to bring together the available literature of expired drug products as well as historical pharmaceutical relicts with an age of more than 80 years and to discuss the actual shelf-life with regard to the respective dosage form and the affiliation of the drug class. It seems to be reasonable for a large portion of drugs to extend the expiry dates far beyond five years.

Abbreviations: API, active pharmaceutical ingredient; FPP, finished pharmaceutical product; Ph. Eur., European pharmacopoeia; USP, United States pharmacopoeia; DoD, department of defense; SNS, strategic national stockpile; SLEP, shelf life extension program; FDA, food and drug administration; ICH, international conference on harmonization; EDOX, epidoxycycline; DHE, dihydroergotamine mesilate; HPLC, high performance liquid chromatography; RP, reversed-phase.

1. Introduction

Pharmaceutical manufacturers have to provide stability data of the active drug substance (API) and the finished pharmaceutical product (FPP) when they ask regulatory authorities for market authorization. The stability studies must be in accordance with the International Conference on Harmonization (ICH) guidelines on testing of new drug substances and products Q1A(R2) [1, 2]. The content of the API has to be within the specification of 95-105% during the time available on the market. Based on the results of real-time and accelerated stability tests, a shelf-life is assigned to the FPP, typically set in a range of 1–5 years [3, 4]. In general, expiry dates are estimated conservatively by the manufacturers because the performance of such comprehensive studies occasions high costs and takes time [5, 6].

Short expiry dates of drug products are a costly challenge for hospitals, nursing homes, and agencies which stockpile large quantities of medicines, e.g. the German armed forces (Bundeswehr) and US Department of Defense (DOD) [7, 8]. According to a report in the Mayo Clinical Proceedings, the replacement of expired drugs costs about $200,000 for a typical American hospital annually [9]. Tons of outdated medicines are reported to be discarded in Germany every year [10, 11]. The health care systems around the world would highly benefit if outdated medicines are extended beyond the expiry date and thus, the replacement of stockpiled drugs could be deferred [12].

In 1986, the Shelf-Life Extension Program (SLEP) was established which was sponsored by the U.S. DOD and performed by the American Food and Drug Administration (FDA) [7]. Since that time, the FDA laboratories have qualified federally stockpiled drug products using analytical methods of the U.S. Pharmacopeia (USP) and the original manufacturers [12, 13]. Other factors like the API content, the presence of degradation products, the dissolution rate, and the appearance of the drug were tested. If successfully passing the tests, the shelf-lives of the FPPs were extended. Calculation to determine the new expiry date is carried out by means of individual statistical extrapolations. Courtney reported costs spent for testing of about $350,000 in 2005, whereby the value of drug products analyzed was about $33 million [14]. Hence, every dollar spent by the DOD for testing the medicines led to substantial savings of $94.

Since 2004, the United States Department of Health and Human Services of the executive branch of the U.S. Federal Government has been involved in the program “Strategic National Stockpile” (SNS). This federal agency stockpiles large quantities of medicines to protect the American population for the case that a public health emergency, e.g. chemical, biological, or radiation emergencies as well as terrorism attack occurs. Usually, the drugs stockpiled are still unused when reaching their labeled expiry dates. The financial benefit of SLEP for SNS

was about $28 for every dollar spent in the period of 2008–2010 [14]. The federal agencies have already realized the possible savings in retesting drugs close to their expiry, consequently extending the shelf-lives if feasible. In 2006, Lyon et al. reported the results of 122 different drug products that had participated in SLEP since 1986. Of note, since 2006, results of SLEP have not been published in the literature again [7]. State and local stockpiles are excluded from SLEP due to limited resources of the FDA [15]. Nevertheless, in recent years the FDA issued guidance for federal agencies and state and local governments on testing to extend shelf-life of antivirals, doxycycline, nerve agent antidotes, and potassium iodide [16, 17]. However, non-federal or civilian agencies may not avail themselves to SLEP initiative.

In the European Union, a program being similar to SLEP is non-existent [18]. Nevertheless, a debate has been started about the feasibility of shelf-life extension of FPPs in Germany and in other European countries as well [19–21]. Generally, the prevailing legal norms prohibit the sale and the donation of expired medicines. However, the Section 71 of the German Medicinal Products Act issues an exception concerning some federal agencies: “The indication of the expiry date stipulated in Section 10 sub-section 1 number 9 is not necessary in the case of medicinal products which are supplied to the Federal Armed Forces, the Federal Police, as well as to the Federal Government and Laender for the purpose of civil protection and disaster control.” [22]. Hence, the Federal State Ministries or the competent state authorities are responsible for the quality, efficacy, and safety of these medicines.

When being approved, the FPP has to meet the specifications stated by the manufacturers and the regulatory authorities during the time being on the market. Stability ensures the quality with regard to purity and content of a FPP. The occurrence of chemical, physical, and microbiological instabilities is affected by environmental factors during storage, e.g. heat, relative humidity, light, and oxygen [3]. The chemical instability is characterized by the decay of the API. Typical degradation reactions occurring during long-term storage are hydrolysis, oxidation, photolysis, polymerization, and isomerization [23, 24]. A very common pathway of API degradation is hydrolysis of esters, amides, and carbamates, which are prone to hydrolysis resulting in compounds containing a carboxylic acid group. Especially, the drug class of local anaesthetics as well as atropine and acetylsalicylic acid show such reactions (Fig. 1). Further common pathways are oxidation and reduction processes that are usually triggered by oxygen and exposure to light. Typically, sympathomimetics containing a catechol structure like epinephrine, norepinephrine, and isoprenaline are susceptible to oxidation processes, finally leading to discoloration of the injection solution due to the formation of adrenochrome and black polymerization particles (Fig. 2) [25]. Manufacturers

often try to prevent or reduce these reactions by adding antioxidants, e.g. sulfites, which may react with the benzylic OH group in a substitution reaction.

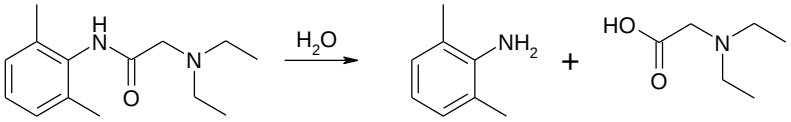

Fig. 1. Lidocaine hydrolysis of amide-type

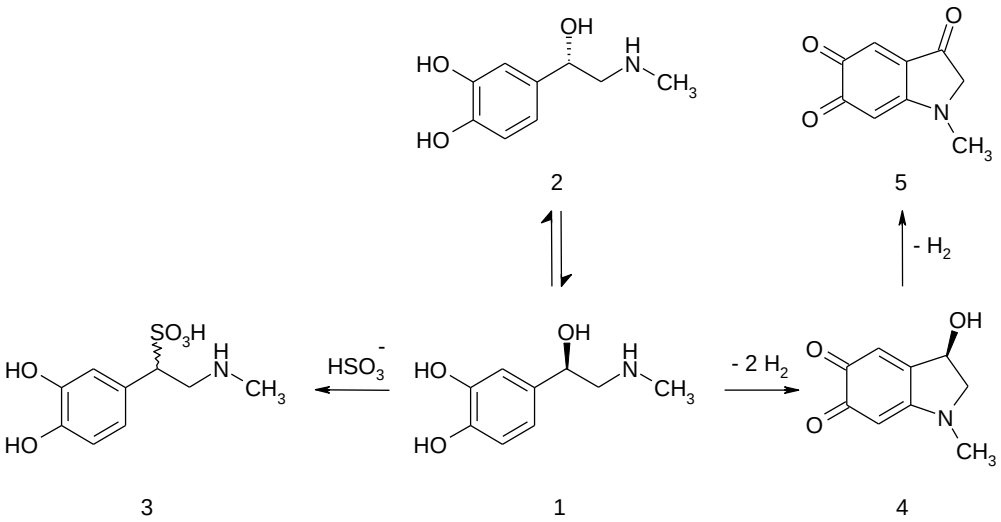

Fig. 2. Degradation pathways of epinephrine: (*R*)-epinephrine (1), (*S*)-epinephrine (2), sulfonic acid derivative (3), adrenochrome (4), oxoadrenochrome (5)

Photolysis is a light induced decomposition process, such as an oxidation, an isomerization, etc., whereby any photon affects the chemical bonds of a molecule, likely happening in dipyridamole, nifedipine, and aztreonam solutions [23, 26, 27]. Furthermore, racemization and epimerization are typical degradation reactions being usually observed when FPPs are manufactured with isomerically pure compounds. In adrenaline injection solutions, the pharmacologically active *R*-enantiomer of epinephrine is commonly used, while the *S*-enantiomer is less potent [28]. However, epimerization is described for the class of the tetracyclines and the ergot alkaloids family [23]. Doxycycline can easily undergo epimerization processes, forming epidoxycycline (EDOX) derivatives such as 4-EDOX, 6-EDOX, and 4,6-EDOX (see Fig. 3) [29, 30].

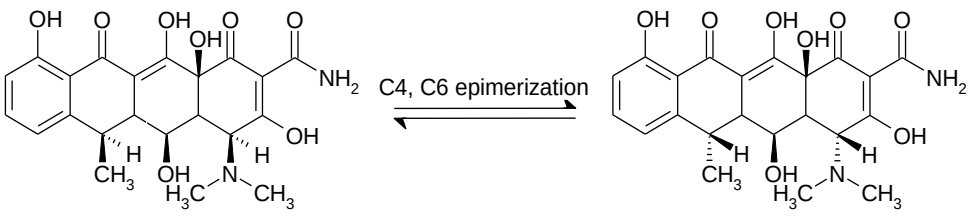

Fig. 3. C4 and C6 epimerization of doxycycline

In addition, the physical stability is crucial for the quality of FPPs. For liquid dosage forms, the appearance, the pH, and the presence of any precipitation must be proven. Suitable attributes like the dissolution rate, the hardness, and the friability are very important for solid dosage forms, e.g. tablets. The efficacy and safety of sustained release tablets and capsules or depot dosage forms could be affected in a highly negative manner because of too much or too little of API release per unit time. Furthermore, microbiological contamination especially of sterile pharmaceutical solutions endangers the safety and efficacy for patients. Microbiological purity of sterile liquids is strictly required according to any pharmacopoeias.

2. Analytical approaches for API quantitation and purity testing

For analysis of FPPs and drug substances, the pharmacopoeias provide methods to verify the identity, purity, and the content [13, 31]. High performance liquid chromatography (HPLC) is the state of the art, enabling the simultaneous determination of the API content, identification and quantitation of impurities, called related substances [32]. For quantitation of expired FPPs, most authors utilized a chromatographic system equipped with photo-diode-array detector and Reversed-Phase (RP) column [33–36]. Ideally, validation processes and data were reported in some studies concerning linearity, precision, accuracy, limit of detection, and quantitation corresponding to the ICH guidelines Q2R(1) [34–37]. Cantrell et al. analyzed eight long-expired medications with 15 different APIs by means of a Time-of-Flight Mass Spectrometer using Electrospray Ionization in negative and positive polarities [38]. In another study, the same author used liquid chromatographic-tandem mass spectrometry for epinephrine auto-injector analysis [39]. Nesmarek et al. and Kudaleck et al. identified unknown and unspecified impurities and degradations products of some old pharmaceutical relicts using a RP-HPLC and HILIC-HPLC system connected with Mass Spectrometry [40, 41]. The assay of the drug substances was carried out by means of titrations, respectively. For determination of purity, the methods for related substances were used according to the European Pharmacopoeia (Ph. Eur.) [34]. In the case of SLEP, the FDA laboratories used methods which were requested from the in-house quality control of the manufacturers.

Table 1 Summary of expired drug products and drug substances experiment data

Trade name	Active pharmaceutical ingredient	Dosage form	Lots tested	Labeled amount	Age	Analysis past expiry	Extended lots	Extension Time in months	Measured Content (%)	Degradation products and impurities > 0.5%	Reference
Liquid dosage forms, e.g. injection solutions, auto-injector, etc.											
EpiPen	Epinephrine	Auto-injector	31	1.0 mg/ml	-	5-50 mos.	-	-	81-100	-	Cantrell et al. 2017
EpiPen Junior	Epinephrine	Auto-injector	9	0.5 mg/ml	-	1-30 mos.	-	-	81-99	-	
EpiPen	Epinephrine	Auto-injector	28	1.0 mg/ml	-	1-90 mos.	-	-	51-102 (79 ± 3)	-	Simons et al. 2000
EpiPen Junior	Epinephrine	Auto-injector	6	0.5 mg/ml	-	1-90 mos.	-	-	55-93 (72 ± 7)	-	
-	Ampicillin sodium	Injection sol.	8	-	-	-	8/8	57 (29-87)	-	-	Lyon et al. 2006
-	Atracurium besylate	Injection sol.	3	-	-	-	2/3	29 (27-30)	-	-	
-	Atropine sulfate	Auto-injector	687	-	-	-	495/687	57 (12-135)	-	-	
-	Atropine sulfate	Injection sol.	27	-	-	-	24/27	101 (19-216)	-	-	
-	Atropine sulfate-pralidoxime chloride	Auto-injector	5	-	-	-	5/5	31 (25-38)	-	-	
-	Bretylium tosylate	Injection sol.	4	-	-	-	4/4	49 (15-71)	-	-	
-	Bupivacaine HCl	Injection sol.	3	-	-	-	3/3	88 (79-95)	-	-	
-	Calcium chloride	Injection sol.	8	-	-	-	8/8	81 (66-106)	-	-	
-	Calcium glucepate	Injection sol.	8	-	-	-	8/8	49 (23-82)	-	-	
-	Chloroquine HCl	Injection sol.	4	-	-	-	4/4	64 (27-98)	-	-	
-	Chlorpromazine HCl	Injection sol.	3	-	-	-	3/3	74 (59-88)	-	-	
-	Cimetidine HCl	Injection sol.	7	-	-	-	7/7	42 (15-67)	-	-	
-	Clindamycin phosphate	Injection sol.	31	-	-	-	25/31	44 (18-77)	-	-	
-	Dexamethasone sodium phosphate	Syringe needle	7	-	-	-	7/7	61 (24-93)	-	-	
-	Dextrose 5%	Injection sol.	22	-	-	-	22/22	65 (13-128)	-	-	
-	Dextrose 10%	Injection sol.	4	-	-	-	4/4	25 (23-29)	-	-	
-	Dextrose and sodium chloride	Injection sol.	4	-	-	-	4/4	64 (51-73)	-	-	
-	Diazepam	Auto-injector	67	-	-	-	66/67	63 (12-100)	-	-	
-	Diazepam	Syringe needle	35	-	-	-	25/35	53 (12-105)	-	-	
-	Diphenhydramine HCl	Spray	2	-	-	-	0/2	No extension	-	-	
-	Diphenhydramine HCl	Syringe needle	12	-	-	-	12/12	76 (33-126)	-	-	
-	Dobutamine HCl	Injection sol.	3	-	-	-	3/3	47 (29-79)	-	-	
-	Edrophonium chloride	Injection sol.	4	-	-	-	4/4	65 (33-114)	-	-	
-	Enflurane	Liquid	8	-	-		8/8	48 (15-94)	-	-	
-	Epinephrine	Cartridge needle	33	-	-	-	17/33	22 (17-24)	-	-	
-	Ephedrine sulfate	Injection sol.	5	-	-	-	5/5	46 (21-80)	-	-	
-	Fentanyl citrate	Injection sol.	6	-	-	-	6/6	84 (70-96)	-	-	
-	Furosemide	Injection sol.	7	-	-	-	7/7	57 (31-90)	-	-	
-	Halothane	Liquid	12	-	-		12/12	67 (51-92)	-	-	
-	Heparin sodium	Injection sol.	16	-	-	-	14/16	52 (22-82)	-	-	
-	Hetastarch in sodium chloride	Injection sol.	5	-	-	-	5/5	44 (30-61)	-	-	
-	Hydrocortisone sodium succinate	Injection sol.	3	-	-	-	3/3	43 (37-56)	-	-	
-	Iothalamate meglumine	Injection sol.	7	-	-	-	7/7	51 (20-78)	-	-	
-	Isoproterenol HCl	Injection sol.	8	-	-	-	2/8	45 (37-53)	-	-	
-	Ketamine HCl	Injection sol.	6	-	-	-	6/6	64 (42-87)	-	-	
-	Levarterenol HCl	Injection sol.	8	-	-	-	1/8	22	-	-	
-	Lidocaine HCl	Injection sol.	15	-	-	-	14/15	58 (28-126)	-	-	
-	Lidocaine HCl and epinephrine	Injection sol.	9	-	-	-	1/9	29	-	-	
-	Mannitol	Injection sol.	10	-	-	-	10/10	66 (21-109)	-	-	
-	Mepivacaine HCl	Cartridge needle	3	-	-	-	3/3	41 (33-45)	-	-	
-	Meperidine HCl	Injection sol.	6	-	-	-	6/6	89 (32-128)	-	-	
-	Metaraminol bitartrate	Syringe needle	4	-	-	-	4/4	40 (33-47)	-	-	
-	Morphine sulfate	Auto-injector	3	-	-	-	3/3	32 (29-37)	-	-	

Trade name	Active pharmaceutical ingredient	Dosage form	Lots tested	Labeled amount	Age	Analysis past expiry	Extended lots	Extension Time in months	Measured Content (%)	Degradation products and impurities > 0.5%	Reference
-	Morphine sulfate	Injection sol.	10	-	-	-	9/10	79 (21-115)	-	-	
-	Morphine sulfate	Syringe needle	13	-	-	-	13/13	89 (35-119)	-	-	
-	Naloxone HCl	Injection sol.	10	-	-	-	10/10	77 (60-95)	-	-	
-	Neostigmine methylsulfate	Injection sol.	4	-	-	-	4/4	60 (31-78)	-	-	
-	Ophthalmic irrigating	Solution	6	-	-	-	6/6	52 (19-77)	-	-	
-	Pancuronium bromide	Injection sol.	13	-	-	-	13/13	79 (54-108)	-	-	
-	Phenobarbital sodium	Cartridge needle	4	-	-	-	2/4	56 (32-79)	-	-	
-	Phenylephrine HCl	Injection sol.	4	-	-	-	4/4	60 (53-78)	-	-	
-	Phenytoin sodium	Injection sol.	5	-	-	-	5/5	63 (29-100)	-	-	
-	Physostigmine salicylate	Injection sol.	14	-	-	-	4/14	31 (21-44)	-	-	
-	Povidone-iodine	solution	20	-	-	-	16/20	74 (29-144)	-	-	
-	Pralidoxime chloride	Auto-injector	412	-	-	-	399/412	120 (19-266)	-	-	
-	Prochloroperazine edisylate	Injection sol.	4	-	-	-	4/4	43 (28-66)	-	-	
-	Promethazine HCl	Injection sol.	9	-	-	-	9/9	51 (28-73)	-	-	
-	Ringer's lactated	Injection sol.	59	-	-	-	56/59	52 (23-125)	-	-	
-	Ringer's, lactated and dextrose	Injection sol.	13	-	-	-	13/13	53 (20-87)	-	-	
-	Sodium bicarbonate	Injection sol.	37	-	-	-	37/37	55 (14-101)	-	-	
-	Sodium chloride	Injection sol.	41	-	-	-	41/41	50 (12-113)	-	-	
-	Sodium chloride	Irrigation	16	-	-	-	16/16	72 (40-108)	-		
-	Sodium nitrite	Injection sol.	10	-	-	-	10/10	89 (35-180)	-	-	
-	Sodium thiosulfate	Injection sol.	14	-	-	-	14/14	131 (24-151)	-	-	
-	Tubocurarine chloride	Injection sol.	4	-	-	-	4/4	59 (47-69)	-	-	
Suprarenin	Epinephrine HCl	Injection sol.	1	1.0 mg/ml	83 yrs.	-	-	-	70.4	Sulfonic acid derivative (25.9%), norepinephrine (0.9%), unknown impurity (3.7%)	Zilker et al. 2018
Adrenalin in Oil	Epinephrine	Injection sol.	1	0.5 mg/ml	47 yrs.	-	-	-	74.3	Small traces of adrenochrome (0.4%)	
Effortil®	Etilefrine HCl	Injection sol.	1	0.03 g/ml	≥ 55 yrs.	-	-	-	98.1	-	
Sympatol®	Synephrine tartrate	Injection sol.	1	0.06 g/ml	≥ 55 yrs.	-	-	-	97.5	-	
Impletol	Procaine HCl	Injection sol.	1	20.0 mg/ml	68 yrs.	-	-	-	79.3	4-Aminobenzoic acid (15.3%)	
	Caffeine			14.2 mg/ml	68 yrs.	-	-	-	101.0	-	
Coffeinum Na. salicylicum	Caffeine and Sodiumsalicylicum	Injection sol.	1	0.2 g/ml	≥ 72 yrs.	-	-	-	100.4	-	
Persantin®	Dipyridamole	Injection sol.	1	5 mg/ml	50 yrs.	-	-	-	85.7	Seven unspecified impurities (0.5-5.7%)	
Lasix®	Furosemide	Injection sol.	1	10 mg/ml	53 yrs.	-	-	-	99.3	Saluamine (1.3%)	
Novalgin®	Metamizole sodium	Injection sol.	1	0.5 g/ml	≥ 53 yrs.	-	-	-	99.7	-	
Scopolaminium hydrobromide	Scopolamine hydrobromide	Injection sol.	1	1.0 mg/ml	50 yrs.	-	-	-	70.6	Tropic acid (30.2%)	Manuscript submitted, December 18th 2018
Dihydroergo-tamine mesilate	Dihydroergotamine mesilate	Injection sol.	1	1.0 mg/ml	≥ 43 yrs.	-	-	-	20.5	2'epi-9,10-Dihydroergotamine (71.0%), unknown impurities (4.1%, 3.4%)	
Cardiazol-Chinin	Quinine	Injection sol.	1	250 mg/ml	79 yrs.	-	-	-	87.2	Quinotoxine (12.8%)	Kudlacek et al. 2017
	Pentamethylenetetrazole			100 mg/ml		-	-	-	101.2	-	
Chinin-Calcium	Quinine	Injection sol.	1	30 mg/ml	77 yrs.	-	-	-	92.0	Quinotoxine (8.0%)	
	Calcium gluconate			100 mg/ml		-	-	-	97.6	-	
Strophosan	Quabain	Injection sol.	1	0.25 mg/ml	80 yrs.	-	-	-	56	Oxidized compound not quantified	Kudlacek et al. 2018
Eroina	Heroin	Injection sol.	1	5 mg/ml	76 yrs.	-	-	-	0	Morphine (96.1%), codeine (3.9%)	Nesmerak et al. 2010
Cocainum hydrochlor.	Cocaine	Injection sol.	1	10 mg/ml	71 yrs.	-	-	-	26.9	Benzoylecogomine (31.5%), ecogonine (17.4%), ecogonine methylester (24.2%)	

Trade name	Active pharmaceutical ingredient	Dosage form	Lots tested	Labeled amount	Age	Analysis past expiry	Extended lots	Extension Time in months	Measured Content (%)	Degradation products and impurities > 0.5%	Reference
Solid dosage forms, e.g. tablets, capsules, powders, and pure drug substances											
Somnafac	Methaqualone	Tablet or capsule	1	200.0 mg	-	28-40 yrs.	-	-	120	-	Cantrell et al. 2012
Fiorinal	Codeine	Tablet	1	7.5 mg	-	28-40 yrs.	-	-	99	-	
	Butalbital			50.0 mg	-		-	-	102	-	
	Aspirin			200.0 mg	-		-	-	1	-	
	Phenacetin			130.0 mg	-		-	-	110	-	
	Caffeine			40.0 mg	-		-	-	128	-	
Codempiral	Codeine	Tablet or capsule	1	32.4 mg	-	28-40 yrs.	-	-	90	-	
	Phenobarbital			16.2 mg	-		-	-	94	-	
	Aspirin			226.8 mg	-		-	-	1	-	
	Phenacetin			162.0 mg	-		-	-	54	-	
Bamadex	Meprobamate	Tablet or capsule	1	300.0 mg	-	28-40 yrs.	-	-	130	-	
	Amphetamine			15.0 mg	-		-	-	54	-	
Obocell	Amphetamine	Tablet	1	5.0 mg	-	28-40 yrs.	-	-	44	-	
Nebralin	Phenobarbital	Tablet or capsule	1	90.0 mg	-	28-40 yrs.	-	-	117	-	
Seconal	Secobarbital	Capsule	1	100.0 mg	-	28-40 yrs.	-	-	91	-	
Hycomine	Hydrocodone	Tablet or capsule	1	5.0 mg	-	28-40 yrs.	-	-	104	-	
	Homatropine			1.5 mg	-		-	-	-	-	
	Chlorpheniramine			2.0 mg	-		-	-	305	-	
	Acetaminophen			250.0 mg	-		-	-	100	-	
	Caffeine			30.0 mg	-		-	-	101	-	
Capoten	Captopril	Tablet	1	12.5 mg	54 mos.	18 mos.	-	-	100	-	Stark et al. 1997
Flucloxin	Flucloxacin	Capsule	1	250 mg	62 mos.	50 mos.	-	-	104 (initial value 113)	-	
Theo-Dur	Theophylline	Tablet	1	200 mg	149 mos.	113 mos.	-	-	98	-	
Mefoxin	Cefoxitin	Powder for injection	1	-	94 mos.	70 mos.	-	-	109 (initial value 111)	-	
-	Acetaminophen pseudophedrine	Capsules	3	-	-	-	3/3	24 (24-24)	-	-	Lyon et al. 2006
-	Albuterol	Inhalant	2	-	-	-	0/2	No extension	-	-	
-	Aluminium acetate	Tablets	12	-	-	-	10/12	52 (16-70)	-	-	
-	Amoxicillin sodium	Tablets	21	-	-	-	21/21	23 (22-23)	-	-	
-	Ampicillin	Capsules	5	-	-	-	5/5	49 (22-64)	-	-	
-	Amyl nitrite	Inhalant	6	-	-		6/6	59 (37-76)	-	-	
-	Benzonatate	Capsules	4	-	-	-	4/4	44 (12-73)	-	-	
-	Cephalexin	Capsules	6	-	-	-	6/6	57 (28-135)	-	-	
-	Cefazolin sodium	Powder	10	-	-	-	8/10	82 (63-110)	-	-	
-	Cefoperazone sodium	Powder	4	-	-	-	4/4	46 (25-57)	-	-	
-	Cefoxitin sodium	Powder	10	-	-	-	5/10	24 (24-55)	-	-	
-	Ceftriaxone sodium	Powder	4	-	-	-	4/4	60 (44-69)	-	-	
-	Cephapirin sodium	Powder	13	-	-	-	13/13	74 (50-114)	-	-	
-	Chloroquine phosphate	Tablets	38	-	-	-	36/38	40 (20-86)	-	-	
-	Chlorpromazine HCl	Tablets	15	-	-	-	15/15	52 (23-78)	-	-	
-	Cimetidine HCl	Tablets	5	-	-	-	5/5	67 (59-75)	-	-	
-	Ciprofloxacin	Tablets	242	-	-	-	242/242	55 (12-142)	-	-	
-	Ciprofloxacin	Suspension	7	-	-	-	7/7	32 (25-40)	-	-	
-	Codeine sulfate	Tablets	9	-	-	-	7/9	89 (16-114)	-	-	
-	Doxycycline hyclate	Capsules	13	-	-	-	13/13	76 (33-126)	-	-	
-	Doxycycline hyclate	Tablets	169	-	-	-	166/169	27 (15-91)	-	-	
-	Doxycycline hyclate	Powder	27	-	-	-	27/27	27 (14-52)	-	-	
-	Enalapril maleate	Tablets	3	-	-	-	2/3	34 (27-42)	-	-	
-	Ergotamine tartrate and caffeine	Tablets	8	-	-	-	4/8	24 (14-35)	-	-	
-	Erythromycin lactobionate	Powder	4	-	-	-	4/4	60 (38-83)	-	-	

Trade name	Active pharmaceutical ingredient	Dosage form	Lots tested	Labeled amount	Age	Analysis past expiry	Extended lots	Extension Time in months	Measured Content (%)	Degradation products and impurities > 0.5%	Reference
-	Flurazepam HCl	Capsules	3	-	-	-	3/3	35 (27-44)	-	-	
-	Guaifenesin	ER Tablets	7	-	-	-	7/7	85 (39-122)	-	-	
-	Mebendazole	Tablets	8	-	-	-	8/8	58 (28-89)	-	-	
-	Mefloquine HCl	Tablets	21	-	-	-	7/21	36 (17-94)	-	-	
-	Naproxen	Tablets	4	-	-	-	4/4	52 (46-62)	-	-	
-	Oxacillin sodium	Powder	13	-	-	-	13/13	56 (28-116)	-	-	
-	Penicillin G	Powder	15	-	-	-	14/15	49 (22-95)	-	-	
-	Penicillin G procaine	Powder	7	-	-	-	2/7	70 (67-72)	-	-	
-	Potassium iodide	Granules	5	-	-	-	5/5	254 (225-278)	-	-	
-	Potassium iodide	Tablets	12	-	-	-	12/12	69 (28-184)	-	-	
-	Pralidoxime chloride	Powder	80	-	-	-	78/80	88 (23-186)	-	-	
-	Primaquine phosphate	Tablets	12	-	-	-	8/12	55 (41-80)	-	-	
-	Protamine sulfate	Powder	4	-	-	-	4/4	64 (57-77)	-	-	
-	Pyridostigmine bromide	Tablets	152	-	-	-	141/152	61 (19-143)	-	-	
-	Sodium polystyrene sulfonate	Powder	3	-	-	-	3/3	55 (45-74)	-	-	
-	Succinylcholine chloride	Powder	9	-	-	-	9/9	72 (58-95)	-	-	
-	Sulfadoxine and pyrimethamine	Tablets	8	-	-	-	7/8	67 (34-93)	-	-	
-	Sulfisoxazole	Tablets	4	-	-	-	4/4	56 (45-68)	-	-	
-	Tetracycline HCl	Capsules	11	-	-	-	11/11	50 (17-133)	-	-	
-	Thiopental sodium	Powder	12	-	-	-	12/12	54 (23-96)	-	-	
-	Triamterene and hydrochlorothiazide	Capsules	6	-	-	-	6/6	19 (18-19)	-	-	
-	Undecylenic acid and zinc salt	Powder	9	-	-	-	9/9	68 (43-82)	-	-	
Metocard	Metoprolol	Tablet	1	50 mg	-	1 yr.	-	-	93	-	Jasinska et al. 2009
Metohexal	Metoprolol	Tablet	1	50 mg	-	1.5 yrs.	-	-	95	-	
Propranolol	Propranolol	Tablet	2	10 mg	-	1 yr., 4 yrs.	-	-	95, 90	-	
Asthmo-Karnit	Theophylline	Tablet	1		35 yrs.		-	-	91	-	Regenthal et al. 2002
-	Oxytetracycline	Drug sub.	1	-	54 yrs.	-	-	-	103.2	-	German et al. 2010
-	Oxytetracycline	Drug sub.	1	-	43 yrs.	-	-	-	99.6	-	
-	Oxytetracycline	Drug sub.	1	-	29 yrs.	-	-	-	99.7	-	
-	Doxycycline	Drug sub.	1	-	36 yrs.	-	-	-	97.5	-	
-	Colistin	Drug sub.	1	-	41 yrs.	-	-	-	101.0	-	
-	Spiramycin	Drug sub.	1	-	47 yrs.	-	-	-	-	-	
-	Amantadine	Drug sub.	2	-	≥ 20, 25 yrs.	-	-	-	-	-	Scholtissek et al. 1998
-	Rimanatadine	Drug sub.	1	-	≥ 25 yrs.	-	-	-	-	-	
-	Acebutolol	Drug sub.	2	-	27, 22 yrs.	-	-	-	100.7, 100.6	-	Manuscript submitted, December 18th 2018
-	Alprenolol	Drug sub.	1	-	24 yrs.	-	-	-	99.1	-	
-	Atenolol	Drug sub.	3	-	8, 22, 24 yrs.	-	-	-	99.7, 99.9, 99.3	-	
-	Bisoprolol	Drug sub.	1	-	24 yrs.	-	-	-	99.9	-	
-	Carteolol	Drug sub.	1	-	23 yrs.	-	-	-	100.7	-	
-	Metipranolol	Drug sub.	1	-	30 yrs.	-	-	-	101.2	Desacetylation (0.7%)	
-	Metoprolol	Drug sub.	2	-	24, 30 yrs.	-	-	-	100.2, 99.1	-	
-	Nadolol	Drug sub.	1	-	≥ 19 yrs.	-	-	-	98.7	-	
-	Oxprenolol	Drug sub.	1	-	≥ 19 yrs.	-	-	-	99.5	-	
-	Penbutolol	Drug sub.	1	-	≥ 19 yrs.	-	-	-	100.0	Three impurities (≤ 6%)	
-	Pindolol	Drug sub.	2	-	≥ 19, 25 yrs.	-	-	-	99.4, 99.0	-	
-	Timolol	Drug sub.	3	-	≥ 19 yrs.	-	-	-	99.7, 99.9, 100.2	-	

Trade name	Active pharmaceutical ingredient	Dosage form	Lots tested	Labeled amount	Age	Analysis past expiry	Extended lots	Extension Time in months	Measured Content (%)	Degradation products and impurities > 0.5%	Reference
-	Dobutamine HCl	Drug sub.	2	-	≥ 19, 25 yrs.	-	-	-	100.1, 100.1	-	
-	Etilefrine HCl	Drug sub.	1	-	46 yrs.	-	-	-	99.8	-	
-	Fenoterol HBr	Drug sub.	1	-	26 yrs.	-	-	-	99.5	Diastereomer (1.5%)	
-	Salbutamol sulfate	Drug sub.	6	-	≥ 19, ≥ 19, ≥ 23, ≥ 23, 28, 30 yrs.	-	-	-	99.9, 99.9, 99.9, 100.3, 98.4, 99.7	-	
-	Atropine sulfate	Drug sub.	1	-	≥ 19 yrs.	-	-	-	99.4	Tropic acid (0.6%)	
-	Hyoscine HCl	Drug sub.	1	-	22 yrs.	-	-	-	100.0	-	
-	Aciclovir	Drug sub.	2	-	28, 31 yrs.	-	-	-	100.6, 100.5	-	
-	Ampicillin sodium	Drug sub.	1	-	21 yrs.	-	-	-	92.0	Unknown impurity (2.4%)	
-	Ciprofloxacin	Drug sub.	1	-	25 yrs.	-	-	-	100.1	-	
-	Ofloxacin	Drug sub.	2	-	22, 23 yrs.	-	-	-	100.0, 100.0	-	
-	Bufexamac	Drug sub.	3	-	22, 22, 24 yrs.	-	-	-	98.6, 98.9, 100.3	-	
-	Flurbiprofen	Drug sub.	1	-	≥ 19 yrs.	-	-	-	100.3	-	
-	Mefenamic acid	Drug sub.	1	-	33 yrs.	-	-	-	100.2	-	
-	Naproxen	Drug sub.	1	-	29 yrs.	-	-	-	100.0	-	
-	Chlorprothixene HCl	Drug sub.	1	-	33 yrs.	-	-	-	99.8	-	
-	Perphenazine	Drug sub.	1	-	≥ 19 yrs.	-	-	-	100.4	-	
-	Dihydroergotamine mesilate	Drug sub.	1	-	26 yrs.	-	-	-	98.6	-	
-	Diphenhydramine in dimenhydrinat	Drug sub.	1	-	24 yrs.	-	-	-	54.6	-	
-	Diphenhydramine	Drug sub.	1	-	36 yrs.	-	-	-	100.3	-	
-	Propafenone HCl	Drug sub.	3	-	≥ 19, 21, 22 yrs.	-	-	-	100.1, 99.7, 99.0	-	
					Other dosage forms						
-	Cellulose, oxidized	Dermal	23	-	-	-	23/23	79 (28-137)	-	-	Lyon et al. 2006
-	Hexachlorophene cleansing	Emulsion	8	-	-	-	8/8	81 (58-106)	-	-	
-	Mafenide acetate	Cream	3	-	-	-	3/3	59 (56-63)	-	-	
-	Methylprednisone acetate	Suspension	3	-	-	-	2/3	38 (25-51)	-	-	
-	Neomycin and polymyxin B sulfates and bacitracin zine	Opht. ointment	5	-	-	-	4/5	28 (12-40)	-	-	
-	Penicillin G benzathine	Suspension	4	-	-	-	4/4	70 (61-84)	-	-	
-	Povidone-iodine	Ointment	7	-	-	-	7/7	65 (35-134)	-	-	
-	Spectinomycin HCl	Suspension	8	-	-	-	7/8	83 (55-109)	-	-	
-	Sulfacetamide sodium	Opht. ointment	4	-	-	-	3/4	39 (35-44)	-	-	
-	Sulfadiazine silver	Cream	37	-	-	-	37/37	57 (28-104)	-	-	

3. Stability of various dosage forms

There is scattered literature information addressing the long-term stability of recently expired drug products and historical pharmaceutical relicts. The studies found upon literature survey showed a high variability in analysis of tested dosage forms, quantities of lots, and drug classes. In the present overview, drug products were categorized into liquid dosage forms, which are mainly solutions for injection, as well as solid dosage forms, such as tablets, capsules, powders, and pure drug substances. Notable results of individual FPPs and selected drug classes that typically occur in the respective dosage forms were discussed in the following chapters. All results are listed in Table 1.

3.1. Liquid dosage forms

Especially parenteral dosage forms are widely used in emergency medication, so they are stockpiled in large quantities in hospitals as well as federal, state, and local health agencies.

3.1.1. Adrenaline and related sympathomimetics

In recent years, pharmacists and stockpile managers have questioned the quality of epinephrine auto-injectors beyond their expiry dates. Cantrell et al. analyzed 31 expired EpiPens containing 1.0 mg/ml of epinephrine and nine EpiPen Junior containing 0.5 mg/ml of epinephrine [39]. The devices were 1–50 months beyond their expiration dates. 19 of the EpiPens and five of the EpiPens Junior contained at least 90% of the labeled epinephrine concentration. No content was found less than 80%. In a former study by Simons et al., 28 EpiPens and six EpiPens Junior with an age of 1–90 months after their expiration date were assayed [33]. The content range was 51–102% for the expired EpiPens with a content of 105–111% referenced to in-date pens. In the EpiPens Junior, a content range of 55–93% was measured compared to 86–114% for fresh solutions. In two devices a pinkish-brown discoloration was observed. Both authors supposed that in the case of a life-threating situation like anaphylaxis there would be a greater benefit of using an expired EpiPen than taking the risk of low dose application or no epinephrine treatment if only an outdated injector being available.

33 lots of epinephrine injection solution were part of SLEP as reported by Lyon et al., whereby an extension of usability was issued for 17 lots by an average of 22 months [7]. The extension of related sympathomimetics like isoprenaline (isoproterenol) and norepinephrine (levarterenol) was denied in 75% and 88% of the lots tested. The reason was the decrease of the API contents. In the case of metaraminol, which is another potent sympathomimetic drug, four lots were tested, no instability was observed.

Furthermore, one very old Suprarenin ampoule manufactured by Bayer in 1934 was analyzed by us [34]. The content was found to be 70.4%. Additionally, the sulfonic acid derivative of epinephrine was identified to be the main degradation product. It has been formed by a nucleophilic substitution of the antioxidant sodium metabisulfite during long term storage, assayed at 25.9% (Figure 2) [25]. The content of another epinephrine ampoule, Adrenalin in Oil, decreased to 74.3% and traces of adrenochrome were noticed. No decomposition was observed in related sympathomimetics like Sympatol®, containing synephrine, and Effortil®, containing etilefrine. Both ampoules had an age of at least 55 years, assayed at 97.5% and 98.1% of the labeled concentrations, respectively.

The chemical stability of members without catechol moiety, such as etilefrine, synephrine, and metaraminol, was found to be higher in comparison with derivatives like epinephrine, norepinephrine, and isoprenaline.

3.1.2. Analgesics

The opioid analgesics morphine, fentanyl, and meperidine (pethidine) were SLEP participants [7]. Noteworthy, 13 lots of morphine sulfate syringe needles were extended by 89 months in average, without any failure in lot testing. Ten morphine injection solutions were extended by 79 months, but only one lot failed due to particulates found in the FPP. For fentanyl and meperidine injection solutions, shelf-lives of all lots were prolonged to an average of 84 and 89 months, respectively. We examined one metamizole (Novalgin®) ampoule with an age of at least 53 years, resulting in 99.7% of the claimed concentration of metamizole sodium [34]. In a 72 years old ampoule containing sodium salicylate combined with caffeine, a content of 100.4% was measured without observing any degradation.

3.1.3. Anaesthetics

3.1.3.1. Local anaesthetics

Characteristically, local anaesthetics consist of an ester or an amide moiety. These functional groups are prone to hydrolysis. An Impletol ampoule produced in 1949 was declared to contain 2% of procaine hydrochloride and 1.42% of caffeine [34]. Procaine content decreased to about 79.3% and 15.3% of 4-aminobenzoic acid has been formed after 68 years of storage. Various local anaesthetics were part of SLEP [7]. Nearly all lots of bupivacaine, lidocaine, and mepivacain were on average extended by 88 months, 58 months, and 41 months, respectively. Eight out of nine lidocaine HCl in combination with epinephrine injection solutions were identified to be less stable due to deviations in initial content. Hence, extension of shelf-life was denied.

3.1.3.2. Inhalative anaesthetics

The two general anesthetics enflurane and halothane were revealed to be non-sensitive to instability by Lyon et al. [7]. Eight lots of enflurane and twelve lots of halothane were tested. The extension of shelf-lives beyond their original expiry dates was determined to be in mean of 48 months and 67 months, respectively.

3.1.4. Antidotes

The DOD shelved large amounts of antidotes, e.g. atropine, pralidoxime, neostigmine, physostigmine, and sodium thiosulfate, which are used for the treatment of poisoning in emergency situations [7]. 687 lots of atropine sulfate auto-injectors were tested and subsequently 72% were extended by an average of 57 months (12–135 months). The extension of lots was denied in some cases because of failures in assay and appearance. An 89% extension rate was reported for atropine injection solutions (19–216 months). 399 out of 412 lots of pralidoxime auto-injectors were extended to over 10 years. Amongst other, a failure criterion was not meeting specifications of content and appearance of the solution and in one case an injector misfire occurred. Also, five combination preparations of atropine sulfate and pralidoxime chloride auto-injectors were found to be stable. Likewise, neostigmine methylsulfate and physostigmine salicylate, both agents blocking acetylcholinesterase, were tested without any failure. Sodium thiosulfate which is used for the treatment of cyanide poisoning was identified to be stable; consequently, the shelf-lives of all 14 lots were extended by a range of 24–131 months.

3.1.5. Anti-infectives

Only a few antibiotic injection solutions were part of SLEP [7]. Despite of being susceptible for instability, pharmaceutical preparations consisting of ampicillin sodium or clindamycin phosphate could be used beyond their original expiry dates for averagely 57 and 44 months, respectively. Chloroquine used for the treatment of malaria was extended by 64 months based on stability tests.

3.1.6. Historical natural compounds

Identification of the degradation products was the main focus of the authors reporting about investigations of some historical pharmaceutical relicts. We investigated two old ampoules with an age of about 50 years, still containing 70.6% of scopolamine hydrobromide and 20.5% of dihydroergotamine mesilate (DHE) (manuscript submitted, Drug Testing and Analysis, December 18th 2018). Scopolamine was hydrolyzed to about 30.2% of tropic acid. The impurity 2'-*epi*-9,10-dihydroergotamine, being specified in the Ph. Eur., has been formed

of about 70.5% in the DHE injection solution. In addition, two unknown impurities of 3.4% and 4.1% were detected.

Kudlacek et al. assayed two quinine ampoules, resulting in 87.2% of the 1937 ampoule and 92.0% of the 1939 ampoule [42]. Quinine was found to be partially decomposed to quinotoxine in both sample solutions. When considering the age of the ampoules, the degree of degradation seems to be very low. Furthermore, Kudlacek et al. quantified an 80-year-old Strophosan ampoule by means of HPLC-MS, initially consisting of 0.025% of ouabain [40]. The content of the cardiacally active compound decreased to about 56% of the declared concentration. Oxidation of the hydroxyl group in position 10 of the steroid ring was postulated to be responsible.

Nesmerak et al. analyzed two historical anestesistic pharmaceutical preparations consisting of heroin and cocaine [41]. A heroin injection solution from 1933 was completely decomposed by deacetylation to 96.1% of morphine. 3.9% of codeine was formed as well. The long-term stability of morphine was confirmed in other investigations by Lyon et al. and Roksvaag et al. [7, 43]. In the second ampoule, 26.9% of cocaine was left in the sample manufactured between 1932 and 1938. Cocaine was decomposed to 31.5% of benzoylecgonine, 17.4% of ecgonine, and 24.2% of ecgonine methyl ester.

3.2. Solid dosage forms

Several solid dosage forms, such as tablets, capsules, and powders, as well as pure drug substances were part of stability investigations.

3.2.1. Analgesics

Cantrell et al. analyzed tablets and capsules with an age of 28–40 years beyond expiry, including eight prescription drug products consisting of acetaminophen (paracetamol), acetylsalicylic acid, codeine, hydrocodone, and phenacetin. The agents were combined in the FPPs with other APIs of various drug classes [38]. Chemical instability was observed for acetylsalicylic acid that was contained in Fiorinal® and Codempiral® and was completely decomposed by hydrolysis of the acetylic moiety. The resulting API content was about 1%, respectively. In one out of two FPPs, the phenacetin content was out of specification. Altogether, twelve out of 14 drug compounds were found to be present in concentrations of at least 90%, even 28–40 years after production. Also, Lyon et al. reported SLEP investigations of acetaminophen combined with pseudoephedrine capsules, naproxen tablets, and codeine sulfate tablets; the lots of the drug products were extended by an average period of 24, 52, and 89 months [7]. In an additional study dealing with several non-steroidal anti-inflammatory drugs like bufexamac, flurbiprofen, mefenamic acid, and

naproxen by us, all batches complied with pharmacopoeial requirements with regard to content and degradation products after at least 19–33 years of storage (manuscript submitted, Drug Testing and Analysis, December 18th 2018).

3.2.2. Anti-infectives

3.2.2.1. Tablets and capsules

Antibiotics were a most common drug class of solid oral dosage forms which participated in SLEP. Large quantities of ciprofloxacin tablets, doxycycline tablets, and doxycycline-hyclate capsules were tested [7]. All of the 242 ciprofloxacin lots were extended beyond their initial expiry dates by an average period of 55 months, ranging between 12 and 142 months. 166 out of 169 lots of doxycycline tablets were extended by a mean of 27 months (15–91 months) and the use period of the 13 lots of doxycycline-hyclate capsules was prolonged for 76 months (33–126 months). Furthermore, a small number of tablet and capsule lots of amoxicillin sodium, ampicillin, cephalexin, sulfisoxazole, and tetracycline was tested successfully and consequently, extended. Several drug products such as chloroquine, primaquine, and sulfadoxine in combination with pyrimethamine, all used for the treatment of malaria diseases, were identified to be stable. The exceptions were mefloquine tablets because extension of use period was issued only for less than 50% of the lots. Here, a variation of dissolution rates of the tablets was responsible for not meeting the specification criteria.

Stark et al. analyzed the chemical and physical stability of Flucloxin® capsules consisting of 250 mg of flucloxacillin sodium [35]. Initially, the average flucloxacillin amount of 113% was noticed in the capsules. After 62 months of storage, the content was still about 104%. Nevertheless, a lower release rate of the Flucloxin® capsules was observed over time, not complying with the demanded specification.

3.2.2.2. Powders and drug substances

Several antibiotic classes, e.g. penicillin, cephalosporines, and tetracyclines, were part of SLEP [7]. The shelf-life of all powder lots of cefoperazone sodium, ceftriaxone sodium, cephapirin sodium, doxycycline hyclate, erythromycin lactobionate, and oxacillin sodium was extended, respectively. Cefazolin sodium, cefoxitin sodium, penicillin G, and penicillin G in combination with procaine were found to be less stable. Only two out of seven lots of penicillin G in combination with procaine met the specification requirements in an initial test. For cefoxitin sodium, the expiry dates of five out of ten lots were updated by an average of 24 months. A further cefoxitin batch was analyzed by Stark et al., resulting in a content of 109% after 94 months of storage [35]. The initial value measured was 111%.

In a study by German et al. in 2010, four antibiotic standards with an age of 29–54 years were assayed [44]. All standards were kept in a refrigerator or a freezer and were found to be stable. The contents ranged between 97.5 and 103.7% by means of HPLC. Results obtained by an alternative microbiological method were in the range of 96.4–101.5%. Oxytetracycline, doxycycline and colistin complied with Ph. Eur. 6.0 limits, respectively. Only spiramycin did not meet the Ph. Eur. monograph requirements.

A collection of some 21–31 years old anti-infectives consisting of acyclovir, ampicillin sodium, ciprofloxacin, and ofloxacin were examined by us (manuscript submitted, Drug Testing and Analysis, December 18th 2018). With the exception of ampicillin sodium, the agents met pharmacopoeial criteria with regard to API content and degradation products.

In 1998, two antiviral agents stored for a least 25 years at ambient temperature were studied by Scholtissek et al. [45]. The activity of amantadine and rimantadine was determined by means of a biological assay. Here, Madin Darby Canine Kidney cells were utilized throughout the study and were infected with the A/Singapore/1/57 (H2N2) influenza virus. No loss of activity of both substances was measured referenced to fresh ones. The authors suggested that large quantities of amantadine and rimantadine could be stored over a long time period in order to be prepared for possible future influenza A pandemic in humans.

3.2.3. Betablockers

The content of four tablet batches of betablockers like metoprolol and propranolol was determined in the study of Jasinska [36]. The tablets were 1–4 years beyond expiry. All tablets analyzed were observed to be in the demand range of the declared value. The high stability of this drug class was confirmed by an investigation of twelve various betablockers, such as acebutolol, alprenolol, atenolol, bisoprolol, carteolol, metipranolol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, and timolol (manuscript submitted, Drug Testing and Analysis, December 18th 2018). A low degree of deacetylation of metipranolol, used in eye drops to treat glaucoma, and the formation of unspecified impurities in the case of penbutolol, applied for the treatment of high blood pressure, were described. No degradation could be ascertained for all the rest.

3.2.4. Xanthine derivatives

In a case report of a theophylline poisoning, the tablets showed a stable content of 91% after 35 years of storage [46]. Furthermore, Stark et al. measured a theophylline content of about 98% in a 12-year-old Theo-Dur tablet [35]. Also, a high stability was reported for caffeine in other studies [34, 38]. Generally, xanthine derivatives are considered to be stable.

3.2.5. Psychotropic drugs

The group of antipsychotics was represented by chlorpromazine, chlorprothixene, and perphenazine. The shelf-life extension of chlorpromazine was determined to averagely 52 months [7]. A low degree of sulfoxide degradation was reported for chlorprothixene and perphenazine, even after 33 and 19 years of storage, respectively (manuscript submitted, Drug Testing and Analysis, December 18th 2018). Furthermore, the hypnotics thiopental, butalbital, methaqualone, phenobarbital, and secobarbital were investigated by Lyon et al. and Stark et al., without finding a significant decay of any API [7, 35]. In recent years, the drug shortage of thiopental, a key anaesthetic in lethal injection, has delayed executions because of the export ban by the European Union severing U.S. prisons from the manufacturers of sodium thiopental.

3.2.6. Inhalative sympathomimetics

In one of our studies, six batches of salbutamol drug substance were analysed (manuscript submitted, Drug Testing and Analysis, December 18th 2018). With the exception of one lot, salbutamol batches met the Ph. Eur. criteria regarding the content and related substances. Small amounts of synthetic by-products were found in all samples. No significant degradation was revealed for fenoterol hydrobromide, too. Two lots of albuterol (salbutamol) inhalants being part of SLEP were not extended due to deviations in content results [7].

4. Extension programs

4.1. American SLEP

In 2006, Lyon and his colleagues reviewed and analyzed data from 122 drug products generated by SLEP since 1986 [7]. 3005 lots were included in the analysis over a timeframe of 20 years. 2652 (88%) of all lots were initially extended for at least one year after their original expiration dates. The average extension period was 66 months. A classification system was used by the authors for categorization of the drug products into five groups, depending on the incidence of initial extension failures and termination failures when retesting initially extended lots. Drug products belonging to the group without any failure in the stability screening of more than ten lots were, among others, amoxicillin sodium tablets, ciprofloxacin tablets, doxycycline capsules, naloxone HCl injection solutions, halothane liquids, diphenhydramine syringe-needles, morphine syringe-needles, and various saline injection solutions. On the other hand, less stable drug products were verified, such as albuterol inhalants, mefloquine HCl tablets, lidocaine HCl and epinephrine injection solutions, penicillin G in combination with procaine powders, and physostigmine salicylate injection solutions. These drug products were all assigned to the group in which less than 50% of the

tested lots were extended. The author concluded that the actual shelf-life of many FPPs was much longer than the original shelf-life which is in accordance with many additional studies reported in this review. A high lot-to-lot variability was observed. Furthermore, periodic testing and systematic assessment of each lot is required when the extension of shelf-life is intended. After 30 years of SLEP, the program just comprises of federal agencies. Participants are the DoD, SNS, Department of Veterans Affairs, Bureau of Federal Prisons. Civilian agencies are not authorized to take part in the program [14].

4.2. Medicines of the german Military (Bundeswehr)

As mentioned before, the Bundeswehr and other federal agencies are not required to indicate terminated expiry dates for medicines corresponding to section 71 of the German Medicinal Products Act [22]. However, all stockpiled drug products of the Bundeswehr undergo long-term stability tests in their laboratories under extreme climate conditions such as found in potential crisis areas around the world [8]. Based on the results, individual expiry dates or re-test dates for these items are determined. A report in the German journal of military medicine (Wehrmedizin and Wehrpharmazie) claimed that 25-year-old morphine auto-injectors were stored under controlled conditions without showing a decrease in quality. Furthermore, huge amounts of atropine and atropine-obidoxime auto-injectors used in military or civil crisis were part of their depots. All drug products were tested in a two-year period to assess whether to comply with the predefined specifications. Unfortunately, no systematically long-term stability data are scientifically published, being different from SLEP.

4.3. Drug shortages and supply interruptions

In 2010, the FDA responded to the H1N1 influenza and seasonal influenza with approved supplemental new drug applications for Relenza®, containing zanamivir, and Tamiflu®, containing oseltamivir, capsules and tablets. In a first issue, the use period of both drug products was extended for a timeframe of seven years after their date of manufacture. However, three years later, the extension period was updated for a maximum of 10 years [47, 48].

Although the pharmaceutical manufacturers are obliged to notify the FDA about current or expected supply interruptions, the number of drug shortages has increased in recent years [49]. The FDA responded with the extension of expiry dates of FPPs that are already in the market. In an announcement addressing to health care professionals and patients, the use dates of selected parenteral lots of aminophylline, epinephrine, atropine sulfate, sodium bicarbonate, and dextrose 50% provided by Pfizer were updated [50]. Based on stability data provided by the manufacturer which were reviewed by the agency, the expiration dates were extended by 4–12 months (see Table 2). In a further issue, the FDA alerted health care

professionals of new extended shelf-lives for various injection solutions manufactured by Baxter Healthcare corporation, e.g. saline, sterile water, potassium chloride, etc. [51].

In addition, the FDA updated the expiry dates of auto-injectors used as nerve agent antidotes in emergency situations. Certain lots of AtroPen (atropine), CANA (diazepam), DuoDote, containing atropine and pralidoxime chloride, morphine sulfate, and pralidoxime chloride injectors all manufactured by Meridian Medical Technologies were declared to be eligible for use by up to six years beyond their original expiry dates (see Table 3) [50].

Table 2
Extended drug products in 2018 (provided by Pfizer)

Extended drug products due to supply interruptions	Extension time (months)	Number of lots extended
Aminophylline injection	5	4
Epinephrine injection, USP 0.3 mg Auto-Injectors	4	25
EpiPen® 0.3 mg Auto-Injectors	4	29
Atropine sulfate injection, USP 0.1 mg/ml; 5 ml Abboject syringe	6	4
Atropine sulfate injection, USP 0.1 mg/ml; 10 ml Abboject syringe	12	27
Atropine sulfate injection, USP 0.1 mg/ml; 10 ml Ansyr Plastic syringe	6	13
Dextrose 50% injection, USP, 50 mL Abboject syringe	12	20
Dextrose injection 50% (0.5 g/ml); 25 g/50 ml Ansyr Plastic syringe	6	70
Epinephrine injection, USP 0.1 mg/ml; 10 ml Abboject syringe	9	98
Sodium bicarbonate injection, USP 8.4%; 50 mEq/50 ml Abboject Glass	6	46
Sodium bicarbonate injection, USP 8.4%; 50 mEq/50 ml single dose glass fliptop vial	5	51
Sodium bicarbonate injection, USP 8.4%; 50 mEq/50 ml single dose glass fliptop vial (labeled as Novaplus)	5	2

Table 3
Extended antidotes for emergency use (FDA)

Drug products eligible for use for nerve agent emergencies beyond the manufacturer-assigned expiry date	Extension time (months)	Number of lots extended
AtroPen (atropine)	36	8
CANA (diazepam)	60	23
DuoDote (Atropine and pralidoxime chloride Injection)	72	26
Pralidoxime chloride	60	3
Morphine sulfate	24	8

4.4. Guidance drafts

Large quantities of potassium chloride tablets as well as doxycycline tablets and capsules are stockpiled by federal agencies, by state and local governments, and by some private sectors in order to ensure that critical medicines remain available in sufficient quantities during emergencies, i.e. radiation catastrophes or flu outbreaks. The Center for Drug

Evaluation and Research of the U.S. Department of Health and Human Services FDA published guidance drafts on testing to extend the shelf-life of the mentioned drug products. If the medicines mentioned before are successfully tested, additional shelf-life extensions in increments of two years can be issued [16, 17].

5. Conclusion

This review has collected data showing that the shelf-life of most FPPs can be extended beyond their labeled expiry dates. It was not uncommon that the actual shelf-life exceeded the manufacturer assigned one by three- or four-fold. Noteworthy, surprisingly long shelf-lives were noticed for injection solutions because of being susceptible to instability in a particular high manner. Except for the occasional one, solid dosage forms appeared to be most stable when reaching their expiry dates. However, the stability of an API is crucially depending on the chemical structure of the molecule, whereas the presence of reactive moieties leads to degradation. Furthermore, critical factors affecting the stability of the API are the dosage form, the interference with present excipients and antioxidants, as well as the prevailing environmental storage conditions. Hence, the surveyed studies showed high product-to-product and lot-to-lot variability.

However, it is difficult for consumers to assess whether a FPP could have an extension of shelf-life or not. The responsibility of the manufacturer for safety and efficacy ends on the first day after the expiry date. Due to financial interests, expiry periods are arbitrarily set as short as possible; so stockpiles are discarded and new ones are purchased regularly. Disposing of still potent medicines which reach their expiry dates is not acceptable any longer, as long as populations of some industrial and developing countries are unable to afford urgently needed medications. Hence, use dates should not be exceeded only in an exceptional case like a public health pandemic as issued by the FDA. The obligation of testing all pharmaceutical products during longer periods and the publication of the actual shelf-life according to scientific knowledge may be considered by the regulatory authorities, when the manufacturers submit application for market authorization. Maybe it is an option to replace the expiry dates by re-testing dates.

Conflict of interest

The authors declare that they have no conflict of interest.

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2. A long-time stability study of 50 drug substances representing common drug classes of pharmaceutical use

Zilker, M., Sörgel, F., Holzgrabe, U.

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Abstract

For assurance of the quality of active pharmaceutical ingredients used for manufacturing medicines, the European Pharmacopoeia has a binding character. Within a particular timeframe a substance is considered to comply with predefined specifications. Hence, it is applicable to manufacture a finished pharmaceutical product. The objective of the study presented here was to assess the long-term stability of 50 drug substances with an age of 20–30 years or even older in some cases. The substances are part of a collection of old pharmaceuticals at the Institute for Pharmacy in Würzburg, Germany, and represent commonly used drug classes containing β-blockers, β-sympathomimetic drugs, anticholinergics, anti-infectives, non-steroidal anti-inflammatory drugs, antipsychotics, antihistaminic drugs, and one antiarrhythmic drug. The content and the degradation profile of the items were determined by means of potentiometric titration and liquid chromatography techniques based on pharmacopoeial approaches for impurity profiling covering all process and degradation related substances. The results of the study show that 44 out of 49 tested substances still complied with specifications of the current pharmacopoeias. For metipranolol which is not monographed in any pharmacopoeia, small degradation by hydrolysis was observed. In one lot of ampicillin sodium, atenolol, atropine, penbutolol, and salbutamol, at least one impurity did not meet the acceptance criteria, respectively. Some impurities were not related to degradation. However, most of the agents could be used for manufacturing of finished pharmaceutical products, even after more than two decades of storage.

Abbreviations: API, active pharmaceutical ingredient; FPP, finished pharmaceutical product; Ph. Eur., European Pharmacopoeia; USP, United States Pharmacopoeia; EDQM, European Directorate for the Quality of Medicines & HealthCare; CEP, Certificate of suitability to the monograph of the European Pharmacopoeia; TLC, thin layer chromatography; NSAID, non-steroidal anti-inflammatory drug; ICH, International Conference on Harmonization guideline; RRT, relative retention time;

1. Introduction

A pharmaceutical manufacturer can use an active pharmaceutical ingredient (API) for manufacturing of finished pharmaceutical products (FPPs) as long as the stability of the drug substance is ensured and no decrease in quality occurs. Therefore, the regulatory authorities ask for quality standards according to the current pharmacopoeias [1]. It is common pharmaceutical practice to set a retest date on an API, not an expiry date [2]. The purpose of a retest period which is based on stability tests is to ensure that the drug substance is still suitable for use [3]. If it remains within established specifications after this time, the shelf-life can be extended by setting a subsequent timeframe. Lots can be retested multiple times unless they continue to comply with the respective specifications, but the total shelf-life of a drug substance should not exceed five years following industry and pharmacy laboratory practice [4, 5]. However, corresponding to the European Pharmacopoeia (Ph. Eur.) the content of an API is most commonly specified as 100% ± 1%. Unspecified impurity is usually limited to 0.1% and specified impurity mostly to 0.1–0.5% depending on the daily intake [1]. The manufacturer of a FPP is committed to submit stability studies of the API and of the FPP to regulatory authorities when submitting the application of approval [6]. For new active substances not described in the Ph. Eur., quality data of API and FPP are required [7]. However, in a growing number of cases, the manufacturer of the finished drug product is not the manufacturer of the drug substance itself. If an existing active substance is already described in the Ph. Eur., the European Directorate for the Quality of Medicines & HealthCare (EDQM) is authorized to grant a “Certificate of suitability to the monograph of the European Pharmacopoeia” (CEP) to the manufacturer of the drug substance, justifying that all potential process impurities and degradation products are adequately controlled in relation to the manufacturing method actually used [8]. In this case the manufacturer of the FPP can rely on the CEP and no further stability data of the drug substance are required [7]. Nevertheless, the manufacturer must set a shelf-life for the FPP based on stability tests. Corresponding to ICH guidelines Q1A(R2) on stability testing, a significant change in FPP is defined as a 5% deviation in assay from its labeled value [6]. Initially, an approval is limited to five years according to the national medicinal products act, consequently the shelf-life is set to five years at the maximum, but typically shorter [9, 10]. When submitting an application for prolongation, the shelf-life is not reevaluated.

There is rare information reported about the long-term stability of drug substances. One study revealed that reference standards of oxytetracycline, doxycycline, colistin, and spiramycin remained stable for longer than 40 years and one for over 50 years of storage [11]. However, only a few more studies investigating the long-term stability of FFPs beyond

their expiration dates can be found in the literature. Most of these studies conclude that actual shelf-lives are often beyond the expiration dates [12–17].

In a previous study nine parenterals manufactured in the last century were chromatographically investigated with regard to content and degradation products [18]. In spite of their high age of 53–72 years, five out of nine ampoules were still within the content limits of 95–105%, e.g. metamizole (Novalgin®), furosemide (Lasix®), etilefrine (Effortil®), synephrine (Sympatol®), and caffeine and sodium salicylicum (Caffeinum Salicylicum).

Within this systematic study, 50 drug substances representing various drug classes, e.g. β-blockers, β-sympathomimetic drugs, anticholinergics, anti-infectives, non-steroidal anti-inflammatory drugs (NSAIDs), antipsychotics, antihistaminic drugs, and one antiarrhythmic drug were investigated considering physical and chemical changes that could have occurred during long-term storage of at least 20 years. The drug substances were part of a collection of old pharmaceuticals at the Institute of Pharmacy and Food Chemistry in Würzburg. The storage is assumed to be under ambient temperature and light protection that may have been fluctuating. Generally, some of the agents selected are susceptible to instabilities by hydrolysis, e.g. atropine and scopolamine, by dimerization, e.g. ampicillin sodium, and by epimerization, e.g. dihydroergotamine [19]. Especially the sulfur of the perphenazine and chlorprothixene could be oxidized easily [20]. However, the class of β-blockers, β-sympathomimetic drugs, and NSAIDs were expected to be stable before beginning with the experiments.

2. Experimental

2.1. Chemicals and reference substances

All reagents were of analytical grade. Phosphoric acid 85%, sodium dodecylsulfate, anhydrous sodium acetate, ammonium acetate, tetrahydrofuran, acetic acid 99%, glacial acetic acid, anhydrous acetic acid, sodium hydroxide 50%, methylene chloride, ammonium phosphate monobasic, sodium phosphate monobasic, potassium phosphate monobasic, nitric acid, lithium, ammonium ferric sulfate dodecahydrate, sodium octanesulfonate, ammonium hydroxide solution 28–30%, dimethyl sulfoxide, tetrabutylammonium hydrogen sulfate, tetrabutylammonium bromide, hyoscine hydrobromide, diphenhydramine hydrochloride, ampicillin anhydrous, 4-methoxybenzaldehyde, 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid, potassium hydrogen phthalate, benzoic acid, and ethanol were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany), sodium hexanesulfonate and tetrahydrofuran from Alfa-Aesar GmbH & Co. KG (Karlsruhe, Germany), anhydrous acetic acid, acetic anhydride, dimethyl sulfoxide, disodium hydrogen phosphate, potassium hydrogen sulfate, sodium heptanesulfonate, HPLC grade

acetonitrile, HPLC grade methanol, Hydranal™ - Solvent, and Hydranal™ - Titrant 5 from VWR International GmbH (Darmstadt, Germany), trimethylamine, dimethylformamide, methylene chloride, and toluene HPLC grade from Fisher Scientific (Loughborough, United Kingdom), dihydroergotamine mesilate, dihydroergotamine for peak identification CRS, hyoscine hydrobromide impurity B CRS, and salbutamol sulfate for system suitability form EDQM (Strasbourg, France), anhydrous formic acid, 0.1 M silver nitrate, 0.1 M ammonium thiocyanate, 0.1 M perchloric acid, 0.1 M sodium hydroxide, and 0.1 M hydrochloric acid from Bernd Kraft GmbH (Duisburg, Germany). Water for HPLC was purified using Milli-Q purification system by Merck Millipore (Schwalbach, Germany).

2.2. Apparatus

HPLC experiments were performed on a HPLC system 1100 series from Agilent Technologies (Waldbronn, Germany) consisting of a vacuum degasser (G1322A), binary pump (G1312A), autosampler (G1313A), thermostated column oven (G1316A), diode array detector (G1315B). Chromatograms were recorded and integrated using the Agilent ChemStation® software (Rev B.03.02). Titrations were performed on a TitroLine® 7000 and water content was determined on a TitroLine® 7500KF from SI Analytics (Mainz, Germany). For pH-measurments a Metrohm 744 pH-Meter from Deutsche METROHM GmbH Co. KG (Filderstadt, Germany) was used.

2.3. Tested substances

All drug substances were manufactured between 1972 and 1999. An alphabetical list of all compounds analyzed can be found in supporting information. In addition, information about the batch label, the year of manufacturing, and the chemical structure are given.

2.4. Methods

The methods utilized were in accordance with the Ph. Eur. 9.3 and the United States Pharmacopoeia 40 - NF 35 (USP) as far as monographs were available [1, 21]. Titrations and HPLC-UV were used for assay. Liquid chromatographic methods were applied for the assessment of related substances. Thin layer chromatography (TLC) methods were carried out whenever it was required in the Ph. Eur. for detecting of potential degradation products. Water content was determined by means of Karl-Fischer titration. Respective monograph numbers, specifications, titrants, as well as chromatographic conditions are listed in Table 1.

Table 1

Methods

Drug substance	Assay	Chromatographic conditions		
Monograph number	Specification and titrant	Column	Mobile Phase	Settings
Acebutolol HCl., 01/2008:0871	99.0–101.0 0.1 M NaOH	Agilent Zorbax Eclipse Plus C18 (150x4.6 mm; 5 µm)	**A:** 2.0 ml of H_3PO_4 and 3.0 ml of TEA diluted to 1000 ml with water; **B:** A, ACN (50:50 V/V)	Gradient, 1.9 ml/min, 240 nm, 40 °C, 25 µl
Aciclovir, 01/2014:0968	98.5–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	**A:** ACN, buffer (3.48 g/L of K_2HPO_4, pH 3.1 (H_3PO_4)) (1:99 V/V); **B:** ACN, buffer (3.48 g/L of K_2HPO_4, pH 2.5 (H_3PO_4)) (50:50 V/V)	Gradient, 1.0 ml/min, 254 nm, 25 °C, 10 µl
Alprenolol HCl., 04/2010:0876	99.0–101.0 0.1 M NaOH	Nucleodur® 100-3 C8ec (150x4.6 mm; 3 µm)	0.656 g of SOS mixed with 150 ml of ACN, diluted to 500 ml with buffer solution (0.9 g H_3PO_4 and 7.8 g NaH_2PO_4 dissolved in 1000 ml with water)	Isocratic, 1.3 ml/min, 280 nm, 25 °C, 20 µl
Ampicillin sodium, 01/2008:0578	91.0–102.0 HPLC	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	**A:** 0.5 ml of acetic acid, 50 ml of 0.2 M KH_2PO_4, 50 ml of ACN, diluted to 1000 ml with water; **B:** 0.5 ml of acetic acid, 50 ml of 0.2 M KH_2PO_4, 400 ml of ACN, diluted to 1000 ml with water	Gradient, 1.0 ml/min, 254 nm, 25 °C, 50 µl
Atenolol, 04/2009:0703	99.0–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (150x4.6 mm; 5 µm)	1.0 g of SOS and 0.4 g of TBAHS dissolved in 1 L of mixture of THF, MeOH, and buffer (3.4 g/L of KH_2PO_4, pH 3 (H_3PO_4)) (2:18:80 V/V/V)	Isocratic, 1.0 ml/min, 226 nm, 25 °C, 10 µl
Atropine sulfate, 04/2008:0068	99.0–101.0 0.1 M $HClO_4$	Phenomenex® Luna C18 (100x4.6 mm; 3 µm)	**A:** 3.5 g of SDS dissolved in 606 ml of buffer (7.0 g/L of KH_2PO_4, pH 3.3 (H_3PO_4)), 320 ml of ACN; **B:** ACN	Gradient, 1.0 ml/min, 210 nm, 25 °C, 10 µl
Bisoprolol fumar., 01/2012:1710	99.0–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	**A:** 10 g/L solution of H_3PO_4; **B:** 10 g/L solution of H_3PO_4 in ACN	Gradient, 1.0 ml/min, 225 nm, 20 °C, 10 µl
Bufexamac, 07/2015:1179	98.5–101.5 0.1 M CH_3LiO	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	**A:** 1.4 g/L of K_2HPO_4, MeOH, pH 3.6 (H_3PO_4) (30:70 V:V) **B:** MeOH	Gradient, 1.0 ml/min, 275 nm, 25 °C, 20 µl
Carteolol HCl., 01/2008:1972	99.0–101.0 0.1 M NaOH	Microsorb-MV 100-5 C18 (250x4.6 mm; 5 µm)	MeOH, ACN, solution of 2.82 g/L of SHXS (1:20:79 V/V/V)	Isocratic, 1.0 ml/min, 252 nm, 25 °C, 20 µl
Chlorprotixene HCl., 01/2015:0815	99.0–101.0 0.1 M NaOH	Agilent Zorbax Eclipse Plus C18 (150x4.6 mm; 5 µm)	6,0 g/L of KH_2PO_4, 2.9 g/L of SDS, 9,0 g/L of TBAB in a mixture of MeOH, ACN, water (5:40:55 V/V/V)	Isocratic, 2.5 ml/min, 254 nm, 25 °C, 20 µl
Ciprofloxacin, 04/2015:1089	99.0–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	ACN, solution (2.45 g/L of H_3PO_4, pH 3.0 (TEA)) (13:87 V/V)	Isocratic, 1.5 ml/min, 278 nm, 40 °C, 50 µl
DHE mesilate, 04/2016:0551	98.0–101.0 0.1 M $HClO_4$	Phenomenex® Luna C18 (100x4.6 mm; 3 µm)	**A:** 3 g/L of SHS, pH 2 (H_3PO_4); **B:** A and ACN (20:80 V/V)	Gradient, 1.0 ml/min, 220 nm, 25 °C, 5 µl
Dimenhydrinate, 07/2009:0601	53.0–55.5 0.1 M $HClO_4$	Agilent Eclipse Plus C18 (250x4.6 mm; 5 µm)	**A:** 10.0 g of TEA dissolved in 1000 ml with water, pH 2.5 (H_3PO_4); **B:** ACN	Grad.,1.2-2.0 ml/min, 225 nm, 30 °C, 10 µl
Diphenhydramine HCl., 01/2016:0023	99.0–101.0 0.1 M NaOH	Agilent Eclipse Plus C8 (250x4.6 mm; 5 µm)	ACN, solution (5.4 g/L of KH_2PO_4, pH 3 (H_3PO_4)) (35:65 V/V)	Isocratic, 1.2 ml/min, 220 nm, 25 °C, 10 µl
Dobutamine HCl., 07/2010:1200	98.5–101.0 0.1 M $HClO_4$	Phenomenex® Luna C18 (150x4.6 mm; 5 µm)	**A:** 2.60 g of SOS dissolved in 1000 ml with water, 3 ml of TEA, pH 2.5 (H_3PO_4); **B:** ACN, MeOH (18:82 V/V)	Gradient, 1.0 ml/min, 280 nm, 25 °C, 20 µl
Etilefrine HCl., 01/2008:1205	98.0–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C8 (250x4.6 mm; 5 µm)	ACN, solution (1.1 g/L of SDS, pH 2.3 (H_3PO_4)) (35:65 V/V)	Isocratic, 1.0 ml/min, 220 nm, 25 °C, 20 µl
Fenoterol HBr., 07/2016:0901	99.0–101.0 0.1 M NH_4SCN	Agilent Zorbax Eclipse Plus C18 (150x4.6 mm; 5 µm)	Solution (24 g/L of Na_2HPO_4), solution (9 g/L of KH_2PO_4 (pH 8.5 (H_3PO_4)), MeOH (69:1:35 V/V/V)	Isocratic, 1.0 ml/min, 215 nm, 25 °C, 20 µl
Flurbiprofen, 01/2017:1519	99.0–101.0 0.1 M NaOH	Agilent Zorbax Eclipse Plus C18 (150x4.6 mm; 5 µm)	Glacial acetic acid, ACN, water (5:35:60 V/V/V)	Isocratic, 0.7 ml/min, 254 nm, 25 °C, 10 µl
Hyoscine HBr., 01/2008:0106	99.0–101.0 0.1 M NaOH	Nucleodur 100-3 C8ec (150x4.6 mm; 3 µm)	ACN, solution (2.5 g/L of SDS, pH 2.5 (H_3PO_4)) (33:67 V/V)	Isocratic, 2.4 ml/min, 210 nm, 25 °C, 5 µl
Mefenamic acid, 01/2010:1240	99.0–101.0 0.1 M NaOH	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	THF, solution (5.75 g/L of (NH_4)H_2PO_4, pH 5.0 (NH_3)), ACN (14:40:46 V/V/V)	Isocratic, 1.0 ml/min, 254 nm, 25 °C, 10 µl
Metipranolol, (see Acebutolol)	- 0.1 M $HClO_4$	Phenomenex® Luna C18 (150x4.6 mm; 5 µm)	**A:** 2.0 ml of H_3PO_4 and 3.0 ml of TEA diluted to 1000 ml with water; **B:** A, ACN (50:50 V/V)	Gradient, 1.0 ml/min, 240 nm, 40 °C, 25 µl
Metoprolol succ., 01/2014:1448; Metoprolol tart., 01/2014:1028	99.0–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (150 x 4.6 mm; 5 µm)	3.9 g of NH_4OAc dissolved in 810 ml of water, 2.0 ml of TEA, 3.0 ml of H_3PO_4, 10.0 ml of glacial acetic acid, and 146 ml of ACN	Isocratic, 1.4 ml/min, 280 nm, 25 °C, 20 µl
Nadolol, 04/2011:1789	98.5–101.0 0.1 M $HClO_4$	LiChroCART 250-4 Merck (250x4.0 mm; 5 µm)	**A:** 5.6 g/L of SOS, pH 3.5 (H_3PO_4); **B:** ACN	Gradient, 1.0 ml/min, 206 nm, 40 °C, 20 µl
Naproxen, 01/2017:0731	99.0–101.0 0.1 M NaOH	Phenomenex® Luna C18(2) (100x4.6 mm; 3 µm)	ACN and buffer (1.36 g/L of KH_2PO_4, pH 2.0 (H_3PO_4) (42:58 V/V)	Isocratic, 2.0 ml/min, 230 nm, 50 °C, 20 µl
Ofloxacin, 01/2011:1455	99.0–101.0 0.1 M $HClO_4$	Phenomenex® Luna C18 (150x4.6 mm; 5 µm)	4.0 g of NH_4OAc and 7.0 g of $NaCLO_4$ dissolved in 1300 ml of water, pH 2.2 (H_3PO_4), and 240 ml of ACN	Isocratic, 0.6 ml/min, 294 nm, 45 °C, 10 µl
Oxprenolol HCl., 01/2008:0628 (see Alprenolol)	98.5–101.5 0.1 M NaOH	Nucleodur 100-3 C8ec (150x4.6 mm; 3 µm)	0.656 g of SOS mixed with 150 ml of ACN, diluted to 500 ml with buffer solution (0.9 g H_3PO_4 and 7.8 g NaH_2PO_4 dissolved in 1000 ml with water)	Isocratic, 1.0 ml/min, 280 nm, 25 °C, 20 µl
Penbutolol sulfate, 01/2017:1461	99.0–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (250x4.6 mm; 5 µm)	**A:** ACN, MeOH (39:61 V/V); **B:** 11 g of SHS dissolved in 1000 ml of water, 5.0 ml of TEA, pH 2.7 (H_3PO_4)	Gradient, 1.0 ml/min, 270 nm, 25 °C, 10 µl
Perphenazine, 01/2009:0629	99.0–101.0 0.1 M $HClO_4$	Nucleodur 100-3 C8ec (150x4.6 mm; 3 µm)	**A:** ACN, solution of 7 g/L of NaH_2PO_4 (35:65 V/V); **B:** ACN	Gradient, 0.8 ml/min, 245 nm, 30 °C, 10 µl
Pindolol, USP and 01/2017:0634	99.0–101.0 0.1 M HCL	Nucleosil® 100-5 CN RP (125x4.0 mm; 5 µm)	ACN, solution (0.05 M sodium acetate, pH 5 (glacial acetic acid)) (35:65 V/V)	Isocratic, 0.7 ml/min, 219 nm, 25 °C, 10 µl
Propafenone HCl., 01/2008:2103	99.0–101.0 0.1 M $HClO_4$	Nucleodur 100-3 C8ec (150x4.6 mm; 3 µm)	**A:** 3.42 g/L solution of K_2HPO_4 adjusted to pH 2.5 with H_3PO_4; **B:** ACN	Gradient, 1.0 ml/min, 220 nm, 30 °C, 20 µl
Salbutamol sulfate, 07/2011:0687	98.0–101.0 0.1 M $HClO_4$	Nucleodur 100-3 C8ec (150x4.6 mm; 3 µm)	**A:** 3.45 g of $NaH_2PO_4xH_20$ dissolved in 1 L of 0.05% V/V of TEA, pH 3.0 (H_3PO_4); **B:** MeOH, ACN (35:65 V/V)	Gradient, 1.0 ml/min, 273 nm, 30 °C, 20 µl

Monograph number	Specification and titrant	Column	Mobile Phase	Settings
Salbutamol, 01/2011:0529	98.0–101.0 0.1 M $HClO_4$	Nucleodur 100-3 C8ec (150x4.6 mm; 3 µm)	ACN, solution (2.87 g/L of SHS and 2.5 g/L of KH_2PO_4, pH 3.65 (H_3PO_4)) (22:78 V/V)	Isocratic, 1.4 ml/min, 220 nm, 25 °C, 20 µl
Timolol maleate, 01/2014:0572	98.5–101.0 0.1 M $HClO_4$	Agilent Zorbax Eclipse Plus C18 (150x4.6 mm; 5 µm)	**A:** MeOH, solution (4.32 g/L of SOS, pH 3.0 (glacial acetic acid)) (50:50 V/V); **B:** MeOH	Gradient, 1.7 ml/min, 295 nm, 25 °C, 20 µl

Abbreviations: ACN, acetonitrile; DHE, dihydroergotamine; MEOH, methanol; SDS, sodium dodecyl sulfate; SHXS, sodium hexanesulfonate; SHS, sodium heptanesulfonate; SOS, sodium octanesulfonate; TBAHS, tertrabutylammonium hydrogen sulfate; TBAB, tetrabutylammonium bromide; TEA, triethylamine;

2.5. Sample preparation

All samples were prepared according to the instructions of the respective monographs. Each of the substances was titrated three times. Three titrations were applied for determination of water content as well. For chromatographic purity the test solutions of all substances and the diluted references were prepared three times, each was injected once. A test solution of metipranolol of 1 mg/ml and a diluted reference 1:1000 were prepared.

2.6. Peak identification and quantitation of impurities

Peak identification of the drug substance and its related substances was realized by means of either reference standards/impurities from EDQM, representative chromatograms available at knowledge database, and stated relative retention time (RRT) values. The content of the impurities was calculated regarding the diluted reference solution. All impurities exceeding the disregard levels according to the monographs were considered in analysis of the chromatograms. The impurities were termed according to the trivial names or the International Non-Proprietary Names as far as possible. Otherwise, the impurities had to be declared corresponding to the Ph. Eur. 9.3 labeling, i.e. impurity A, B, C, etc. IUPAC nomenclature of all impurities found is reported in supporting information.

3. Results and Discussion

The age of the drug substances was determined as far as possible by means of the lettering on the primary and secondary packaging regarding the lot label, the year of manufacturing, and the expiry date. Because of the lack of thorough labeling, detailed information had to be obtained from the original manufacturer in some cases. All results of the substances investigated are combined in Tables 2-8 and the chemical structure of the impurities exceeding the required limit criteria are depicted in Table 9.

3.1. β-Blockers

The first group of β-blockers consisted of twelve different agents such as acebutolol, alprenolol, atenolol, bisoprolol, carteolol, metipranolol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, and timolol. 11 out of 12 β-blockers were analyzed by means of

methods corresponding to the Ph. Eur. or USP monographs, but no monograph of metipranolol was existent.

For content determination the lots were titrated with either 0.1 M perchloric acid or 0.1 M sodium hydroxide. Assay results were in the range from 98.7% to 100.7%, complying with pharmacopoeial specifications, respectively. Except for atenolol Azupharma and penbutolol, the impurities found in the API lots were well within the Ph. Eur. acceptance criteria (Table 2).

In the lot of Azupharma two impurities were verified exceeding the limits, the impurity A, which is an intermediate product of synthesis, present in a content of 0.26% as well as the impurity J, which is a synthetic by-product, being without the propane moiety linked to the secondary amine and present in a content of 0.23% (see Table 9). Thus, they are not related to degradation. Furthermore, small amounts of two impurities were observed. Subsequently, a second atenolol lot was examined and compared to the atenolol reference of Wörwag, the observed impurities met the Ph. Eur. requirements. No degradation could be ascertained in atenolol lots. In the penbutolol lot six unspecified impurities were quantified of about 0.15%, 0.35%, 0.40%, 0.64%, 1.36%, and 6.01%. However, three impurities had contents less than 0.5%, which is the limit for unspecified impurities in this monograph. Identification of the impurities was not feasible because only one impurity is specified in the monograph but was not observed in this lot.

Oxprenolol hydrochloride lot was analyzed on the one hand by means of the Ph. Eur. TLC method without detecting any impurity and on the other hand by means of liquid chromatography corresponding to the alprenolol hydrochloride monograph. The retention time of oxprenolol was about 12 min, no impurity was observed in both cases.

Since metipranolol is not monographed in any pharmacopoeia, there are no acceptance criteria given, but chromatographic conditions were applied as stated in the monograph of acebutolol hydrochloride due to the related chemical structure of both molecules. For determination of the content, an anhydrous titration with 0.1 M perchloric acid was carried out and found 101.2%. Metipranolol has been decomposed by to the 0.65% desacetylmetipranolol being the expected degradation product (Table 9).

Taken together, with exception of metipranolol, the drug substances of the β-blockers are stable.

Table 2

β-Blockers

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Acebutolol hydrochloride	1991	Unspecified (0.92) = 0.10%	100.7 (0.3)
	1996	No impurity ≥ 0.05%	100.6 (0.7)
Alprenolol hydrochloride	1994	No impurity ≥ 0.05%	99.1 (0.4)
Atenolol	1994	**A (0.45)** [a] **= 0.26%**, B (0.33) = 0.09%, I (0.87) = 0.11%, **J (0.77)** [a] **= 0.23%**	99.3 (0.3)
	1996	A (0.43) = 0.05%, B (0.34) = 0.05%, J (0.76) = 0.06%	99.9 (0.2)
	2010 [b]	B (0.33) = 0.07%, I (0.86) = 0.05%, J (0.78) = 0.07%	99.7 (0.2)
Bisoprolol fumarate	1994	G (1.04) = 0.27%	99.9 (0.1)
Carteolol hydrochloride	1995	H (0.85) = 0.02%	100.7 (0.7)
Metipranolol	1988	Desacetylmetipranolol (0.52) = 0.65%	101.2 (1.0)
Metoprolol succinate	1988	No impurity ≥ 0.05%	99.1 (0.4)
Metoprolol tartrate	1994	"	100.2 (0.1)
Nadolol	≤ 1999	A (0.21) = 0.16%, D (1.51) = 0.16%	98.7 (1.1)
Oxprenolol hydrochloride	≤ 1999	No impurity ≥ 0.4%	99.5 (1.2)
Penbutolol sulfate	≤ 1999	Unspecified (0.15) = 0.35%, **(0.22)** [a] = **0.64%,** (0.27) = 0.40%, (0.40) = 0.15%, **(0.43)** [a] **= 6.01%, (0.93)** [a] **= 1.36%**	100.0 (0.5)
Pindolol	1993	No impurity ≥ 0.05%	99.0 (0.4)
	≤ 1999	"	99.4 (0.3)
Timolol maleate	≤ 1999	No impurity ≥ 0.05%	99.7 (0.1)
(Hexal, Merck, unknown manuf.)	≤ 1999	"	99.9 (0.2)
	≤ 1999	"	100.2 (0.3)

[a] being out of specification according to Ph. Eur. monograph
[b] reference substance

3.2. β-Sympathomimetic drugs

The class of β-sympathomimetic drugs contained two cardiovascular drugs, dobutamine and etilefrine, and two inhalative sympathomimetics, fenoterol and salbutamol. The results are summarized in Table 3.

Table 3

β-Sympathomimetics

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Dobutamine hydrochloride	1993	C (1.31) = 0.09%	100.1 (0.1)
	≤ 1999	C (1.31) = 0.10%	100.1 (0.2)
Etilefrine hydrochloride	1972	No impurity ≥ 0.02%	99.8 (0.7)
Fenoterol hydrobromide	1992	A (1.28) = 1.45%	99.5 (1.0)
Salbutamol sulfate (Glaxo, 3M Medica, Stada, Kettelhack Ricker, unkown manuf.)	≤ 1995	C (1.69) = 0.07%, D/N (1.79) = 0.20%, F (1.83) = 0.30%, O (1.93) = 0.11%	99.9 (0.3)
	≤ 1999	D/N (1.78) = 0.10%, F (1.83) = 0.07%, O (1.94) = 0.19%	99.9 (0.6)
	≤ 1995	C (1.68) = 0.12%, **F (1.83)** [a] **= 0.48%**, D/N (1.78) = 0.24%	100.3 (1.2)
	1988	C (1.70) = 0.05%, D/N (1.78) = 0.11%, O (1.95) = 0.16%, unspecified (1.60) = 0.07%	99.7 (0.6)
	≤ 1999	C (1.68) = 0.08%, D/N (1.77) = 0.08%, O (1.93) = 0.13%	99.9 (0.4)
Salbutamol	1990	No impurity ≥ 0.05%	98.4 (0.3)

[a] being out of specification according to Ph. Eur. Monograph

3.2.1. Cardiovascular drugs

The content of dobutamine hydrochloride and etilefrine hydrochloride was determined by means of anhydrous titration with 0.1 M perchloric acid. The results were in accordance with the Ph. Eur. limits, respectively. In both dobutamine lots small traces of an intermediate product specified as impurity C of respective 0.10% were noticed. The cleavage of three methoxy moieties of this compound leads to dobutamine in the final reaction of synthesis. The Ph. Eur. limits impurity C to 0.5%. In addition, no impurity peak was observed in the chromatogram of the etilefrine hydrochloride test solution.

3.2.2. Inhalative sympathomimetics

The assay of fenoterol hydrobromide was carried out by means of Volhard-Titration and resulted in 99.5%. The fenoterol hydrobromide is a chiral molecule, presented as the (*R,R*) and (*S,S*) racemate. The other enantiomeric pair (*R,S*) and (*S,R*) is specified as impurity A and limited to a maximum 4%. 1.45% of this impurity was found.

The contents of all salbutamol batches complied with the Ph. Eur. specifications. Nevertheless, in the salbutamol lot of Stada the impurity F, being an ether-linked dimeric impurity occurring as a synthetic by-product, exceeded the demanded limit of 0.3% (see Table 9). Further impurities were identified in the salbutamol lots. The peaks of the impurity N and D could not be identified unambiguously because of closed separation of both compounds. In summary, even though the compound contains feature which might be prone to degradation, e.g. the benzylic OH group which might split off water, no stability issues were observed.

3.3. Anticholinergics

The group of anticholinergic drugs consisted of drug substances of atropine and hyoscine (see Table 4). The content of the atropine sulfate was found to be 99.4% by means of anhydrous titration with 0.1 M perchloric acid. The lot contained 0.57% of tropic acid specified as impurity C which exceeded the limit of 0.3%. Even in the reference lot of 2012 impurities were quantified of 0.36% 7-hydroxyhyoscyamine and 0.74% littorine, stated as impurity E and G (see Table 9). Both compounds were isolation by-products of atropine formed by biosynthetic pathway of tropane alkaloids in plants including Datura stramonium and Atropa belladonna. Determination of water content yielded about 2.5% in both lots being within the required range of 2.0–4.0%, respectively. Two hyoscine salts analyzed met the Ph. Eur. specifications. Moreover, contents of tropic acid were below the limit of quantitation, respectively.

Table 4
Anticholinergics

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Atropine sulfate	≤ 1999	**C (0.17)** [a] **= 0.57%**, unspecified (0.32) = 0.08%	99.4 (0.4)
	2012 [b]	**E (0.65)** [a] **= 0.36%**, **G (1.15)** [a] **= 0.74%**	99.5 (0.6)
Hyoscine hydrochloride	1996	No impurity ≥ 0.05%	100.0 (0.5)
Hyoscine hydrobromide	2015 [b]	"	100.8 (0.8)

[a] being out of specification according to Ph. Eur. monograph
[b] reference substance

3.4. Anti-infectives

The group of anti-infectives consisted of aciclovir as well as three antibiotics, i.e. ampicillin, ciprofloxacin, and ofloxacin, the results are listed in Table 5.

3.4.1. Antiviral drug

The content of aciclovir was determined by means of a titration with 0.1 M perchloric acid, gave 100.5% for the 1987 and 100.6% for the 1990 lot related to the anhydrous substance. The water content of the substances was 5.4% and 5.3%, respectively. A characteristic impurity profile of aciclovir was observed in the chromatograms, but no impurity was out of specification. Both lots met the criteria required in the Ph. Eur.

3.4.2. Antibiotics

The content of ampicillin sodium was determined by means of HPLC-UV, meeting the required specification of 91.0-101.0%. For quantitation fresh anhydrous ampicillin was used. One unspecified impurity present in a content of 2.4%, and several impurities not exceeding the limit of 2.0% were observed. Hence, the substance did not comply with Ph. Eur. specification. The content of water was about 1.5%. For testing of system suitability of the method, the ampicillin dimer, being a typical degradation product, was formed by heating of reference substance at 60 °C for 1 h, but this compound was not observed in the historical 1997 lot.

The group of fluoroquinolones was found to be stable. The content of ciprofloxacin and ofloxacin was determined by titration with 0.1 M perchloric acid and found 100.1% and 100.0%, respectively. In the ciprofloxacin lot the impurity C, the ethylenediamine compound formed by degradation of the piperazine ring moiety, was observed in a content of 0.05%. The presence of the impurity A, being a fluoroquinolone without the piperazine ring moiety, was excluded by application of the TLC method according to Ph. Eur. In the ofloxacin lot the impurities found were by-products of synthesis but no oxidative degradation products were observed.

Table 5
Anti-infectives

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Aciclovir	1987	A (1.85) = 0.03%, B (0.46) = 0.44%, F (1.78) = 0.07%, N (1.36) = 0.04%, O (1.39) = 0.07%, P (0.82) = 0.03%, unspecified (2.54) = 0.05%	100.5 (0.2)
	1990	B (0.47) = 0.27%, F (1.79) = 0.05%, N (1.35) = 0.05%, O (1.40) = 0.06%, P (0.81) = 0.04%, unspecified (2.54) = 0.05%	100.6 (1.4)
Ampicillin sodium	1997	**Unspecified (2.92) [a] = 2.4%**	92.0 (0.9)
Ciprofloxacin	1993	C (0.73) = 0.05%	100.1 (0.1)
Ofloxacin	1996	C (0.52) = 0,10%, E (0.91) [b] = 0,03%	100.0 (0.3)
	1995	C (0.51) = 0,06%, D (0.77) = 0,06%, E (0.92) [b] = 0,14%	100.0 (0.3)

[a] being out of specification according to Ph. Eur. monograph

3.5. NSAIDs

The drug class of NSAIDs included four drug substances. Flurbiprofen, mefenamic acid, and naproxen were titrated with 0.1 M sodium hydroxide and bufexamac was assayed by titration with 0.1 M lithium methoxide because of containing a NH-acid moiety (Table 6). The contents of the APIs as well as of impurities fell within the specification intervals, respectively. Impurity A of flurbiprofen, lacking a fluoro-substituent on the phenyl ring, was noticed in a content of 0.31%, deceeding the Ph. Eur. limit of 0.5%.

Table 6
NSAIDs

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Bufexamac (Sigma, Heu. lot 07861 and 07860)	1994	No impurity ≥ 0.05%	100.3 (1.5)
	1996	"	98.6 (1.2)
	1996	C (4.83) = 0.09%	98.9 (0.9)
Flurbiprofen	≤ 1999	A (0.73) = 0.31%	100.3 (0.3)
Mefenamic acid	1985	No impurity ≥ 0.05%	100.2 (0.5)
Naproxen	1989	No impurity ≥ 0.05%	100.0 (0.9)

3.6. Antipsychotics

The group of antipsychotics contained chlorprothixene and perphenazine which were titrated with 0.1 M sodium hydroxide and 0.1 M perchloric acid, respectively. The determination of contents resulted in 99.8% for chlorprothixene hydrochloride and 100.4% for perphenazine (Table 7). In the chlorprothixene lot the sulfoxide degradation product was found being below the disregard limit whereas the isomer, denoted as impurity F, was present in 0.15%. In the case of perphenazine, the oxidation of the sulfur has occurred in 0.07% stated as impurity A.

Table 7
Antipsychotics

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Chlorprothixene hydrochloride	1985	F (1.31) = 0.15%	99.8 (0.1)
Perphenazine	≤ 1999	A (0.36) = 0.07%, B (0.80) = 0.05%	100.4 (0.3)

3.7. Further representatives

Representatives of various drug classes were investigated, containing one ergot alkaloid, one antihistaminic agent, and one anti-arrhythmic drug (Table 8).

The content of dihydroergotamine mesilate was ascertained by titration with 0.1 M perchloric acid to 98.6%. Small amounts of impurities were measured in the lot, not exceeding the limits stated in the monograph. Contents of related substances were found 0.09% of 9,10-dihydroergostine, 0.07% of 8-hydroxy-9,10-dihydroergotamine, and 0.11% dihydroergocristine, stated according to the Ph. Eur. 9.3 as impurity B, C, and E, respectively. No epimerization of the agent was observed, and no impurity was related to decomposition.

Dimenhydrinate is a combination of two drugs, 54.3% of diphenhydramine and 45.7% of 8-chlorotheophylline. The xanthine derivative is added because of two reasons: First, the solubility is increased due to the ionic interaction and second, a reduction of adverse effects like fatigue is achieved. The content of diphenhydramine determined by means of potentiometric titration with 0.1 M perchloric acid was found 54.6% being within the demanded range. The content of theobromine stated as impurity A, as well as impurity F, the demethylated diphenhydramine compound, were found within the required limits.

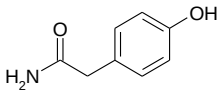
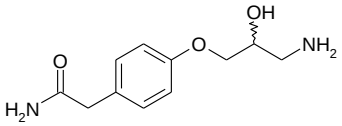
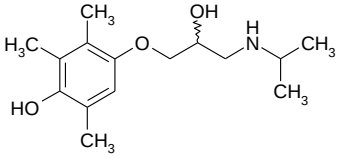
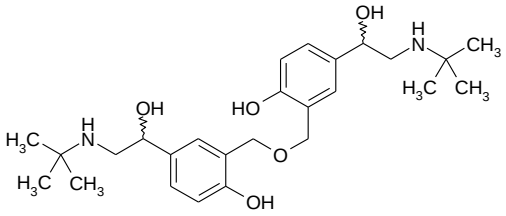
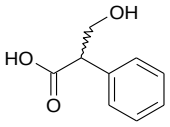
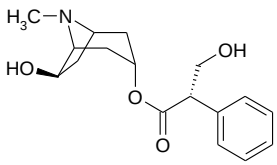
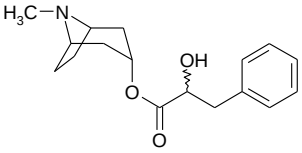
Three lots of propafenone hydrochloride were part of the collection, assaying by means of titration with 0.1 M perchloric acid. The contents were within the range of 99.0–100.1%. Not any impurity exceeding the 0.03% limit was measured in the batch samples.

Table 8
Various representatives

Drug substance	Year of manuf.	Impurities according to the Ph. Eur. specification (RRT)	Content in % (RSD)
Dihydroergotamine mesilate	1992	B (1.22) = 0.09%, C (0.84) = 0.07%, E (1.46) = 0.11%	98.6 (0.8)
Diphenhydramine in Dimenhydrinat	1994	A (0.27) = 0.05%, F (0.96) = 0.18%	54.6 (0.2)
Diphenhydramine	1982	A (0.92) = 0,08	100.3 (0.3)
Propafenone hydrochloride	≤ 1999	No impurity ≥ 0.03%	100.1 (1.4)
	1996	“	99.0 (0.6)
	1997	“	99.7 (0.3)

Table 9

Impurities exceeding the limit criteria

Atenolol:			
	Imp. A	Imp. J	
Penbutolol:	Impurities with unknown structure		
Metipranolol:			
	DesacetylmetipranolOl		
Salbutamol:			
	Imp. F		
Ampicillin:	Impurity with unknown structure		
Atropine:			
	Tropic acid (Imp. C)	7-Hydroxyhyoscyamine (Imp. E)	Littorine (Imp. G)

4. Conclusion

In conclusion, drug substances remained stable even after 20 years of storage at room temperature. 44 out of 49 APIs were within the acceptance criteria of the respective pharmacopoeial monograph regarding the content and chromatographic purity. No acceptance criteria were given for metipranolol, but a low degree of degradation of about only 0.7% was found. However, one lot of atenolol, penbutolol, salbutamol, and ampicillin sodium, respectively, did not meet the limits of related substances, but the impurities observed were rather synthetic by-products and intermediates of synthesis than degradation products. They were just above the required limits; it can be assumed that the impurities were presented in the lots from the beginning [22, 23]. The decomposition by hydrolysis of the atropine batch was found. The resulting tropic acid has been formed of about 0.7% in the atropine lot, but no instability was observed in atropine reference substance and hyoscine lots, respectively.

Hydrolysis, epimerization, and oxidation have been occurred just to a minor extend in the solids. Furthermore, no discoloration of any lot was observed. This study confirmed the

predicted chemical stability of pure drug substances, for more than two decades. Accordingly, they could be used for manufacturing of FPPs. A similar long-term stability is presumed for tablets, capsules, and powders. Physical instability should be expected rather than chemical instability of the APIs. Generally, liquid dosage forms are inherently more sensitive to degradation because of dissolved APIs are susceptible to react with water and other excipients. There is little literature information addressing the long-term stability of FPPs beyond their expiry dates [12, 14-18]. However, these studies reported that the shelf-lives are often longer than the declared expiry dates, even exceeding the maximum timeframe of five years. The comprehensive and systematic “Shelf-Life Extension Program” investigated the stability of drugs beyond their expiry dates was installed by the Food and Drug Administration and the United States Department of Defense [12]. Based on stability assessment of 122 different drugs of 3005 lots, 88% of the lots were extended by at least one year beyond their stated expiry date. Due to the current occurrence of drug supply interruptions, the FDA and Pfizer have extended the shelf-life of epinephrine, atropine, and sodium bicarbonate parenterals based on stability tests up to one year beyond their labeled expiry dates [24].

Considering the results of this study, the previous investigation of the ampoules, and the data in the literature, the extension of shelf-lives of FPPs, in particular for tablets and capsules, should be aimed, based on implementation of systematic long-term stability tests by regarding inter-individual batch stability.

Conflict of interest

The authors declare that they have no conflict of interest.

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3. A stability-study of expired ampoules manufactured more than 40 years ago

Zilker, M., Sörgel, F., Holzgrabe, U.

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Abstract

Pharmaceutical manufacturers have to study the stability of drug products before marketing according to ICH guideline Q1A(R2); data of those investigations aim to set expiry dates. The expiry date on the container of a remedy assures the physician and the patient a stability of the drug in its formulation i.e. within a specification of 95–105%. Only few studies show that shelf-lives of pharmaceutical products are often longer than expiration dates. The objective of the study presented here was determining the content of nine expired ampoules manufactured in the last century and identifying the impurity profile by means of HPLC-UV and HPLC-MS, respectively. The ampoules are part of the “PEAK-collection” of long expired finished pharmaceutical products at IBMP, Nürnberg-Heroldsberg, and consists among others of epinephrine (Suprarenin and Adrenalin in Oil), etilefrine (Effortil®), synephrine (Sympatol®), caffeine and procaine (Impletol), caffeine and sodium salicylate (Caffeinum Salicylicum), dipyridamole (Persantin®), furosemide (Lasix®), and metamizole (Novalgin®). For chromatographic investigations methods of the European Pharmacopoeia for related substances were used; for determining the content, they were validated for linearity, precision, and accuracy. The results were compared to current reference ampoules. Five out of nine ampoules were still within the specified content limits. In Suprarenin and Adrenalin in Oil, both containing epinephrine, Impletol (procaine), and Persantin® (dipyridamole) contents were decreased to 70%, 74%, 79%, and 86%, respectively, and therefore out of specification.

Abbreviations: FPP, finished pharmaceutical product; API, active pharmaceutical ingredient; Ph. Eur., European Pharmacopoeia; RRT, relative retention time; RSD, relative standard deviation; QC samples, quality control samples; CI, confidence interval; R^2, Coefficient of determination.

1. Introduction

The shelf-life of a finished pharmaceutical product (FPP) has to be determined by the pharmaceutical manufacturer before marketing. The content of the active pharmaceutical ingredient (API) has to be in a range of 95% to 105% of its labeled value during its shelf-life according to the ICH guideline Q1A(R2) [1, 2]. The manufacturer is required to submit stability studies of the FPP to regulatory authorities and to assign a shelf-life, which is typically five years for stable and shorter for less stable APIs such as some classes of anti-infectives, local anesthetics with ester function, and catecholamines i.e. epinephrine [3, 4]. Regulatory authorities restrict the marketing authorization of FPPs to five years according to the national medicinal products acts [5]. Therefore, no FPP with a shelf-life for more than five years is available on the market. Scattered or little information of long-term drug stability studies beyond their expiry date can be found in the literature. These publications confirm that shelf-lives are often beyond expiration dates. Extensions of the shelf-life are possible, generally depending on the chemistry of the molecule and its dosage form [4 ,6–11]. The shelf-life extension program (SLEP) installed by the FDA for the United States Department of Defense, includes 122 different drugs and 3005 different lots. Based on stability assessments, 88% of the lots were extended by at least 1 year beyond their original expiration date; the average extension was 66 months. The purpose of the program was to determine the actual shelf-life of stockpiled drug products, and has resulted in savings to the military budget by reducing high costs of replacing expired drugs [4]. Another study revealed that captopril tablets, flucloxacillin capsules, cefoxitin powder for injection, and theophylline sustained release tablets to be chemically and physically stable for periods of 1.5–9 years beyond their registered shelf-life [12]. The responsibility of the manufacturer for safety and efficacy of the drug ends on the first day after the expiry date. There are reasons for the conservative manner of shelf-life-determination, like ensuring drug delivery in therapeutic doses, reducing the toxicity potential of decomposition products, and allowing fluctuation of temperature and humidity during transport and storage in real life [12]. Stability problems during long term storage can be distinguished between chemical, physical, microbiological instabilities and vary with storage [13].

Within the frame of this study, nine expired ampoules of a large collection of expired drug products with an age up to 83 years, containing either epinephrine (Suprarenin and Adrenaline in Oil), etilefrine (Effortil®), synephrine (Sympatol®), caffeine and procaine (Impletol), caffeine and sodium salicylate (Caffeinum Sodiumsalicylicum), dipyridamole (Persantin®), furosemide (Lasix®), and metamizole sodium (Novalgin®) were chromatographically investigated with regard to content and degradation products (see Table 1 and Fig. 1).

Table 1

Collection of ampoules

Ampoule	API	Manufacturing year	Minimum age (years)
Suprarenin hydrochl. synth 1:1000 1 cc. Bayer GANF	Epinephrine hydrochloride	1934	83
Adrenalin in Oil 1:500 1 cc. No. 192 CM105 Park Davis & Company	Epinephrine	prior to 1970	47
Effortil® 0.01 g/ccm C.H.Boehringer Sohn Ingelheim am Rhein Ch.B. 6101	Etilefrine hydrochloride	1949–1962	55
Sympatol® 0.06 g/ccm C.H.Boehringer Sohn Ingelheim am Rhein	Synephrine tartrate	1930–1962	55
Impletol 2 cc Bayer (2% procaine hydrochloride, 1.42% caffeine)	Procaine hydrochloride and caffeine	1949	68
Coffeinum-Natriumsalicylicum 0.2 g/ccm (Wehrkreissanitätspark München)	Caffeine and sodiumsalycilate	prior to 1945	72
Persantin® 10 mg/2 ccm Thomae	Dipyridamole	1959–1997	20
Lasix® 20 mg/2 ml HOECHST AG Op.-Nr.: 022	Furosemide	1964	53
Novalgin® 50 % 2 ccm HOECHST AG LWNW	Metamizole sodium	1951–1964	53

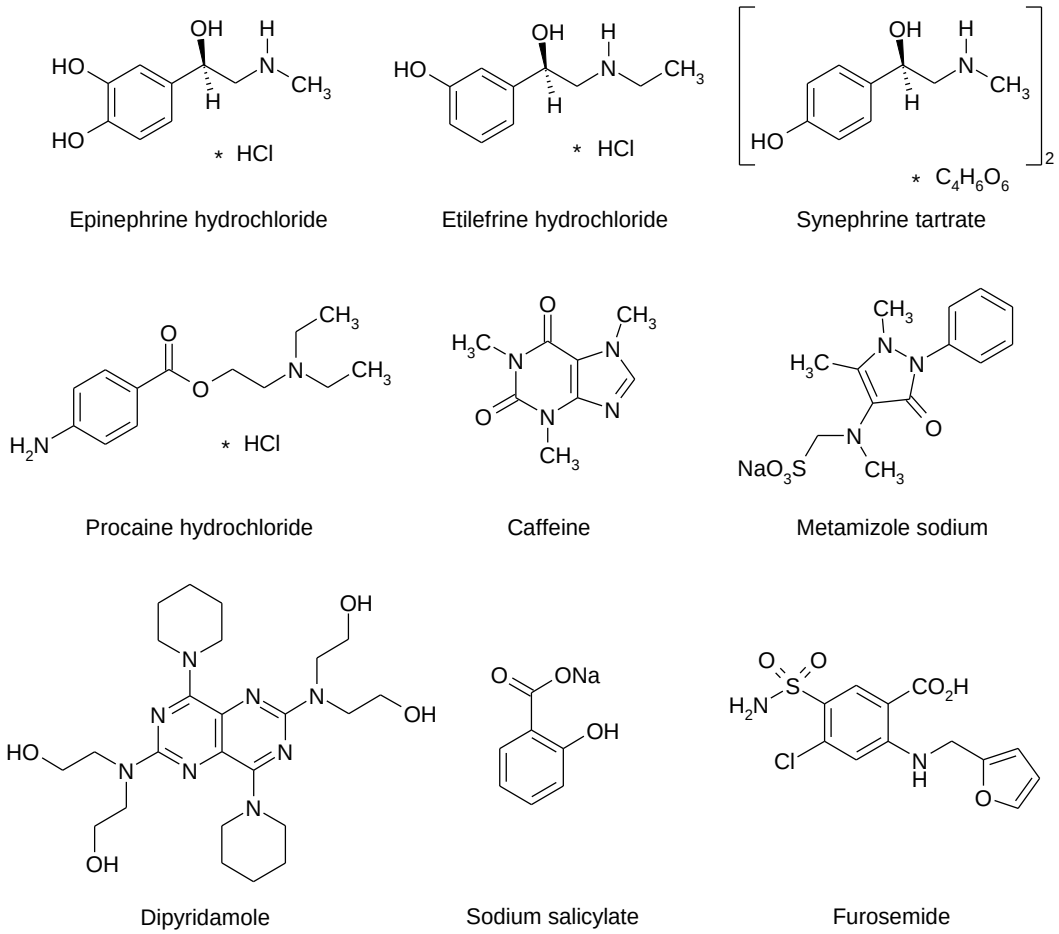

Fig. 1. Active pharmaceutical ingredients analyzed

Especially oxidations and hydrolyses are typical degradation reactions that result in an impairment of efficacy and safety. Phenylalkylamines such as adrenaline **(1)**, etilefrine, and synephrine are susceptible to oxidation reactions catalyzed by light, elevated temperature, and basic conditions. Due to the catechol structure, being only present in epinephrine, colored degradation products such as adrenochrome **(2)** and oxoadrenochrome **(3)** are formed by oxidation, leading to a red color and black particles. Adrenochrome is detectable by means of UV spectroscopy at a wavelength of 490 nm [14]. The oxidation reactions in the formulation can be prevented by the addition of antioxidants such as sulfites (e.g. sodium metabisulfite, $Na_2S_2O_5$). In this case (1*R*)-1-(3,4-dihyroxyphenyl)-2-(methylamino)-ethansulfonic acid (sulfonic acid derivative) **(4)** can be formed by a nucleophilic substitution of the alcohol with the sulfite ions during long-term storage (see Fig. 2) [15–17].

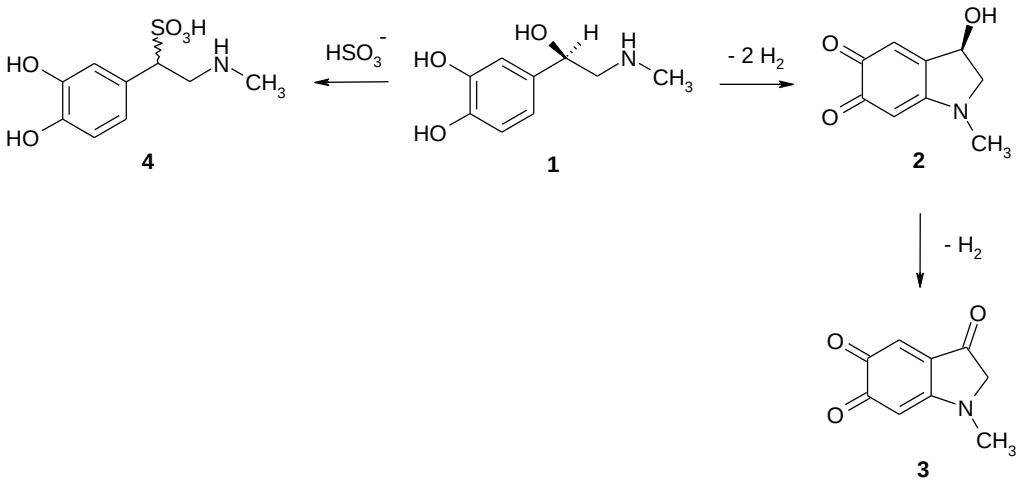

Fig. 2. Degradation pathways of epinephrine

Impletol is a two-component mixture containing procaine and caffeine. Procaine is instable because of its ester function, which is easily hydrolyzed to 4-aminobenzoic acid in aqueous solutions. The presence of caffeine delays the hydrolysis as a complex between the drug and caffeine is formed [15, 18]. Hydrolysis is also expected in expired formulations of furosemide and metamizole which are degraded to 4-chloro-5-sulfamoyl-anthranilic acid (saluamine) and in 4-methylaminophenazone, respectively.

The aim of this study was to develop and validate HPLC-UV methods in order to quantify the APIs and assess the corresponding impurities in the expired ampoules. The methods utilized were based on the methods of the European Pharmacopoeia (Ph. Eur.) for related substances because they ensure selective separation of all specified impurities. An exception was synephrine, because of the lack of a method in current pharmacopoeias. Therefore, the method of etilefrine was applied due to the similarity of both molecules. Peak

identification of APIs and their impurities was realized by relative retention times (RRT) and confirmed by external standards as far as available.

2. Experimental section

2.1. Chemicals and reagents

The ampoules are part of the “PEAK-collection” of expired drugs at IBMP – Institute of Biomedical and Pharmaceutical Research in Nürnberg-Heroldsberg, Germany. Epinephrine hydrochloride, 1-(3,4-dihydroxyphenyl)-2-(methylamino)ethansulfonic acid, adrenochrome, (±)-synephrine, potassium dihydrogenphosphate, phosphoric acid 85%, sodium lauryl-sulfate, octopamine, tyramine, 1-(4-hydroxyphenyl)-2-(methylamino)ethanone, anhydrous sodium acetate, tetrahydrofuran, acetic acid 99%, sodium hydroxide 50%, cetrimide, sodium dihydrogenphosphate, ammonium hydroxide solution 28–30%, 4-aminobenzoic acid, theobromine, procaine hydrochloride, and furosemide were purchased from Sigma Aldrich Chemie GmbH (Schnelldorf, Germany), sodium 1-octanesulfonate and dipyridamole from Alfa-Aesar GmbH & Co. KG (Karlsruhe, Germany), 1-propanol HiPerSolv chromanorm for HPLC, HPLC grade acetonitrile, MS HPLC grade acetonitrile, HPLC grade methanol, and MS HPLC grade methanol from VWR International GmbH (Darmstadt, Germany), triethylamine HPLC grade from Fisher Scientific (Loughborough, United Kingdom), etilefrine hydrochloride from Boehringer Ingelheim (Ingelheim, Rhein), caffeine anhydrous from Fagron GmbH u. Co KG (Barsbüttel, Germany), metamizole sodium from Berlin Chemie (Berlin, Germany), and Suprarenin® ampoules 1 mg/ml from Sanofi (batch no. CY001; expiry date: 07/2018; Frankfurt, Germany), Effortil® drops from Boehringer Ingelheim (batch no. 532633A; expiry date: 09/2019; Ingelheim, Germany), Coffeinum N 0.2 g from MYLAN dura (batch no. 83790A; expiry date: 08/2020; Darmstadt, Germany), Procain 2.0% ampoules from Steigerwald Arzneimittelwerk GmbH (batch no. 590163; expiry date: 08/2018; Darmstadt, Germany), Persantin® ampoules 10 mg/2 ml from Boehringer Ingelheim (batch no. 528654; expiry date: 10/2018; Ingelheim, Germany), Furosemid-ratiopharm® ampoules 20 mg/2 ml from Ratiopharm (batch no. R32445; expiry date: 09/2020; Ulm, Germany), and Novaminsulfon-ratiopharm® ampoules 1 g/2 ml from Ratiopharm (batch no. R35486; expiry date: 09/2019; Ulm, Germany). Water for HPLC was purified using the Milli-Q purification system by Merck Millipore (Schwalbach, Germany).

2.2. Apparatus

HPLC experiments were performed on a HPLC system 1100 series from Agilent Technologies (Waldbronn, Germany) consisting of a vacuum degasser (G1322A), binary pump (G1312A), autosampler (G1313A), thermostated column oven (G1316A), diode array detector (G1315B) and a MS/ESI – ion trap detector (G24450). Chromatograms were

recorded and integrated using the Agilent ChemStation® software (Rev B.03.02). For pH-measurements a Metrohm 744 pH-Meter from Deutsche METROHM GmbH & Co. KG (Filderstadt, Germany) was used.

2.3. Methods

HPLC conditions for each API: **1. a) Adrenaline**: Phenomenex® Luna 3u C18 (100 x 4.6 mm; 3 µm), mobile phase A consisting of a mixture of 95% [V/V] solvent mixture A (consisting of 37 mM potassium dihydrogen phosphate and 12 mM sodium octanesulfonate in water adjusted to pH 2.8 with phosphoric acid) and 5% [V/V] acetonitrile, mobile phase B consisting of a mixture of 55% [V/V] solvent mixture A and 45% [V/V] acetonitrile, gradient: 0 min B = 8%, 15 min B = 50%, 20 min B = 8%, 25 min B = 8%, 50 °C, 2.0 ml/min, 20 µl, 210 nm [19]. **1. b) Adrenochrome quantitation**: Phenomenex® Luna 3u C18 (100 x 4.6 mm; 3 µm), mobile phase A consisting of a mixture of 95% [V/V] solvent mixture A (see above) and 5% [V/V] acetonitrile, mobile phase B consisting of a mixture of 55% [V/V] solvent mixture A and 45% [V/V] acetonitrile, gradient: 0 min B = 0%, 3 min B = 0%, 15 min B = 50%, 24 min B = 0%, 50 °C, 1.0 ml/min, 20 µl, 490 nm. **2. Etilefrine and synephrine**: Agilent Zorbax Eclipse Plus C8 analytical (250 x 4.6 mm; 5 µm), mobile phase consisting of a mixture of 65% [V/V], aqueous 3.8 mM sodium laurylsulfate adjusted to pH 2.3 with phosphoric acid, 35% [V/V] acetonitrile, 30 min isocratic, 20 °C, 1.0 ml/min, 20 µl, 220 nm [20]. **3. Caffeine, procaine, and sodiumsalicylate**: Agilent Zorbax Eclipse Plus C18 (150 x 4.6 mm; 5 µm), mobile phase consisting of a mixture of 2% [V/V] tetrahydrofuran, 2.5% [V/V] acetonitrile, and 95.5% [V/V] aqueous 10 mM anhydrous sodium acetate adjusted to pH 4.5 with glacial acetic acid, 20 min isocratic, 20 °C, 1.0 ml/min, 10 µl, 275 nm [21]. **4. a) Dipyridamole**: Agilent Zorbax Eclipse Plus C18 Analytical (150 x 4.6 mm; 5 µm), mobile phase A consisting of aqueous 7.35 mM potassium dihydrogen phosphate adjusted to pH 7 with 0.5 M sodium hydroxide and mobile phase B methanol, gradient: 0 min B = 60%, 5 min B = 60%, 19 min B = 95%, 24 min B = 60%, 29 min B = 60%, 45 °C, 2.35 ml/min, 5 µl, 295 nm [22]. **4. b) Dipyridamole impurity identification by means of MS/ESI – ion trap:** Agilent Zorbax Eclipse Plus C18 Analytical (150 x 4.6 mm; 5 µm), mobile phase A millipore water and mobile phase B methanol MS grade, gradient: 0 min B = 10%, 10 min B = 40%, 13 min B = 40%, 16 min B = 95%, 20 min B = 10%, 45 °C, 1.0 ml/min, 5 µl; ESI-MS spectra was obtained in the positive ion electrospray mode using the following settings: capillary voltage 3500 V, nitrogen gas for the nebulizer 60 psi, nitrogen gas as drying gas 10.00 l/min and 350 °C, range of scan 100 m/z – 2200 m/z **5. Furosemide**: Agilent Zorbax Eclipse Plus C8 Analytical (250 x 4.6 mm; 5 µm), mobile phase consisting of a mixture of 70% [V/V] aqueous 21.0 mM potassium dihydrogen phosphate and 9.8 mM cetrimide adjusted to pH 7.0 with ammonia 28–30% and 30% [V/V] 1-propanol, 25 min isocratic, 20 °C, 1.0 ml/min, 20 µl,

238 nm [23]. **5. b) Furosemide impurity identification by means of MS/ESI – ion trap:** Agilent Zorbax SB-CN (50 x 4.6 mm; 3.5 µm), mobile phase A millipore water 0.1 % acetic acid and mobile phase B acetonitril MS grade 0.1% acetic acid, gradient: 0 min B = 5%, 5 min B = 5%, 10 min B = 90%, 15 min B = 90%, 20 min B = 5%, 25 °C, 0.4 ml/min, 5 µl; ESI-MS spectra was obtained in the negative ion electrospray mode using the following settings: capillary voltage 3500 V, nitrogen gas for the nebulizer 40 psi, nitrogen gas as drying gas 8.00 l/min and 350 °C, range of scan 200 m/z – 400 m/z **6. Metamizole sodium**: Agilent Zorbax Eclipse Plus C18 Analytical (250 x 4.6 mm; 5 µm), mobile phase consisting of a mixture of 28% [V/V] methanol and 72% [V/V] buffer solution prepared as follows: 1000 volumes of 50 mM sodium dihydrogen phosphate and 1 volume of trimethylamine adjusted to pH 7.0 with sodium hydroxide solution 50%, 35 min isocratic, 20 °C, 1.0 ml/min, 10 µl, 254 nm [24].

2.4. Preparation of old samples

Suprarenin: 0.5 ml of epinephrine hydrochloride ampoule solution was diluted to 50.0 ml with solvent mixture B consisting of a mixture of 87% [V/V] solvent mixture A (see above) and 13% [V/V] acetonitrile, injecting threefold. Additionally, 0.1 ml of epinephrine hydrochloride ampoule solution was diluted to 1.0 ml with solvent mixture B (1:10 dilution). **Adrenalin in Oil 1:500**: 0.5 ml of epinephrine solution was diluted to 100.0 ml with solvent mixture B and additionally for adrenochrome determination 0.1 ml of ampoule solution was diluted to 1.0 ml with solvent mixture B, injecting threefold. **Effortil®:** 0.5 ml of etilefrine hydrochloride ampoule solution was diluted to 50.0 ml with water. Three times 5.0 ml of the stock solution was diluted to 50.0 ml, each injecting once. **Sympatol®:** 0.5 ml of synephrine tartrate ampoule solution was diluted to 50.0 ml with mobile phase. Three times 1.0 ml of the stock solution was diluted to 100.0 ml, each injecting once. **Impletol:** Three times 0.5 ml of ampoule solution was diluted to 100.0 ml with mobile phase. 1.0 ml of each stock solution was further diluted to 10.0 ml with mobile phase, each injecting once. **Coffeinum salicylicum:** 0.5 ml of ampoule solution was diluted to 50.0 ml with mobile phase. Three times 1.0 ml of the stock solution was diluted to 100.0 ml with mobile phase, each injecting once. **Persantin®:** 0.5 ml of dipyridamole ampoule solution was diluted to 50.0 ml with methanol, injecting threefold immediately after preparation; the solution was stored under light protection and injected after seven days. 25.0 ml of the solution was transferred to a 25.0 ml volume flask, stored under daylight exposure and injected after seven days. 0.2 ml of dipyridamole ampoule solution was diluted to 20.0 ml with methanol MS grade for MS/ESI – ion trap analysis. **Lasix®:** Three times 0.5 ml of furosemide ampoule solution was diluted to 50.0 ml with mobile phase. 5.0 ml of each stock solution was further diluted to 50.0 ml with mobile phase, each injecting once. 0.1 ml of furosemide ampoule solution was

diluted to 20.0 ml with acetonitrile MS grade for MS/ESI–ion trap analysis. **Novalgin®:** Three times 0.5 ml of metamizole sodium ampoule solution was diluted to 50.0 ml with methanol. 1.0 ml of each stock solution was diluted to 100.0 ml with mobile phase, each injecting once. Validation and impurity quantitation are described in the supporting information.

2.5. Validation and impurity quantitation

For details please refer to the supporting information of this article.

3. Results and discussion

The age of the ampoules from the “PEAK-collection” was determined as far as possible by means of the batch label, the brand logo, and the lettering on the primary and secondary packaging, because of the lack of expiry dates or manufacturing dates labeled on the ampoules in former times (Table 1). Further information was obtained from the original manufacturer in some cases. In order to determine the content of the expired ampoules the HPLC methods applied here had to be validated.

3.1. Validation

HPLC methods used in the Ph. Eur. for assessment of related substances have been validated during the development for pharmacopoeia monographs with regard to specificity and separation of all impurities. In order to apply these methods for determinations of drug content the linearity, accuracy, and precision have to be proved in accordance to the International Conference on Harmonization guideline Q2R(1) for analytical methods [25].

3.1.1. Linearity

The linearity was determined within the range from 80% to 120% of API target concentration. For impurity quantitation calibration curves were prepared for (1*R*)-1-(3,4-dihyroxyphenyl)-2-(methylamino)-ethansulfonic acid (sulfonic acid derivative), norepinephrine, adrenochrome, 4-aminobenzoic acid, and theobromine in adequate ranges (see supporting information). Each calibration curve was constructed of five levels whereas each calibration solution was prepared once and injected three times. The relative standard deviation (RSD) on every level of every API and impurity was below 1%. Coefficient of determination (R^2) of every curve was higher than 0.9904, hence the linearity was confirmed (see Table 2).

Table 2
Calibration curves of APIs and observed impurities

Drug	Range (µg/ml)	Equation of Calibration curve	R^2
Epinephrine hydrochloride	9.6–14.4	$y = 30.239x + 10.028$	0.9954
Etilefrine hydrochloride	8.0–12.0	$y = 30.365x + 2.5613$	0.999
Synephrine	3.2–4.8	$y = 57.382x + 6.2178$	0.9944
Procaine hydrochloride	8.0–12.0	$y = 29.749x - 0.4614$	0.9998
Caffeine	5.6–8.4	$y = 29.747x + 1.4178$	0.9997
Sodium salicylate	6.0–12.0	$y = 3.7228x - 3.8777$	0.9995
Dipyridamole	40.0–60.0	$y = 7.7029x - 9.6438$	0.9972
Furosemide	8.0–12.0	$y = 81.734x + 6.7609$	0.9996
Metamizole sodium	40.0–60.0	$y = 13.754x - 24.806$	0.998
Sulfonic acid derivative	1.0–5.0	$y = 36.33x + 2.2727$	0.9998
Norepinephrine	0.3–2.0	$y = 39.159x + 0.5203$	1.000
Adrenochrome	0.3–1.0	$y = 13.546x + 0.2263$	0.9994
4-Aminobenzoic acid	0.5–1.0	$y = 53.979x + 3.0046$	0.9904
Theobromine	0.8–1.2	$y = 40.276x + 2.4923$	0.999

3.1.2. Precision

Precision of the method was determined by measuring six replicates of the reference ampoules or reference drugs by performing the same dilution procedure that was applied for sample preparation of the ampoules. Each of the six samples was injected once. The results were expressed as RSD, given in Table 3, Suprarenin®, Procain 2%, Persantin®, Furosemid-ratiopharm®, and Novaminsulfon-ratiopharm® were used. Different dosage forms had to be used for etilefrine (Effortil® drops 7.5 mg/ml) and caffeine (Caffeinum tablets 0.2 g). No suitable reference drug was available for synephrine. Therefore, a stock solution for substitution was prepared with synephrine concentration of 0.4 mg/ml. The RSDs calculated lower than 1.0% confirmed sufficient precision of the methods. The content of all reference APIs remained in the range from 95 to 105% of the labeled concentration without observing instabilities.

Table 3
Assay of reference ampoules and determination of precision

Reference drug	API content in percent (± SD)	Precision (n=6) RSD (%)
Suprarenin® ampoules 1 mg/ml Sanofi	101.6 (± 0.3)	0.3
Effortil® drops 7,5 mg/ml Boehringer	101.3 (± 1.0)	1.0
Synephrine reference	Ref. ampoule unavailable	0.4
Coffeinum N 0.2 g tablets Mylan dura	97.7 (± 1.0)	1.0
Procain 2.0% ampoules Steigerwald 2 ml	100.0 (± 0.4)	0.4
Persantin® ampoules 10 mg/2 ml Boehringer	97.3 (± 0.8)	0.8
Furosemid-ratiopharm® ampoules 20 mg/2 ml	102.3 (± 0.9)	0.9
Novaminsulfon-ratiopharm® ampoules 1 g/2 ml	100.8 (± 0.9)	0.9

3.1.3. Accuracy

Accuracy was assessed by using nine quality control (QC) samples at 80%, 100%, and 120% levels, three replicates of three concentration levels each, covering the specified range. Accuracy validation parameters are given in Table 4. Values obtained were in the accepted range from 98.6–100.9%. The RSD results were calculated by the variance obtained for accuracy determination throughout the total analytical process including weight and dilution procedure of the references. RSD results obtained are ± 2% indicating sufficient precision values.

Table 4
Determination of accuracy

Drug	Level	Accuracy (%)	RSD (%)
Epinephrine hydrochloride	80%	98.6	0.6
	100%	99.9	0.3
	120%	100.0	0.4
Etilefrine hydrochloride	80%	99.4	1.5
	100%	100.7	1.5
	120%	100.8	0.3
Synephrine	80%	99.6	0.2
	100%	100.5	0.6
	120%	100.9	0.2
Procain hydrochloride	80%	99.3	0.5
	100%	99.3	1.9
	120%	99.0	1.0
Caffeine	80%	99.5	1.1
	100%	99.1	1.5
	120%	98.8	1.3
Dipyridamole	80%	100.3	0.9
	100%	100.2	0.7
	120%	100.5	0.8
Furosemide	80%	99.6	0.4
	100%	100.3	0.5
	120%	99.2	1.1
Metamizole sodium	80%	99.9	0.6
	100%	99.8	0.5
	120%	100.6	0.2

3.2. Quantitative analysis of samples

Content of APIs and specified impurities were determined by linear regression according to equations given in Table 2. Quantification by means of normalization procedure was necessarily utilized when impurities were unspecified or no reference available (Table 5). Basically, there are two suitable procedures to quantify unspecified impurities, normalization procedure and dilution of the main peak as reference as usually done in the Ph.Eur. for related substances. In the study presented here both approaches led to the same results.

Table 5

API content in old ampoules and their impurities

Product	API content in percent (± SD)	RSD (%)	Observed impurities
Suprarenin hydrochl. synth 1:1000 Bayer	70.4 (± 0.2) [a]	0.3	Sulfonic acid derivative (0.349 mg/ml ≙ 25.9%) [a], norepinephrine (0.008 mg/ml ≙ 0.9%) [a], unknown impurity (3.7%) [b]
Adrenalin in Oil 1:500 Park Davis & Company	74.3 (± 0.2) [a]	0.3	Adrenochrome (7.4 µg/ml ≙ 0.4%) [a]
Effortil® 0.01 g/ccm C.H.Boehringer Sohn Ingelheim am Rhein	98.1 (± 0.9) [a]	0.9	Unknown impurity (0.3%) [b]
Sympatol® 0.06 g/ccm C.H.Boehringer Sohn Ingelheim	97.5 (± 0.3) [a]	0.3	Unknown impurity (2.8%) [b]
Caffeine in Impletol 1.42% Bayer	101.0 (± 0.8) [a]	0.8	-
Procaine hydrochloride in Impletol 2.0% Bayer	79.3 (± 0.5) [a]	0.6	4-Aminobenzoic acid (1.535 mg/ml ≙ 15.3%) [a]
Coffeinum Natriumsalicylicum 0.2 g/ccm	100.4 (± 0.6) [a]	0.6	Theobromine (0.113 mg/ml ≙ 0.1%) [a]
Persantin® 10 mg/ 2 ml Thomae	85.7 (± 0.4) [a]	0.5	Seven impurities (0.5–5.7%) [b]
Lasix® 20 mg/2 ml HOECHST AG	99.3 (± 1.8) [a]	1.8	Saluamine (1.3%) [b]
Novalgin® 50% 2 ccm HOECHST AG	99.7 (± 1.7) [a]	1.7	-

[a] Quantitation by linear regression
[b] Qauntitation by normalization procedure

3.2.1. Epinephrine ampoules

For determination of content of epinephrine, the range needed to be extended from 55% to 120% by two additional calibration solutions, because of epinephrine concentrations measured were lower than 80%. The following equation obtained was applied for calculation: $y = 30.922x + 2.3384$, $R^2 = 0.9977$. Two ampoules containing epinephrine were assessed for their content and impurity profile: Suprarenin hydrochl. 1:1000 and Adrenalin in Oil 1:500, the latter looking slightly reddish before opening the ampoule. The epinephrine hydrochloride concentration measured was 0.854 mg/ml in the Suprarenin ampoule, being equivalent to 70.4% of the labeled amount. Two specified impurities were verified, the sulfonic acid derivative (RRT: 0.2) present in a concentration of 0.349 mg/ml and norepinephrine (RRT: 0.8) present in a concentration of 0.008 mg/ml, being equivalent to 25.9% and 0.9% of epinephrine target assay concentration. The contents of the API and the impurities summed up resulted in 97.2% of the labeled amount. In addition, an unknown impurity (RRT: 3.6) of about 3.7% was quantified by normalization procedure in the sample solution (1:10 dilution) (see Fig. 3).

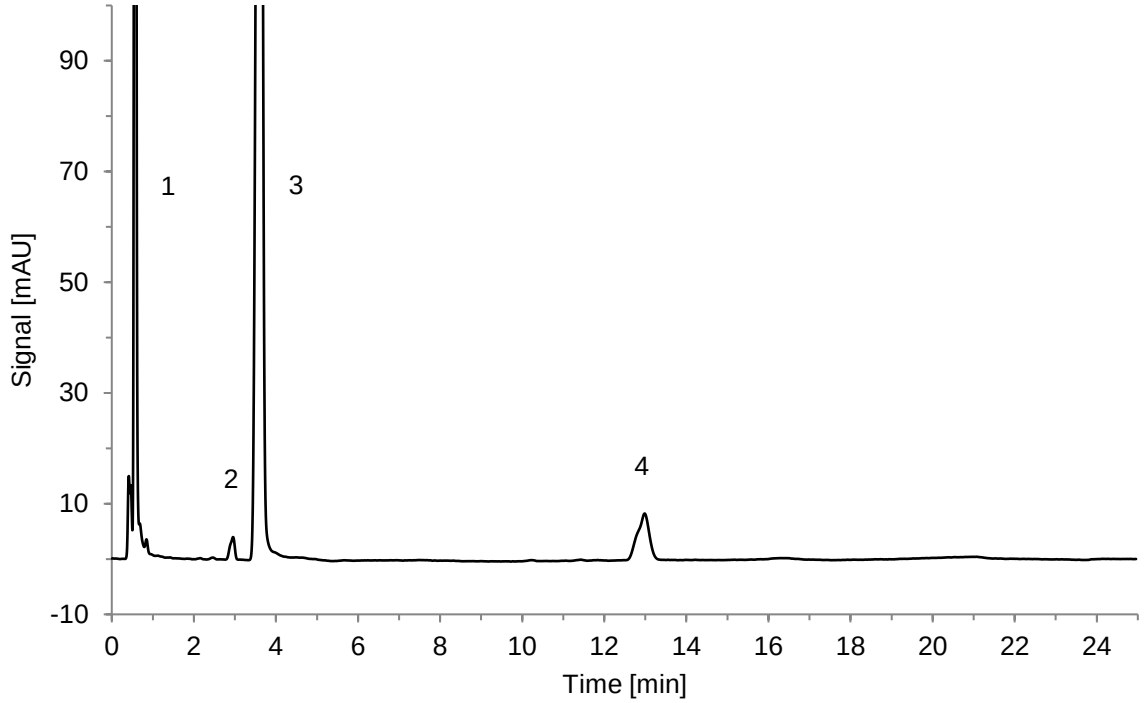

Fig. 3. Chromatogram of Suprarenin 1:10 dilution, order of elution: sulfonic acid derivative (1), norepinephrine (2), epinephrine (3), unknown impurity (4)

In the second ampoule “Adrenalin in Oil 1:500” a concentration of 1.486 mg/ml epinephrine was found, being equivalent to 74.3% of the labeled adrenaline concentration. Additionally, a peak next to the injection peak was observed, so the method had to be adjusted, as described in method *1.b*, to achieve separation and to identify adrenochrome (see Fig. 4). Consequently, the concentration of adrenochrome, which was responsible for the red color of the solution, was assayed to be 7.4 µg/ml, inferred that 7.5 µg/ml (0.4%) of epinephrine had been reacted to adrenochrome. No further impurities were noticed by means of the HPLC applied. So the epinephrine mass balance found was 1.494 mg/ml. The gap to the labeled amount of epinephrine (2 mg/ml) could not be closed.

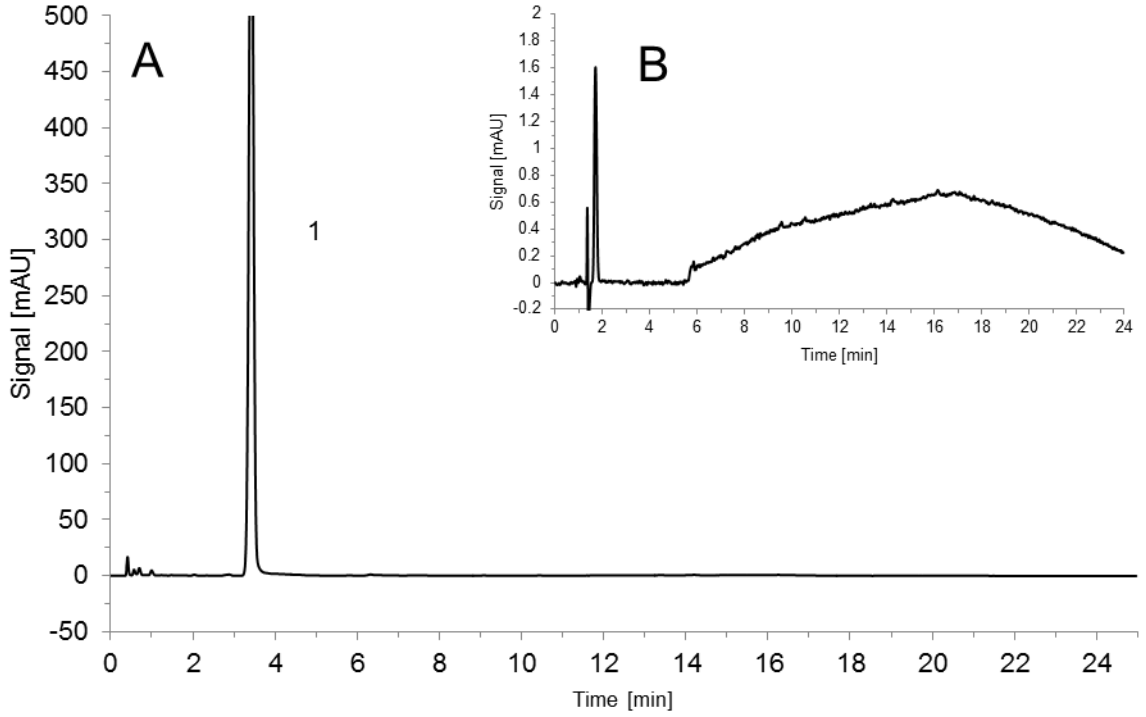

Fig. 4. A: Chromatogram of a dilution of “Adrenalin in Oil” 1:10 dilution monitoring epinephrine (1) at 210 nm according to method *1.a*. B: Chromatogram of determination of adrenochrome at 490 nm according to method *1.b*.

3.2.2. Effortil® and Sympatol®

In the Effortil® ampoule a concentration of 9.805 mg/ml etilefrine hydrochloride was found, corresponding to a content of 98.1%. The chromatogram of the stock solution showed an unknown impurity (RRT: 0.9) of about 0.3% by normalization procedure. Before analyzing Sympatol® separation of synephrine and its related substances such as octopamine, tyramine, and 1-(4-hydroxyphenyl)-2-(methylamino)ethanone had been shown in a pretrial (see Fig. 5). A synephrine concentration of 40.38 mg/ml was found in Sympatol®. It equates to 97.5% of the labeled amount of 41.41 mg/ml synephrine, corresponding to 60 mg/ml synephrine tartrate. Beside the API peak, a tartrate peak, and peak of an unspecified impurity (RRT: 0.7) of 2.8% were present in the stock solution.

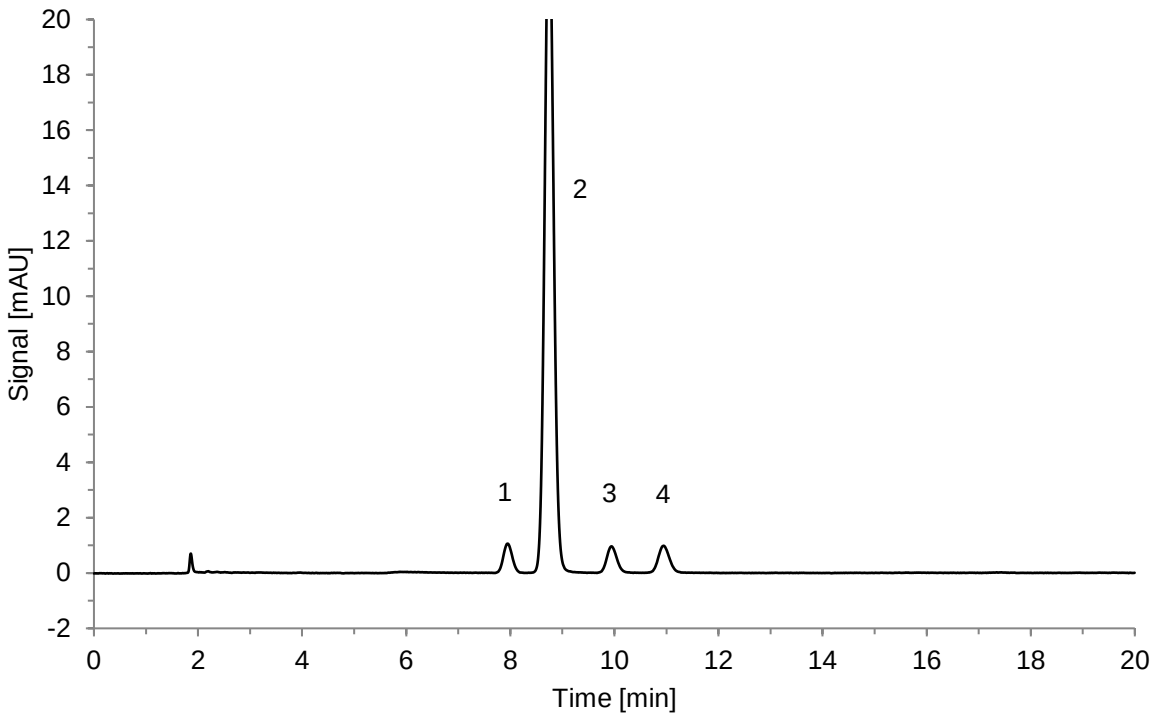

Fig. 5. Chromatogram of synephrine (5 µg/ml) spiked with its related substances (each 0.25 µg/ml), order of elution: octopamine (1), synephrine (2), tyramine (3), 1-(4-hydroxyphenyl)-2-(methylamino)ethanone (4)

3.2.3. Impletol and Coffeinum sodiumsalicylicum

For determination of Impletol co-elution of caffeine, procaine, and their impurities was excluded by using the method of caffeine described in the Ph. Eur. Separation of all compounds was sufficient. Impletol was labeled with 1.42% caffeine and 2% procaine hydrochloride, being equivalent to 14.2 mg/ml and 20 mg/ml, respectively. The caffeine concentration measured was 14.349 mg/ml being a content of 101.0% and the procaine hydrochloride concentration found was 15.861 mg/ml, corresponding to a content of 79.3%. The chromatogram is given in Fig. 6. The concentration of 4-aminobenzoic acid found was 1.535 mg/ml, hence 3.053 mg/ml (15.3%) of procaine hydrochloride was hydrolyzed to 4-aminobenzoic acid. In conclusion the mass balance of caffeine was 101.0% and of procaine hydrochloride was 94.6% of the declared amount.

The content of the “Coffeinum Natriumsalicylicum 0.2 g” ampoule was 88.1 mg/ml (44%) of caffeine and 112.7 mg/ml (56%) of sodiumsalicylate, combined confirming the labeled claim. Small theobromine traces of 0.1 mg/ml were found in the stock solution.

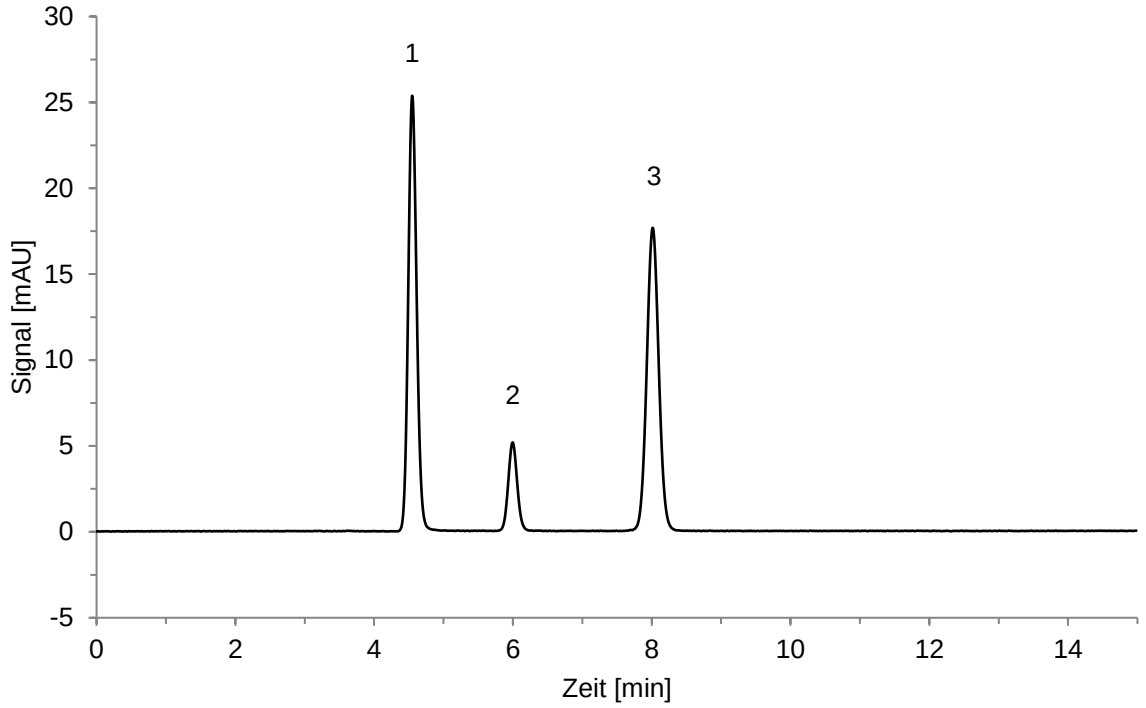

Fig. 6. Chromatogram of Impletol test solution, order of elution: procaine (1), 4-aminobenzoic acid (2), caffeine (3)

3.2.4. Persantin®

The concentration of dipyridamole found in Persantin® was 4.286 mg/ml immediately after opening the ampoule, being equivalent to a content of 85.7%. Using the normalization procedure seven impurities were quantified, one of about 5.7% (RRT: 0.12) and additional impurities (RRT: 0.09, 0.20, 0.39, 0.79, 1.38, 2.00) in a range of 0.5–1.6% (see Fig. 7). The phenomenon of instability to light exposure of dipyridamole was observed by comparison of the dipyridamole content after one-week storage under daylight exposure and light protection. No significant decrease of content occurred in the test solution within one week protected from light: It changed not significantly from 85.7% (95% confidence interval (CI), 84.7–86.7%) to 84.6% (95% CI, 83.2–85.9%). However, the content decreased to 8.9% (95% CI, 8.7–9.1%) under daylight exposure. The same phenomenon was seen in reference ampoules, there the content was found to be 97.3% at the beginning, further decreased to 24.8% (95% CI, 24.3–25.3%) after daylight exposure for one week. In turn, one peak (RRT: 0.09) increased over the term of storage. A photolysis product with a mass of 519 g/mol is described in the literature, formed by an oxidation reaction of piperidine ring moiety [26]. The increase of this photolysis product was confirmed by means of MS/ESI – ion trap analysis, as described in method *4.b,* (see Fig. 8).

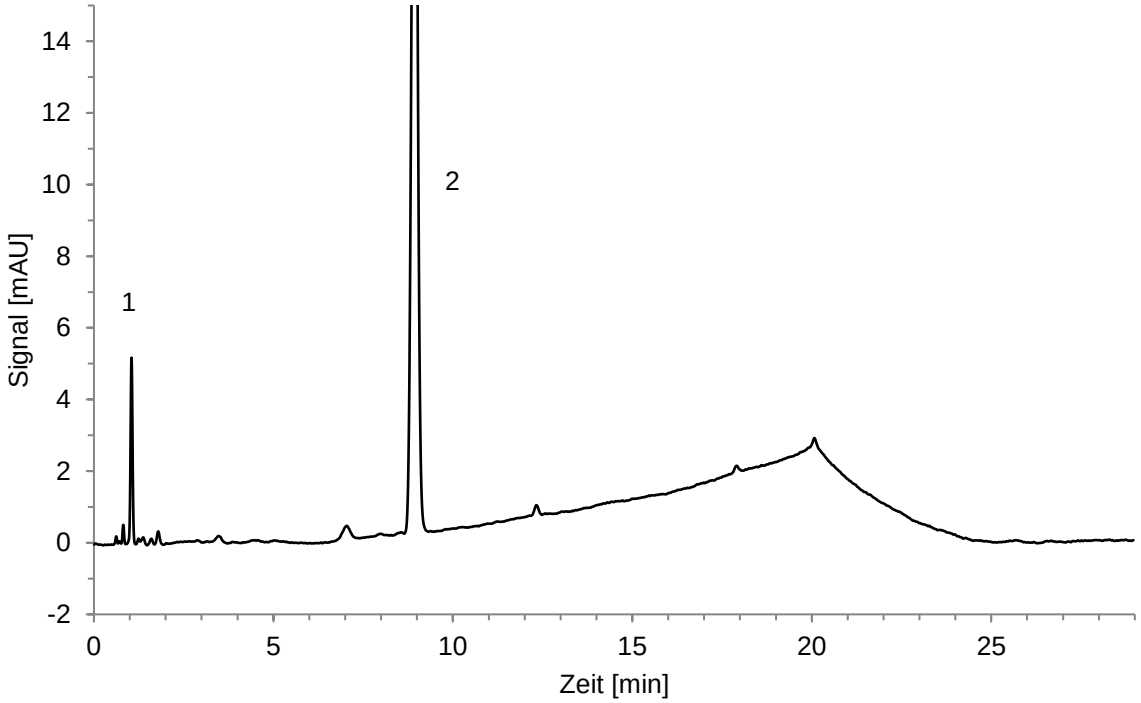

Fig. 7. Chromatogram of Persantin® test solution, order of elution: unknown impurity (1), dipyridamole (2)

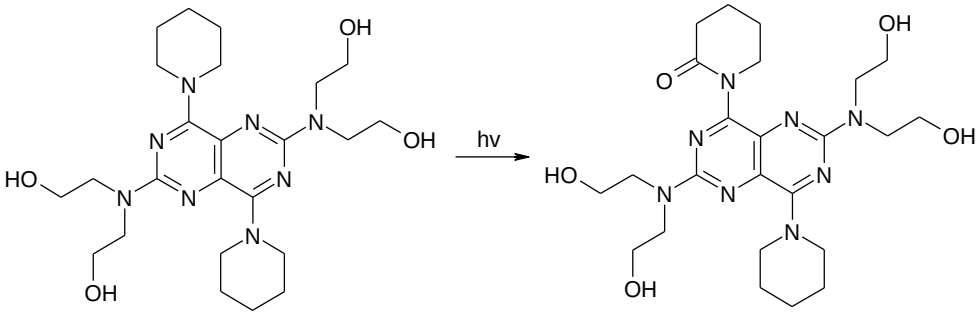

Fig. 8. Photolysis reaction of dipyridamole during day light exposure

3.2.5. Lasix®

In the Lasix® ampoule a concentration of 9.93 mg/ml of furosemide was measured. This concentration corresponds to 99.3% of the labeled claim. A second peak was observed and assigned to saluamine (RRT: 0.5) specified as Impurity C. The amount of saluamine was quantified to be roughly 1.3% by normalization procedure. The mass of 250.7 g/mol of saluamine was confirmed by means of MS/ESI – ion trap analysis, according to method *5.b.*

3.2.6. Novalgin®

Finally, the content of the Novalgin® ampoule was evaluated. A concentration of 498.7 mg/ml of metamizole sodium was measured, corresponding to 99.7% of the label claim (500 mg/ml metamizole sodium).

4. Conclusions

Temperature, humidity, and light exposure are critical parameters in long term stability of FPPs. Today ampoules are manufactured under vacuum or under an inert gas to avoid oxidation reactions and to allow sterile production. For FPPs investigated in this study no information is available on manufacturing process. The storage is assumed to be under ambient temperature and light protection that may have been fluctuating. The source of FPPs tested is from patients or their relatives or private collectors. In this investigation solely liquid dosage forms were studied for two reasons: liquid forms contain the API in solution and is not affected by possibly incomplete release from the FPP e.g. tablet. This would be the case when studying solid forms. Secondly instability may be expected from solutions more likely than from solid forms like tablets, dragees, etc. Decompositions of adrenaline, procaine and dipyridamole were expected. In Suprarenin, Adrenalin in Oil, Impletol, and Persantin® decreased contents of the declared API to about 70%, 74%, 79%, and 86%, respectively, were found. The content of 70% of dose present is a significant decay in active drug. However, taking into account the liquid dosage form, which is generally susceptible for instability, and the year of manufacture 1934 the content is surprisingly high. In addition, expectations of the stability of APIs such as caffeine, phenylalkylamines without catechol structure, sodium salicylate, furosemide, and metamizole were confirmed, because of the absence of reactive moieties in the molecules, furosemide in brown ampoules, and the secondary package prevented the API against degradation. The ampoules have been untouched in all these years.

The results showed that five out of nine ampoules met acceptance criteria for content being valid for pharmaceutical products containing small molecules nowadays. Therefore, those FPPs can be used today in spite of their high age of 53–72 years. SLEP was the most comprehensive study addressing the issue of drug stability beyond labeled expiration dates [4]. 122 different drug products stored under controlled conditions in the military department were assessed in the program. Numerous anti-infectives, analgetics, and antihistaminics were included. A percentage of 88% of all lots were extended at least one year but the conclusion was to carry out regular tests of lots, to evaluate and assess the results for real shelf-life prediction. Even though the present study was retrospective in its nature it can be stated that shelf-lives longer than five years may be considered by the

regulatory authorities for pharmaceutical products with a chemistry that is not inherently sensitive to instability. Further systematic investigations at these institutions are underway to explore potential candidates for extension of shelf-life.

Conflict of interest

The authors declare that they have no conflict of interest.

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4. Analysis of Lobesym, Kombetin, and Atriphos ampoules manufactured more than 50 years ago

1. Introduction

The objective of this study was to determine the content and to identify the degradation profile of the three ampoules Lobesym, Kombetin, and Atriphos by means of HPLC-UV and HPLC-MS. The ampoules are pharmaceutical relicts, being no longer used for the treatment of patients. The Lobesym ampoule was manufactured between 1936 and 1946, the Kombetin and Atriphos ampoules were estimated to be older than 50 years.

Lobesym is a two-component mixture containing synephrine and lobeline which is a piperidine alkaloid. In former times, such an injection solution was applied in the therapy of chronic pneumonia, asthma, or bronchitis. The alkaloid was extracted from a plant named Lobelia inflata L. [1].

Kombetin consists of a mixture of various cardiac glycosides, being natural compounds used for the treatment of cardiac deficiencies. This mixture was extracted from the seeds of Strophantus kombe, stated as strophanthin-K glycosides [2–4]. Typically, the main constituents are 60–80% of k-strophanthoside, 15–25% of erysimoside, and 10–15% of k-strophanthin-β, but they vary in content. Furthermore, about 15 related glycosides were isolated and identified as well [5].

The nucleotide adenosine triphosphate is the active pharmaceutical ingredient (API) of the Atriphos ampoule. It was utilized for the treatment of pain and vascular disease. In general, the nucleotide adenosine triphosphate is susceptible to hydrolysis leading to adenosine monophosphate and adenosine in aqueous solutions or under physiological conditions [6, 7].

Since the APIs are not monographed in any pharmacopoeia, appropriate HPLC-UV and HPLC-ESI/MS techniques had to be applied for analysis based on methods published in the literature [1, 2, 7].

2. Experimental section

2.1. Chemicals and reagents

The ampoules were part of the “PEAK-collection” of expired drugs at the IBMP (Institute of Biomedical and Pharmaceutical Research in Nürnberg-Heroldsberg, Germany). The Lobesym ampoule is declared to contain 17.5 mg/ml of lobeline phosphate and 81.5 mg/ml of synephrine sulfate (C.H. Boehringer Sohn Ingelheim, 1936–1946), Kombetin 1/8 consists of

0.125 mg/ml mixture of cardenoides (C.F. Boehringer & Soehne GmbH Mannheim, older than 1978), the Atriphos ampoule contains 5.43 mg/ml of adenosine triphosphate disodium (unknown Hungarian manufacturer, older than 50 years). Phosphoric acid 85%, sodium laurylsulfate, anhydrous sodium acetate, acetophenone, ammonium formate, formic acid, potassium hydrogenphosphate, dipotassium hydrogenphosphate, (-)-lobeline hydrochloride, synephrine, k-strophanthidin, adenosine triphosphate, adenosine monophosphate, and adenosine were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany), HPLC grade acetonitrile, HPLC grade methanol, and MS-HPLC grade acetonitrile from VWR International GmbH (Darmstadt, Germany). Water for HPLC was purified using the Milli-Q purification system by Merck Millipore (Schwalbach, Germany).

2.2. Apparatus

HPLC experiments were performed on a HPLC system 1100 series from Agilent Technologies (Waldbronn, Germany) consisting of a vacuum degasser (G1322A), binary pump (G1312A), autosampler (G1313A), thermostated column oven (G1316A), diode array detector (G1315B) and a MS/ESI – ion trap detector (G24450). Chromatograms were recorded and integrated using the Agilent ChemStation® software (Rev B.03.02). For pH-measurements a Metrohm 744 pH-Meter from Deutsche METROHM GmbH & Co. KG (Filderstadt, Germany) was used.

2.3. Methods

HPLC conditions for each ampoule: **1. a) Lobeline and synephrine quantitation:** Agilent Zorbax Eclipse Plus C8 Analytical (250 x 4.6 mm; 5 µm), mobile phase A consisting of a mixture of 65% [V/V], aqueous 1.1 g/L sodium laurylsulfate adjusted to pH 2.3 with phosphoric acid, 35% [V/V] acetonitrile, mobile phase B consisting of a mixture of 25% [V/V], aqueous 2.9 g/L sodium laurylsulfate adjusted to pH 2.3 with phosphoric acid, 75% [V/V] acetonitrile, 40 min gradient: 0 min B = 0%, 9 min B = 0%, 35 min B = 100%, 40 min B = 0%, 20 °C, 1.0 ml/min, 20 µl, 210 nm. **1. b) Lobeline impurity identification by means of MS/ESI:** Agilent Zorbax Eclipse Plus C8 Analytical (250 x 4.6 mm; 5 µm), mobile phase consisting of 70% [V/V], aqueous 30 mM ammonium formate adjusted to pH 2.8 with phosphoric acid, 30% [V/V] acetonitrile MS grade, isocratic, 25 °C, 0.8 ml/min, 5 µl; ESI-MS spectra was obtained in the positive ion electrospray mode using the following settings: capillary voltage 3500 V, nebulizer 60 psi, drying gas 12.00 l/min and 350 °C, range of scan 50–400 m/z, and collision gas helium **2. Kombetin quantitation and impurity profiling:** Agilent Zorbax Eclipse Plus C18 Analytical (150 x 4.6 mm; 5 µm), mobile phase A consisting of a mixture of 76% [V/V] water, 1% [V/V] formic acid, 23% [V/V] acetonitril, mobile phase B consisting of a mixture of 60% [V/V] water, 1% [V/V] formic acid, 39% [V/V] acetonitril MS

grade, 25 min gradient: 0 min B = 0%, 12 min B = 0%, 20 min B = 100%, 25 min B = 0%, 25 °C, 210nm, 0.6 ml/min, 20 µl; ESI-MS spectra was obtained in the negative ion electrospray mode using the following settings: capillary voltage 3500 V, nebulizer 65 psi, drying gas 12.00 l/min and 325 °C, range of scan 400–950 m/z. **3. Atriphos quantitation and impurity profiling:** Agilent Zorbax Eclipse Plus C8 Analytical (250 x 4.6 mm; 5 µm), mobile phase A consisting of aqueous 2.72 g/L of potassium hydrogenphosphate and 5.2 g/L of dipotassium hydrogenphosphate adjusted to pH 2.3 with phosphoric acid, mobile phase B consisting of methanol, 25 min gradient: 0 min B = 0%, 2 min B = 0%, 10 min B = 12.5%, 12 min B = 12.5%, 20 min B = 40.0%, 25 min B = 0%, 25°C, 1.0 ml/min, 5 µl, 220 nm.

2.4. Preparation of samples and standard solutions

Lobesym: 0.5 ml of ampoule solution was diluted to 50.0 ml with mobile phase A. Three times 1.0 ml of the stock solution was diluted to 100.0 ml with mobile phase A. 10.0 ml of the stock solution was further diluted to 100.0 ml with mobile phase A, injecting three times into the HPLC. For analysis by means of MS/ESI-ion trap, 0.2 ml of the ampoule solution was diluted to 20.0 ml with 1% formic acid in water. 1.0 ml of the stock solution was diluted to 100.0 ml with the same solvent. Five standard solutions containing synephrine at 3.0–7.2 µg/ml and lobeline at 6.9–16.7 µg/ml were prepared in solvent mixture consisting of 35%/65% water/ACN, respectively.

Kombetin: The ampoule solution was injected to HPLC without prior dilution procedure. Five calibration solutions containing k-strophanthidin between 2.5 and 50.0 µg/ml were prepared in mobile phase A.

Atriphos: 0.5 ml of the ampoule solution was diluted to 25.0 ml with mobile phase A, injecting three times. Five calibration solutions containing adenosine triphosphate at 14–21 µg/ml were prepared in mobile phase A. Five standard solutions of adenosine monophosphate and adenosine standard solutions were prepared at ranges of 40–60 µg/ml and 12–18 µg/ml, respectively.

3. Results and discussion

Contents of lobeline, synephrine, k-strophanthidin, adenosine triphosphate, adenosine monophosphate, and adenosine were performed by means of the external standard method. The coefficient of determination R^2 of every calibration curve was higher than 0.995, hence the linearity was given. Quantification of unknown impurities was necessarily done by means of normalization procedure. For quantitation of other cardiac glycosides, the aglycone k-strophanthidin was used as reference. The response factors of k-strophanthidin and the further cardiac glycosides were assumed to be identical at wavelength of 220 nm due to the

same core structure which is present in all related glycosides. Based on the respective molecular weight and the corresponding peak area, the concentration of each compound was calculated.

3.1. Lobesym

In the Lobesym ampoule, a synephrine concentration of 62.7 mg/ml was found, which is equal to 99.1% of the declared 63.3 mg/ml synephrine, corresponding to 81.5 mg/ml synephrine sulfate. Furthermore, a concentration of 8.5 mg/ml lobeline was found. It equates to 62.5% of the labeled claim of 13.6 mg/ml lobeline, corresponding to 17.5 mg/ml lobeline phosphate. When applying normalization procedure, 35.5% of an unknown degradation product and about 3% of acetophenone were ascertained in the test solution (see Fig. 1). Likewise, the same main degradant was generated when carrying out forced degradation tests by exposure of heat or intensive light to a reference solution of lobeline. The procedure for identification of the compound is described in the following section.

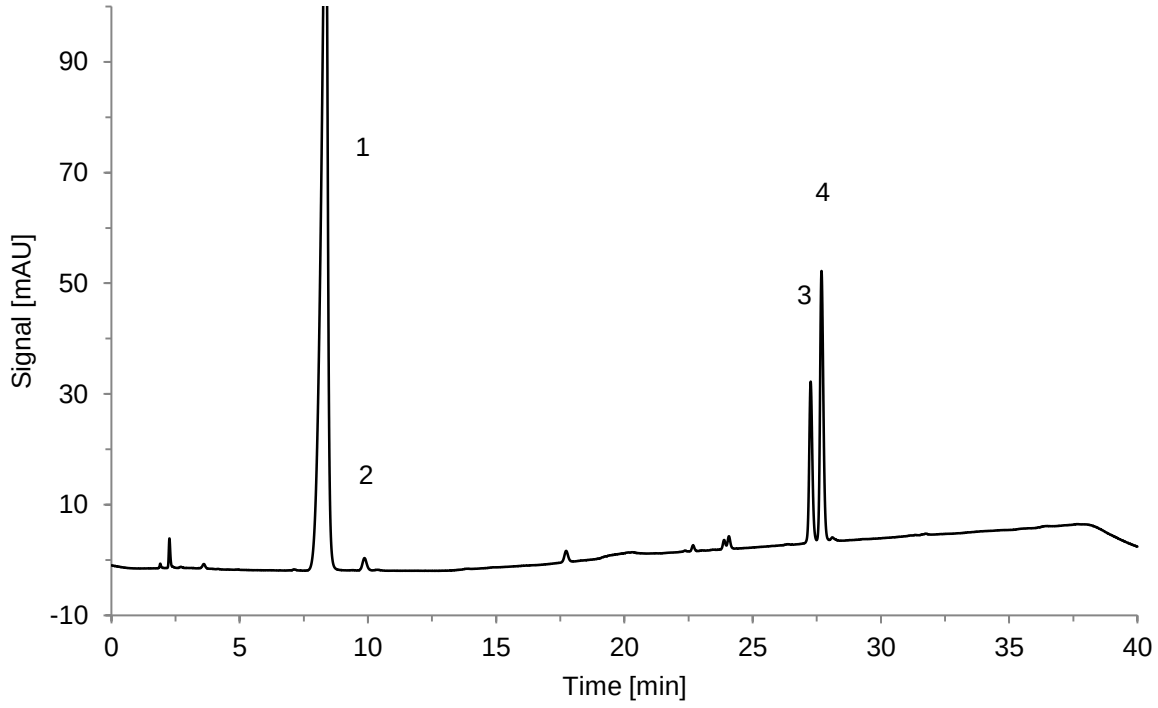

Fig. 1. Chromatogram of Lobesym 1:10000 dilution: Synephrine (1), acetophenone (2), lobeline isomere (3), lobeline (4).

In order to identify the unknown degradant, HPLC-MS/ESI analysis was carried out by applying MS-chromatographic conditions, as described in method *1.b)*. The MS-MS spectra provided information about the characteristic fragment ions of lobeline and the degradant. For both peaks, the protonated product ion $[M+H]^+$ of m/z 338.5 was obtained at mass spectrum (see Fig. 2). Fragmentation of lobeline led to product ions of m/z 96.7, 216.4,

218.1, 320.4, and 340.3 (Table 1). The m/z 320.5 can be explained by a loss of water. The ion at m/z 340.3 might have been formed by reduction of the carbonyl moiety, m/z 218.1 by splitting off the phenyl-2-ketoethyl unit, and m/z 216.4 by loss of phenyl-2-hydroxyethyl side chain. The product ion at m/z 96.7 might be explained by the *N*-methylated piperidine moiety. The identical fragmentation pattern was observed for the degradant. Hence, the formation of a diastereomeric compound can be hypothesized. The results of the aforementioned ESI/MS-MS measurements were in accordance with the mass spectra of lobeline published in the literature [1].

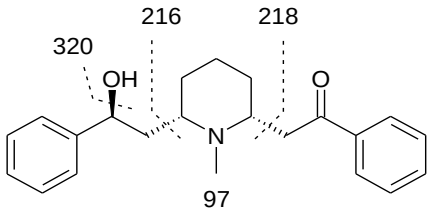

Fig. 2. Fragmentation units of lobeline

Table 1
Characteristic fragmentation ions.

Peak	Retention time (min)	$M+H^+$ (m/z)	MS-MS (m/z)
Lobeline diastereomere	18.7	338.5	96.8; 216.0; 218.1; 320.7; 340.2
Lobeline	19.9	338.5	96.7; 216.4; 218.1; 320.5; 340.3

3.2. Kombetin

The Kombetin ampoule was labeled to contain 0.125 mg/ml of a mixture of cardiac strophanthin-K glycosides, but no specified composition of this injection solution was declared or described elsewhere. Chromatographic separation of the components was sufficiently achieved (see Fig. 3). The chromatographic conditions were based on the method which was applied by Grosa et al. for characterization of strophanthin-K [2]. The order of elution of the compounds was revealed to be in accordance with the one reported by Grosa et al. Neoglucoerysimoside and k-strophantoside were eluated close to each other but overlapping of both peaks could not be avoided. Identification of the components was confirmed by means of ESI/MS analysis. Likewise, abundant adduct ions of strophanthin-K glycosides and formic acid $[M+HCOO]^-$ were observed. K-Strophanthoside was identified as the major constituent of the injection solution (see Fig. 4 and Table 2). All related cardiac glycosides consist of the identical aglycone structure, stated as k-strophanthidin, but they differ in the attached sugar moiety, e.g. cymarose, digitoxose, glucose, or a combination of these. In the sample solution, the concentration of the k-strophanthidin was found 9.0 µg/ml. Maybe it was formed by hydrolysis of the related cardiac glycosides or being part of the

extract. In the ampoule, the main compounds were calculated 55.9 µg/ml of k-strophanthoside, 28.1 µg/ml of erysimoside, 13.8 µg/ml of k-strophanthin-β, and 9.4 µg/ml of neoglucoerysimoside [3]. Traces of further glycosides were obtained in the chromatogram as well. The sum of all compound concentrations was found 125.7 µg/ml. In conclusion, the mass balance of strophanthin-K was 100.6% of the labeled amount.

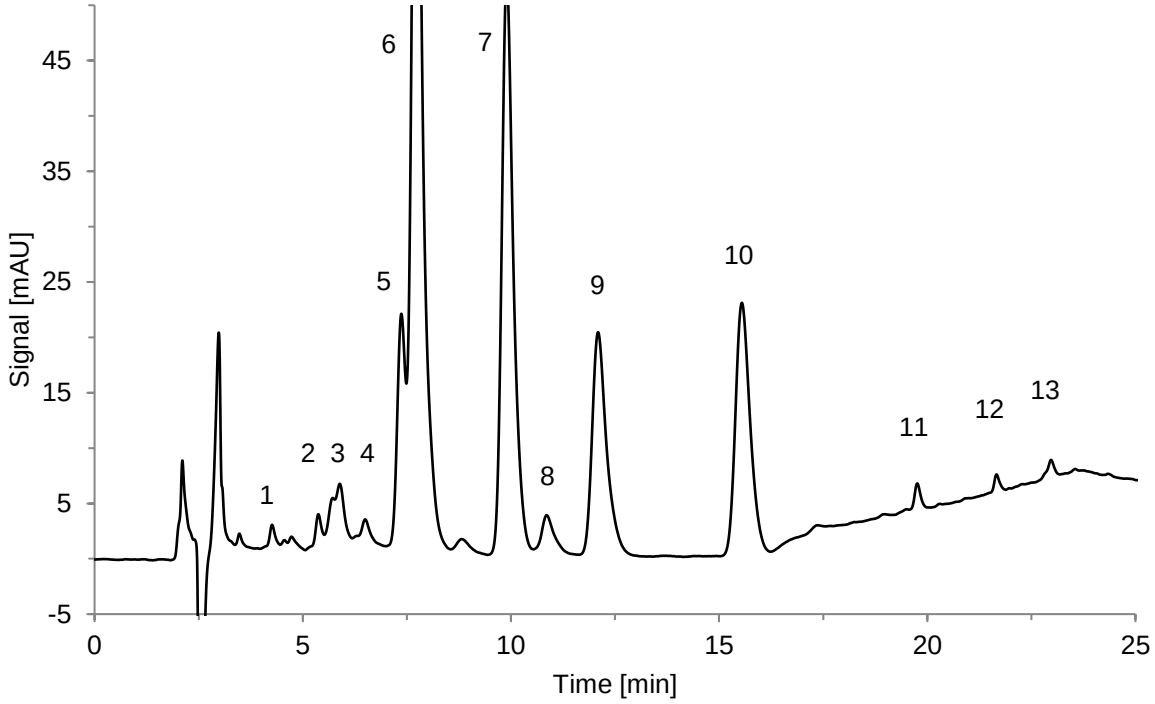

Fig. 3. Chromatogram of the Kombetin injection solution: unknown impurity (1–4), neoglucoerysimoside (5), k-strophanthoside (6), erysimoside (7), unkn. impurity (8), k-strophanthin-β (9), k-strophanthidin (10), helveticoside (11), unkn. impurity (12), cymarin (13).

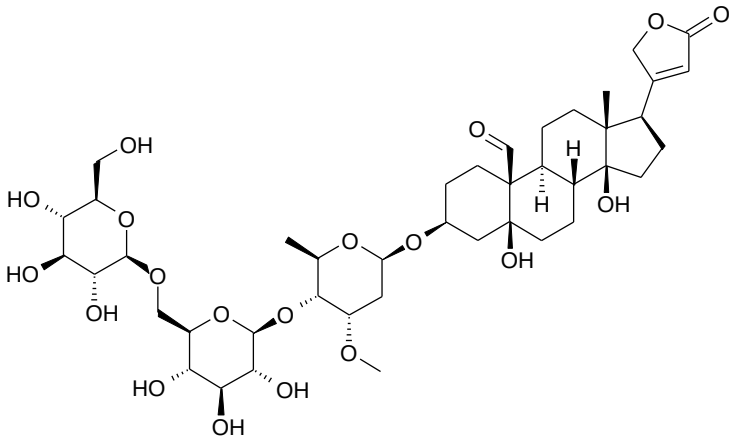

Fig. 4. Structure of k-strophanthoside

Table 2
Cardiac glycosides found in the Kombetin ampoule

Sugar	Substance	Molecular weight	Retention time (min)	Detection $[M+HCOO]^-$	Concentration (µg/ml)
-	Four unknown impurities	-	5.4–6.4	613.5; 611.5; 919.5; 743.6	0.8; 1.3; 3.2; 1.3
Digit-Glu-Glu	Neoglucoerysimoside	858.3	7.4	903.8	9.4
Cym-Glu-Glu	K-Strophanthoside	872.9	7.7	917.8	55.9
Digit-Glu	Erysimoside	696.8	9.9	741.6	28.1
-	Unknown impurity	-	10.9	451.4	1.6
Cym-Glu	K-Strophanthin-β	710.8	12.1	755.6	13.8
-	K-Strophanthidin	404.5	15.6	449.5	9.0
Digit	Helveticoside	534.6	19.8	579.7	0.6
-	Unknown impurity	-	21.7	-	0.3
Cym	Cymarin	548.7	23.0	593.3	0.5
					125.7

Abbreviations: Digit, Digitoxose; Glu, Glucose; Cym, Cymarose.

3.3. Atriphos

In most cases, stability studies of adenosine triphosphate were performed under physiological conditions or in human whole blood, where the compound was stable for just a few minutes or seconds due to excessive enzymatic degradation [7–10]. No data are available on the long-term stability of adenosine triphosphate in drug products, because of not being anymore in therapeutic use. In the old Atriphos ampoule, the adenosine triphosphate disodium concentration was evaluated to 0.80 mg/ml, corresponding to an API content of 14.7%. The chromatogram is depicted in Fig. 5. The concentration of adenosine monophosphate was found 2.56 mg/ml, hence 66.4% of adenosine triphosphate disodium was hydrolyzed to adenosine monophosphate. Furthermore, a concentration of 0.68 mg/ml of adenosine was found in the ampoule, 25.8% of adenosine triphosphate was decomposed to adenosine. In the literature, a high stability of adenosine in infusion solutions was described [6, 11]. In conclusion, the mass balance of adenosine triphosphate was 106.9% of the declared amount.

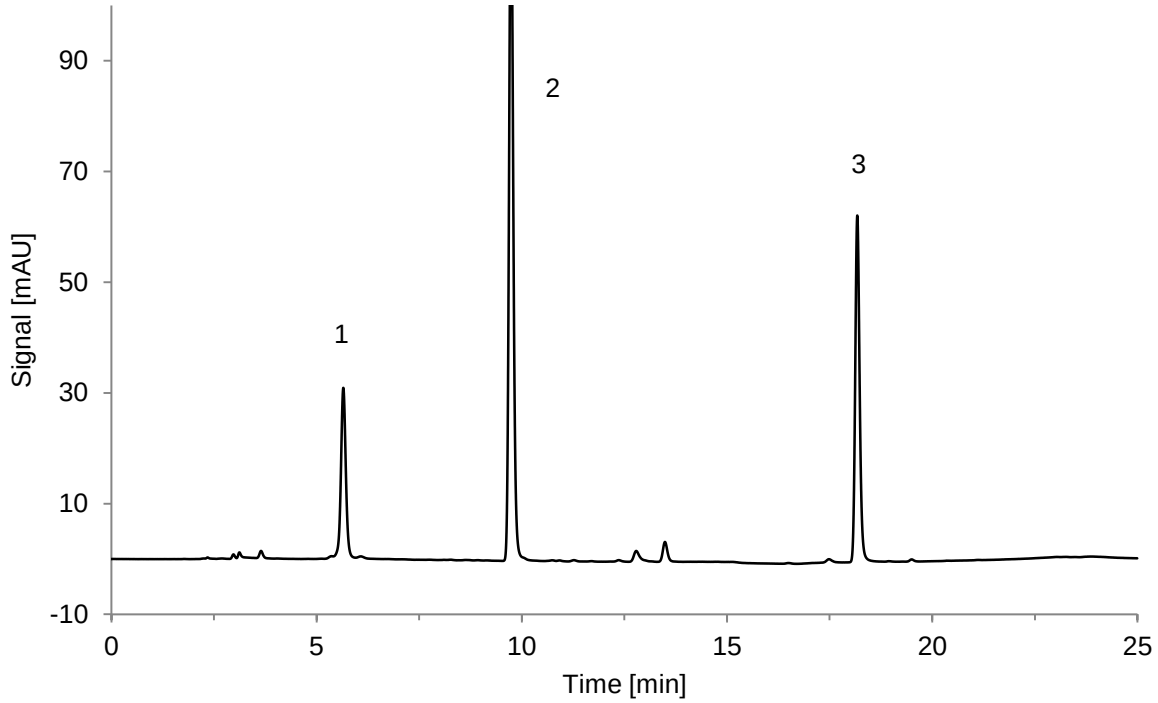

Fig. 5. Chromatogram of Atriphos 1:100 dilution, order of elution: adenosine triphosphate (1), adenosine monophosphate (2), adenosine (3)

4. Conclusion

In the present study the degradation profiles of the old ampoules Lobesym, Komebtin, and Atriphos were identified by means of HPLC-UV and HPLC-ESI/MS approaches. For Lobesym, the content of synephrine was 99.1% and the content of lobeline decreased to 62.5% after more than 72 years of storage. Formation of the isomere was identified to be the main degradation reaction. The composition of the Kombetin ampoule was identified and quantified by means of LC-ESI/MS measurements, but no significant decomposition has been occurred. Massive decay of adenosine triphosphate by hydrolysis to adenosine monophosphate and adenosine was ascertained, decreasing to an API content of 14.7% of the labeled claim.

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5. The stability of old scopolamine and dihydroergotamine ampoules

1. Introduction

The aim of the study presented here was determining the content of a 50 years-old scopolamine ampoule, also known as hyoscine, and a dihydroergotamine ampoule with an age of at least 40 years and identifying the impurity profiles by means of HPLC-UV, respectively. In the hyoscine hydrobromide and dihydroergotamine mesilate monographs of the Ph. Eur. 9.3, the liquid chromatography is utilized for the control of related substances [1, 2]. The quantitative analysis of the ampoules was conducted based on the methods of the monographs because they ensure selective separation of the API and degradation products. In order to apply these methods for content determinations, the linearity, intra-assay precision, and accuracy had to be verified corresponding to the ICH guideline Q2(R1) [3].

Due to the chemistry of the molecules and the dosage form, instabilities were expected before beginning with the experiments. Scopolamine is instable because of its ester function, which is easily hydrolyzed to scopine and tropic acid in aqueous solutions. In the case of the dihydroergotamine injection solution, degradation by epimerization to 2´-*epi*-9,10-dihydro-ergotamine might take place [4, 5]. Typical degradation reactions are depicted in Figure 1.

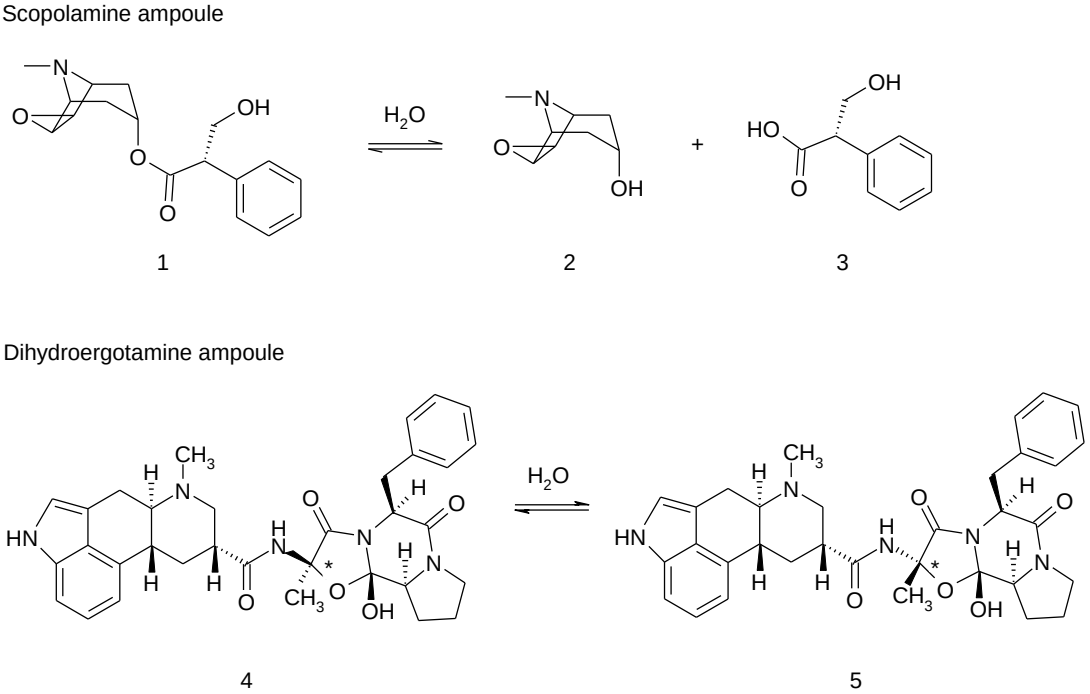

Fig. 1. Degradation reactions in aqueous solutions: scopolamine (1), scopine (2), tropic acid (3), dihydroergotamine (4), 2'-*epi*-9,10-dihydroergotamine (5).

2. Experimental

2.1. Chemicals and reagents

The ampoules were part of a collection of expired drugs at the Institute of Biomedical and Pharmaceutical Research in Nürnberg-Heroldsberg: Scopolamin hydrobromide 1 mg/ml, lot 016808 (Eifelfango Chem. Pharma. Werk, 1968); Dihydroergotamine mesilate 1 mg/ml, no lot number (US Sandoz, older than 1978); Sodium laurylsulfate, phosphoric acid 85%, hyoscine hydrobromide, and HPLC grade acetonitrile were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany), sodium heptanesulfonate from VWR International GmbH (Darmstadt, Germany), dihydroergotamine mesylate, dihydroergotamine for peak identification *CRS*, and hyoscine hydrobromide impurity B *CRS* from EDQM (Strasbourg, France). Dihydroergotamine mesylate reference was part of a collection of old drug substances at the Institute for Pharmacy and Food Chemistry in Würzburg (unknown manufacturer, 1992). This compound complied with current pharmacopoeial specifications. Water for HPLC was purified using the Milli-Q purification system by Merck Millipore (Schwalbach, Germany).

2.2. Apparatus

HPLC experiments were performed on a HPLC system 1100 series from Agilent Technologies (Waldbronn, Germany) consisting of a vacuum degasser (G1322A), binary pump (G1312A), autosampler (G1313A), thermostated column oven (G1316A), diode array detector (G1315B). Chromatograms were recorded and integrated using the Agilent ChemStation® software (Rev B.03.02). For pH-measurements a Metrohm 744 pH-Meter from Deutsche METROHM GmbH Co. KG (Filderstadt, Germany) was used.

2.3. Methods

Scopolamine: Nucleodur 100-3 C8ec (150 x 4.6 mm; 3 µm), mobile phase consisting of a mixture of 67% [V/V], aqueous 2.5 g/L sodium laurylsulfate adjusted to pH 2.5 with phosphoric acid, 33% [V/V] acetonitrile, 12 min isocratic, 25 °C, 2.4 ml/min, 5 µl, 210 nm [1].

Dihydroergotamine: Phenomenex® Luna C18 (100 x 4.6 mm; 3 µm), mobile phase A consisting of 3.0 g/L solution of sodium heptansulfonate monohydrate adjusted to pH 2.0 with phosphoric acid, mobile phase B consisting of a mixture of 20% [V/V] mobile phase A and 80% [V/V] acetonitrile, 18 min gradient (0 min B = 42%, 15 min B = 60%, 18 min B = 42%), 25 °C, 1.0 ml/min, 5 µl, 220 nm [2].

2.4. Sample preparation

2.4.1. Ampoules

0.5 ml of scopolamine hydrobromide ampoule solution was diluted to 5.0 ml with mobile phase and injecting threefold. The dihydroergotamine mesilate ampoule was analyzed by diluting 0.5 ml of the solution to 5.0 ml with mobile phase, injecting three times.

2.4.2. Method validation:

For preparing a five-point calibration curve, five scopolamine hydrobromide references were weighted in equal intervals in the range from 40–60 mg. The weighted portions were dissolved in 50.0 ml mobile phase. 10.0 ml of the primary stock solutions were diluted to 100.0 ml with mobile phase. To show intra-assay precision, one stock solution of 1.0 mg/ml was prepared. Six times 0.5 ml of this solution was diluted to 5.0 ml with mobile phase. For Quality Control (QC) samples stock solutions of 1.2 mg/ml, 1.0 mg/ml, and 0.8 mg/ml were prepared, three solutions at each level. 0.5 ml of each solution was diluted to 5.0 ml with mobile phase. A stock solution of 1.0 mg/ml of tropic acid was prepared and diluted to five calibration solutions in a range of 10–20 µg/ml.

In the case of dihydroergotamine mesilate, five portions of reference were weighted in the interval from 40–60 mg and dissolved in 50.0 ml solvent mixture. 10.0 ml of the stock solutions were further diluted to 100.0 ml with solvent mixture. For determination of intra-assay precision, one stock solution of 1.0 mg/ml was prepared. Six times 0.5 ml was diluted to 5.0 ml with solvent mixture. For QC samples stock solutions of 1.2 mg/ml, 1.0 mg/ml, and 0.8 mg/ml were prepared and diluted according to the procedure of the test solution.

2.5. Peak identification and quantitative analysis of samples

Peak identification of the active substance and its related substances was realized by means of either reference standards/impurities from EDQM, representative chromatograms available at knowledge database, and stated relative retention time values. The content of scopolamine, dihydroergotamine, and specified impurities were determined by linear regression. Quantification by means of normalization procedure was applied when impurities were unspecified or no reference available.

3. Results

3.1. Scopolamine ampoule

3.1.1. Validation of the method

Linearity was validated in a range of 80–120% of the scopolamine target concentration, resulting in a coefficient of determination (R^2) of 0.999. Six determinations at 100% level were verified to confirm the repeatability of the method. The relative standard deviation (RSD) was 0.9%. Accuracy was assessed by using nine quality control samples at 80%, 100%, and 120% level, three replicates of three concentrations levels each. Values obtained were in an accepted range from 99.0–101.3%. Hence, the linearity, precision, and accuracy were confirmed. The R^2 value of the calibration curve of tropic acid was 0.996.

3.1.2. Quantitative analysis of the ampoule

For determination of content, the range was extended to 0–120% by preparing of three additional calibration solutions, because of scopolamine concentrations ascertained were lower than 80%. The following equation obtained from the eight-point calibration curve was applied for calculation: $y = 2.5977x - 0.466$, $R^2 = 0.999$. In the old ampoule a concentration of 706 µg/ml scopolamine hydrobromide was found, corresponding to a content of 70.6% of the labeled amount (Fig. 2).

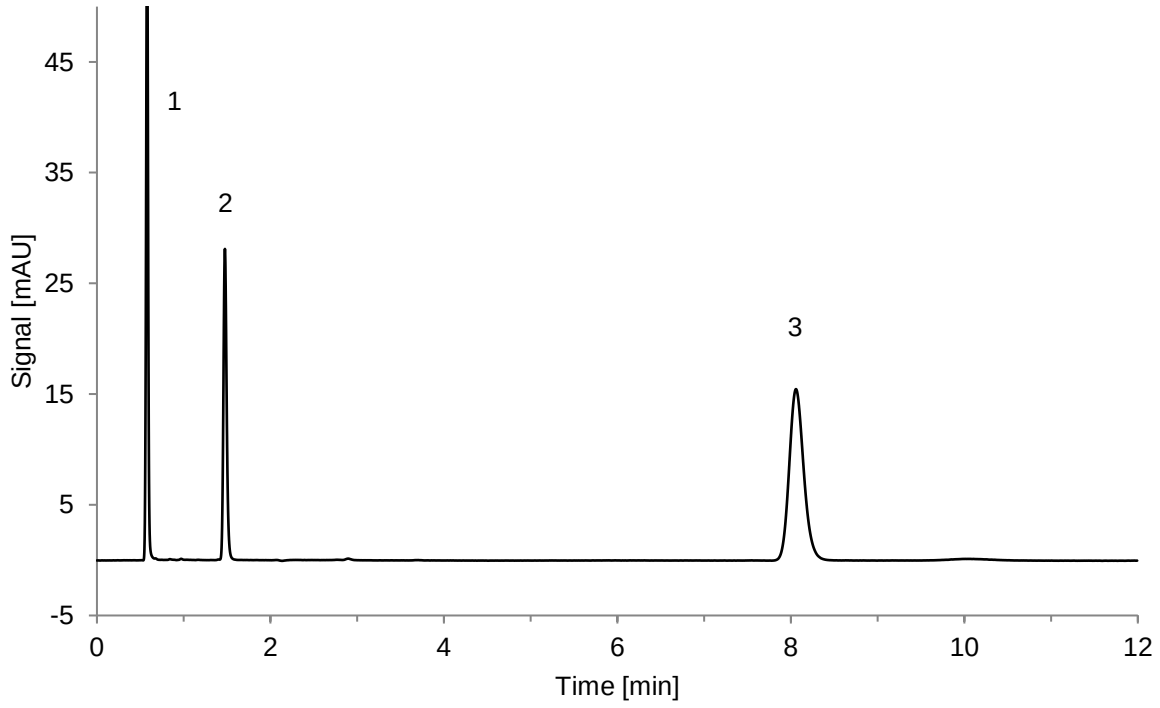

Fig. 2. Chromatogram of the scopolamine hydrobromide test solution, order of elution: bromide (1), tropic acid (2), scopolamine (3).

Tropic acid which is declared as impurity D in the Ph. Eur. monograph was assayed to be 114.5 µg/ml, hence 302.0 µg/ml of scopolamine was hydrolyzed to tropic acid, indicating that degradation of 30.2% of the active substance has been occurred during 50 years of storage.

3.2. Dihydroergotamine

The chromatogram of the old dihydroergotamine injection solution is given in Figure 3.

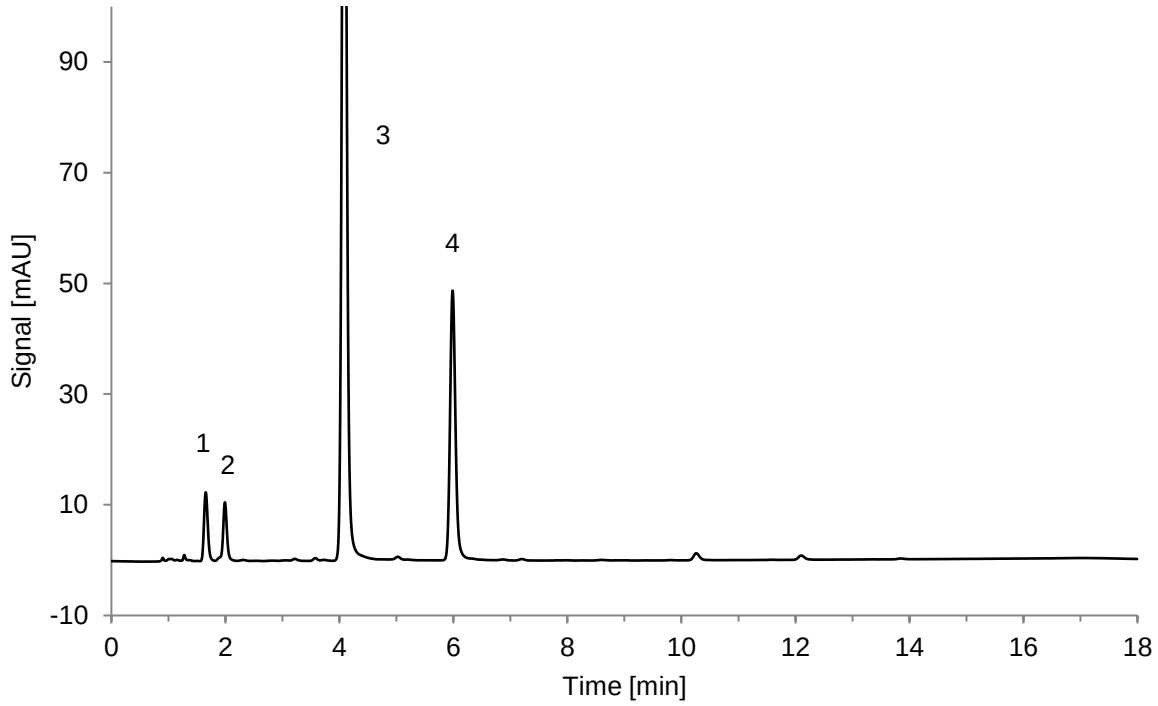

Fig. 3. Chromatogram of the dihydroergotamine mesilate test solution, order of elution: unspecified impurities (1, 2), 2'-*epi*-9,10-dihydroergotamine (3), dihydroergotamine (4).

3.2.1. Validation of the method

The linearity was determined in a range from 80–120% of the stated dihydroergotamine concentration. The R^2 value was calculated 0.999. To show repeatability of the method, six determinations at 100% level were verified. RSD was 0.9%. Accuracy was assessed by using nine quality control samples at 80%, 100%, and 120% levels. Values obtained were in a range of 98.8–100.4%. The results obtained indicate a sufficient validation of the method.

3.2.2. Quantitative analysis of the ampoule

For determination of the content, the range of the calibration curve was extended to 0–120% by three additional calibration solutions, because of observing a decay of about 80% of the active substance dihydroergotamine. The following equation obtained was used for calculation: $y = 15.539x + 3.629$, $R^2 = 0.999$. The concentration of the API was found

205 µg/ml, being equivalent to 20.5% of the labeled amount. Three impurities were quantified using the normalization procedure. The 2´-*epi*-9,10-dihydroergotamine, specified as Impurity D, was formed by epimerization in solution and found to be 70.5%, indicating degradation. Furthermore, two unknown impurities were measured of about 4.1% and 3.4%, respectively.

4. Conclusion

However, the contents of the scopolamine hydrobromide and the dihydroergotamine mesilate ampoules were decreased to 71% and 21% of the labeled amounts, respectively. Consequently, both ampoules did not meet acceptance criteria for content being valid for pharmaceutical products nowadays. Despite of the high age of the scopolamine ampoule, the content of about 70% was surprisingly high. Nevertheless, massive decomposition was found for dihydroergotamine. In contrast to the results of previous investigations of expired drug products, the general high drug stability long beyond the expiry dates could not be confirmed in this study [6, 7].

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D Final discussion

The objective of the studies presented here was to determine and assess the chemical stability of pure drug substances and drug products after extreme long storage. In the experimental studies 50 drug substances manufactured 20–30 years ago and 14 ampoules with an age of more than 40 years, exceeding many times the maximum shelf-life of five years, which is regulated by federal law, were analyzed [1]. The investigations were performed mainly using HPLC.

1. Investigation of drug substances

Several drug substances of commonly used drug classes were chosen, involving β-blockers, β-sympathomimetic drugs, anticholinergics, anti-infectives, non-steroidal anti-inflammatory drugs, antipsychotics, antihistaminic drugs, and representatives of further drug classes. In spite of their age of 20–30 years or even older, 44 out of 49 agents were found to comply with current pharmacopoeial specifications regarding the content and chromatographic purity. In addition, metipranolol which is not monographed in any pharmacopoeia was decomposed by hydrolysis to the 0.7% desacetylmetipranolol. Furthermore, small degradation by hydrolysis of atropine was revealed, the resulting tropic acid has been formed of about 0.7%. In one lot of ampicillin, atenolol, penbutolol, and salbutamol, at least one impurity did not meet the Ph. Eur. monograph requirements, respectively. In some cases, the impurities exceeding the acceptance criteria were not related to decomposition. It is presumed that the impurities found in atenolol and salbutamol batches may have been synthetic by-products or intermediates of synthesis.

In summary, the degradation products found were just above the defined specifications of the Ph. Eur., respectively. Therefore, the long-term storage under ambient conditions has not affected the drug substances in a significant manner. Most of the APIs tested could be used for manufacturing of finished pharmaceutical products even after at least 20 years of storage.

2. Investigation of old ampoules

Several ampoules being part of a collection of long expired FPPs with an age of up to 83 years were investigated with regard to content and impurity profile. In spite of their high age of 53–72 years, the APIs caffeine, etilefrine, synephrine, metamizole sodium, and furosemide, and sodium salicylate were not degraded and can thus, be regarded as stable. The contents of these ampoules were still within the specification of 95–105% of its labeled claim, being in accordance with the ICH Q1A guideline [2].

Average or massive decomposition was expected and consequently observed in the injection solutions containing scopolamine, procaine, adenosine triphosphate, epinephrine, dipyridamole, or dihydroergotamine. Ester hydrolysis occurred in the scopolamine and

procaine ampoule as well as in an excessive degree in the adenosine triphosphate ampoule, the contents were decreased to 71%, 70%, and 15% of the labeled claim, respectively. The content of the analyzed epinephrine ampoules was about 70%, respectively. Just small traces of oxidative degradation products were found, whereas in Suprarenin the nucleophilic substitution with the anti-oxidative agent sodium metabisulfite led to formation of a pharmacologically inactive agent. For the dipyridamole injection solution, oxidative degradation of the API was observed as well. In the dihydroergotamine ampoule, an epimerization has been occurred, resulting in an API content of 21%. Furthermore, two ampoules containing the natural compounds lobeline and strophanthin-K plant extract, respectively, were analyzed. Lobeline was decomposed to the 36% main degradation product, which is formed by isomerization, and no degradation was found in the mixture of cardiac glycosides.

Altogether, taking into account the high age of the ampoules and the general susceptibility to instability of a liquid dosage form, the APIs examined showed surprisingly high stability. The exceptions were dihydroergotamine and adenosine triphosphate which underwent excessive degradation by isomerization or hydrolysis, resulting in API contents of less than a quarter of the labeled value, respectively. For procaine, scopolamine, and epinephrine, decomposition was established by the presence of instable functional groups in the chemical structures, i.e. ester or catechol moiety.

3. Consequences, to be drawn

When assessing the results of our investigation and the data published in the literature, the actual shelf-lives most of the drug substances and drug products were markedly longer than the labeled expiry dates or even exceeding the maximum shelf-life limit of five years. In the “Shelf-Life Extension Program” conducted by the FDA and the United States Department of Defense, nearly 90% of 3005 lots of 122 different drugs were extended by at least one year [3]. Nevertheless, a high product-to-product and lot-to-lot variability was described. A similar high stability was reported in further publications, mostly describing no or low decomposition of expired drugs [4–9].

Typically, an expiry date is set to 2–3 years for a drug product, but often the period seems to be arbitrary assigned. Notably, setting of short expiry dates are a way, the manufacturers try to sell more drug products. The companies justify the assignment of conservative shelf-lives to drug products with their responsibility to ensure the quality and safety of the FPP regarding the drug delivery, the prevention of forming toxic degradants, as well as the resistance against fluctuation of environmental conditions during storage [7]. Such arguments can be easily refuted. Supported by the data of the present thesis and the

literature, a large number of drug products neither decrease in content nor alter the delivery rate after passing the registered expiry dates. Assessing the toxicity of all potential impurities and degradants must be provided at submission of the application for registration. Furthermore, temperature, humidity, and light exposure are critical parameters in long-term stability of FPPs, but storage conditions have to be defined by the manufacturer based on stability studies.

More often, scientists recommend the use of outdated FPPs that has been kept under proper conditions and exceed the expiry dates for a short time. If a patient with anaphylaxis comes to a situation where there is no other choice except for applying an expired epinephrine Pen, the use would entail more benefit than potential risk to the patient [4]. Nevertheless, the use of some medications can be critical, e.g. for biologic products, insulin, injectables, and eye drops after the expiry date, because of being highly prone to chemical, physical, and microbiological instability. Likewise, the use of outdated antibiotics with decreased API content may promote antibiotic resistance, and not be recommended. Drug substances and solid dosage forms like powders, tablets, and capsules are inherently less sensitive to degradation.

Associated with a general extension of shelf-lives, there would be an enormous financial benefit to the health care system, because stockpiling agencies have to dispose and replace outdated but unused medications frequently, despite the high stability of the drugs past their labeled expiry dates. The same applies to patients who cannot afford expensive medicines. Furthermore, it could be considered to permit donations of soon to expire or already expired drug products to developing countries, where medication support is urgently needed.

Even though most studies are retrospectively and non-systematically performed it can be stated that shelf-lives longer than five years should be applicable by the regulatory authorities if a longer period is justified. Hence, the regulatory authorities should oblige the pharmaceutical manufacturer to assess the stability and the expiry dates of their FPP again when being on the market for a defined time period. Maybe the consideration of re-testing dates for drug products as established for the drug substances could be an option in order to prevent waste of still working medications.

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E Summary

Upon approval of a drug, the stability of the API and the FPP has to be studied intensively because it determines the shelf-life. If a drug is found to be stable, the expiry date is arbitrary set to five years at the maximum, if a drug tends to undergo degradation, the expiry date is set shorter. The drug product must comply with predefined specifications in accordance with the ICH guidelines Q6A and Q6B during its entire market life. The content of the active substance is required to be within a specification of 95–105% of its labeled claim until expiry corresponding to the ICH guideline Q1A(R2). However, there is little or scattered literature information addressing the stability of drug products beyond their expiry dates. The objective of this thesis was to study and assess the long-term stability of a collection involving numerous pure drug substances and ampoules manufactured in the 20th century. The content and the impurity profile were examined by means of appropriate analytical methods, mainly using liquid chromatography. The results were compared to data being available in the literature. Assessing the stability regarding the dosage form and the affiliation of the drug class was conducted.

The experimental studies comprise the examination of 50 drug substances manufactured 20–30 years ago and 14 long expired ampoules which were older than 40 years in the time of analysis, exceeding many times the maximum shelf-life of five years.

For investigation of the solid drug substances, pharmacopoeial methods were applied as far as possible. Indeed, results of the study showed that 44 tested substances still complied with the specification of the Ph. Eur. with regard to the content and impurity profile, even after more than two decades of storage.

For analysis of the injection solutions, HPLC-UV and HPLC-ESI/MS techniques were applied, commonly based on liquid chromatography methods of the Ph. Eur. for determination of related substances. Each method was further validated for its application to ensure accurate API quantification corresponding to ICH Q2(R1). Quite a few ampoules were identified to show surprisingly high stability. In spite of their age of 53–72 years, APIs such as caffeine, etilefrine, synephrine, metamizole sodium, furosemide, and sodium salicylate complied with the specified content that is valid nowadays, respectively. Nevertheless, typical degradation reaction, e.g. hydrolysis, oxidation, or isomerization, was observed in all remaining ampoules. Various degrees of hydrolysis were revealed for scopolamine, procaine, and adenosine triphosphate, the contents were decreased to 71%, 70%, and 15% of the declared concentrations, respectively. In the epinephrine and dipyridamole ampoules, oxidative degradation has been occurred, finding respective API contents of more or less 70%. For dihydroergotamine, excessive decomposition by epimerization was observed, resulting in an API content of 21% and degradation by isomerization was found in lobeline, still containing 64% of the labeled claim.

In conclusion, supported by the data of the present studies and the literature, defining and authorizing a longer shelf-life may be applicable to numerous pharmaceuticals which should be considered by pharmaceutical manufacturers and regulatory authorities, if justified based on stability studies. A general extension of the shelf-lives of drug products and the abolishment or extension of the maximum shelf-life limit of five years would prevent disposing of still potent medications and save a lot of money to the entire health care system.

F Zusammenfassung

Bei der Zulassung eines Arzneimittels muss die Stabilität sowohl des Wirkstoffes als auch des Fertigarzneimittels umfassend untersucht werden, da dies für die Festlegung der Haltbarkeit wesentlich ist. Wenn sich herausstellt, dass ein Arzneimittel stabil ist, wird das Verfallsdatum auf höchstens fünf Jahre festgelegt. Neigt ein Arzneimittel zum Abbau, so wird ein kürzeres Verfallsdatum gewählt. Das Arzneimittel muss innerhalb der Haltbarkeitsfrist definierten Spezifikationen entsprechen, welche in den ICH-Richtlinien Q6A und Q6B festgelegt sind. Dabei muss insbesondere der Wirkstoff-Gehalt des Arzneimittels gemäß der ICH-Richtlinie Q1A(R2) innerhalb der Spezifikation von 95–105 % der deklarierten Konzentration liegen. In der Literatur gibt es jedoch wenige Informationen darüber, wie stabil Arzneimittel lange nach Ablauf des Verfallsdatums sind. Das Ziel dieser Arbeit war es, die Stabilität zahlreicher Feststoffe und Ampullen, die aus einer Altarzneimittel-Sammlung stammten und während des 20. Jahrhunderts hergestellt wurden, zu untersuchen und zu bewerten. Der Gehalt und das Verunreinigungsprofil wurden mittels geeigneter instrumenteller Analyseverfahren bestimmt, wobei hauptsächlich flüssigchromatographische Methoden zur Anwendung kamen. Die Untersuchungsergebnisse wurden mit Literaturdaten verglichen und es wurde eine Beurteilung der Stabilität in Abhängigkeit von der Darreichungsform und der Zugehörigkeit zu einer Arzneistoffklasse vorgenommen.

Die experimentellen Studien umfassten die Untersuchung von 50 Feststoffen, die vor 20 bis 30 Jahren hergestellt worden waren, und 14 Alt-Ampullen, die ein Alter von mindestens 40 Jahre aufwiesen und damit die maximale Haltbarkeit von fünf Jahren um ein Vielfaches überschritten hatten.

Zur Untersuchung der Feststoffe wurden meist Arzneibuchmethoden verwendet. Die Ergebnisse zeigten, dass 44 geprüfte Substanzen auch nach mehr als zwei Jahrzehnten hinsichtlich ihres Gehalts und Verunreinigungsprofils den jeweiligen Spezifikationen des Europäischen Arzneibuchs entsprachen.

Zur Analyse der Alt-Ampullen wurden HPLC-UV- und HPLC-ESI/MS-Techniken eingesetzt. Diese basierten häufig auf Arzneibuch-Methoden zur Prüfung auf verwandte Substanzen. Für die Gehaltsbestimmungen wurden entsprechend der ICH-Richtlinie Q2(R1) die erforderlichen Parameter validiert. Einige Ampullen zeigten eine überraschend hohe Stabilität des Wirkstoffs, trotz ihres Alters von 53 bis 72 Jahren. Dabei entsprachen die Wirkstoffe Koffein, Etilefrin, Synephrin, Metamizol-Natrium, Furosemid und Natriumsalicylat dem heute gültigen Spezifikationsbereich von 95–105 %. Nichtsdestoweniger wurden bei einigen Ampullen typische Abbaureaktionen wie Hydrolyse, Oxidation oder Isomerisierung festgestellt. Die Hydrolyse der Arzneistoffe Scopolamin, Procain und Adenosintriphosphat führte zu verringerten Gehalten von 71 %, 70 % bzw. 15 % der jeweiligen gekennzeichneten Wirkstoffkonzentration. Die Epinephrin- und Dipyridamol-Injektionslösungen waren von

oxidativem Abbau betroffen. Der Wirkstoffgehalt dieser Ampullen lag jeweils bei ca. 70 %. In der Dihydroergotamin-Ampulle trat eine massive Epimerisierung auf, wobei ein Gehalt von 21 % bestimmt wurde. Aufgrund der Isomerisierung des Arzneistoffes Lobelin reduzierte sich der Wirkstoffgehalt auf 64 %.

Als Schlussfolgerung der experimentellen Studien und der verfügbaren Daten aus der Literatur sollten die pharmazeutischen Unternehmer und die Aufsichtsbehörden erwägen, die Haltbarkeitsdauer für zahlreiche Arzneimittel zu verlängern, wenn dies basierend auf Stabilitätsuntersuchungen gerechtfertigt ist. Eine generelle Ausweitung der Verwendbarkeit von Arzneimitteln sowie die Abschaffung oder Erweiterung der maximalen Haltbarkeitsdauer von fünf Jahren würde die Entsorgung noch wirksamer Medikamente verhindern und dem Gesundheitssystem viel Geld einsparen.

G Appendix

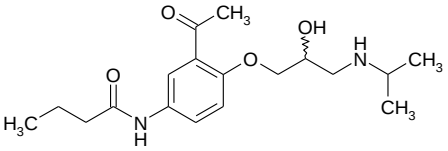
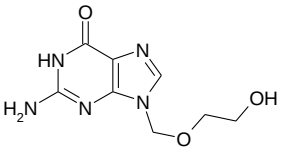
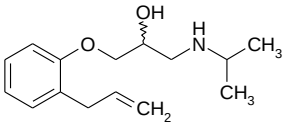
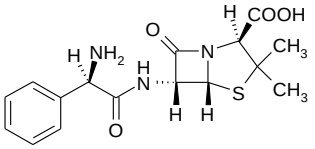
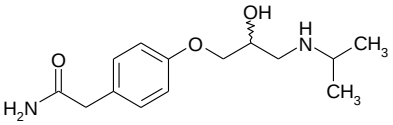
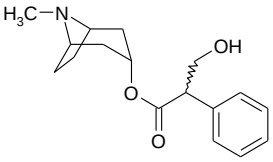
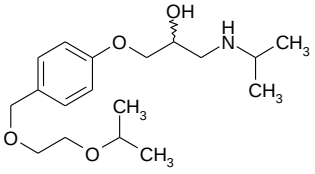
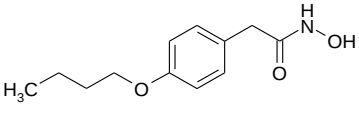
1. Supporting information

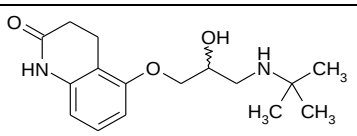
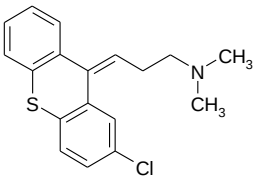
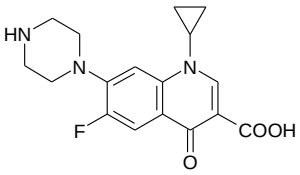
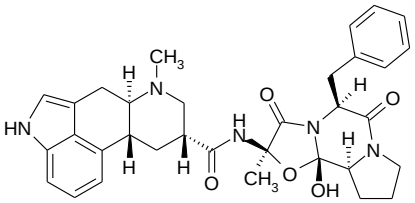
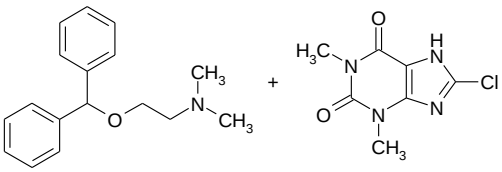
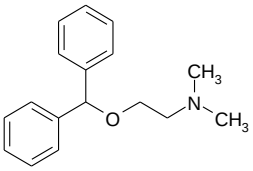
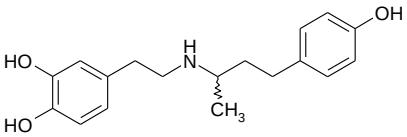
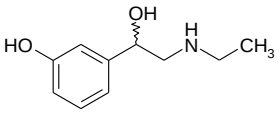
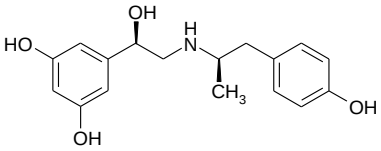
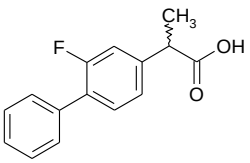
1.1. Supplementary data for chapter C 2.

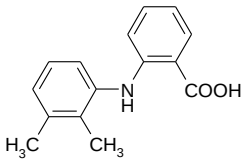
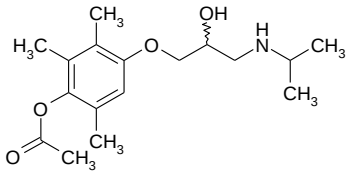
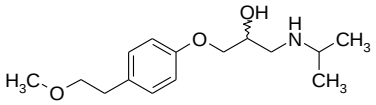
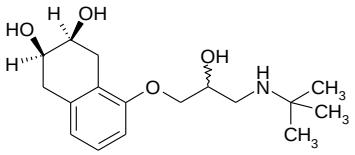
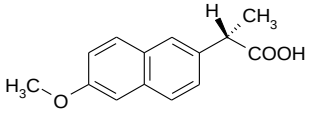
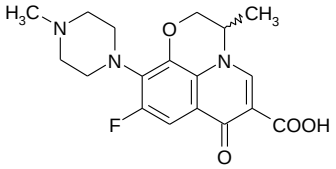
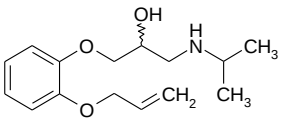
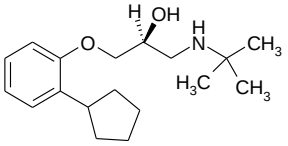
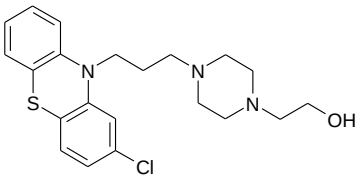
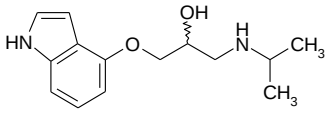
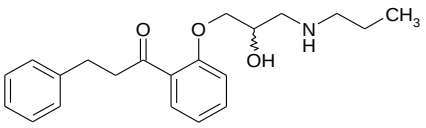
a) Tested substances

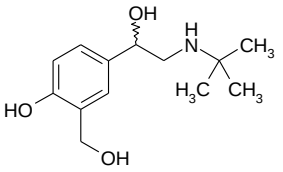
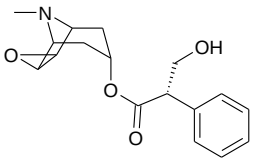
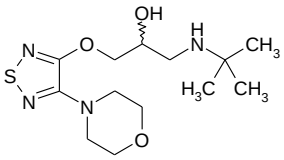
Table 1

Alphabetical list of all compounds analyzed

Drug substance	Data of the manufacturer (lot number, manufacturer and manufacturing year)	Chemical structure
Acebutolol	Acebutolol hydrochloride lot 96-09649 and lot 91-05536 (Heumann Pharma, 1996 and 1991)	
Aciclovir	Aciclovir lot A1400L/UE6 (The Wellcome Foundation Ltd London, 1987), Aciclovir lot 1059 (Apotheek De Liefde Burroughs Wellcome, 1990)	
Alprenolol	Alprenolol hydrochloride no lot number (Astra Chemicals GmbH, 1994)	
Ampicillin	Ampicillin sodium lot CCB6Z0157 (Pfizer GmbH, 1997)	
Atenolol	Atenolol lot 1794301 (Azupharma, 1994), Atenolol lot 96-04647 (Heumann, 1996), Atenolol lot 9346A2RII (Wörwag Artesan, 2010)	
Atropine	Atropine sulfate lot 0000391577 (HEEL, 2010), Atropine sulfate no lot number (unknown manufacturer, older than 1999)	
Bisoprolol	Bisoprolol fumarate lot 20825727 (Merck, 1994)	
Bufexamac	Bufexamac lot 96-07860 (Heumann Pharma, 1996), Bufexamac lot 96-07861 (Heumann Pharma, 1996), Bufexamac lot 84H0797 (Sigma, 1994)	

Carteolol	Carteolol hydrochloride lot 49191 (Madaus AG, 1995)	
Chlorprothixene	Chlorprothixene hydrochloride lot 6111185 (Troponwerke Cologne, 1985)	
Ciprofloxacin	Ciprofloxacin lot R-123-1 (Bayer, 1993)	
Dihydro-ergotamine	Dihydroergotamine mesilate no lot number (unknown manufacturer, 1992)	
Dimenhydrinate	Dimenhydrinate lot 94-03549 (Heumann Pharma, 1994)	
Diphenhydramine	Diphenhydramine hydrochloride no lot number (Dr. Much, 1982)	
Dobutamine	Dobutamine lot 9112033 (Hexal, older than 1999) Dobutamine hydrochloride lot 067H26 (Lilly, 1993)	
Etilefrine	Etilefrine hydrochloride lot 313105/300 (Boehringer Ingelheim, 1972)	
Fenoterol	Fenoterol hydrobromide lot 211787 (Boehringer Ingelheim, older than 1992)	
Flurbiprofen	Flurbiprofen no lot number (unknown manufacturer, older than 1999)	

Mefenamic acid	Mefenamic acid lot 75F0054 (Sigma® Chemical Company, 1985)	
Metipranolol	Metipranolol lot 90047-90 (Dr. Mann Pharma, 1988)	
Metoprolol	Metoprolol tartrate no lot number (Astra, 1988) Metoprolol succinate no lot number (Hexal, 1994)	
Nadolol	Nadolol lot 43846 (Bristol-Meyrs Squibb GmbH, older than 1999)	
Naproxen	Naproxen no lot number (unknown manufacturer, 1989)	
Ofloxacin	Ofloxacin L439 and lot A380 (Hoechst AG, 1996 and 1995)	
Oxprenolol	Oxprenolol hydrochloride no lot number (unknown manufacturer, older than 1999)	
Penbutolol	Penbutolol sulfate lot A126 (Hoechst AG, older than 1999)	
Perphenazine	Perphenazine lot 3207762 (Merck, older than 1999)	
Pindolol	Pindolol lot 3-OG2-205 (Sandoz, 1993), Pindolol lot 40110404 (Lederle, older than 1999)	
Propafenone	Propafenone hydrochloride lot 67207 (Knoll AG, older than 1999), Propafenone hydrochloride lot 96-09744 and lot 97-06736 (Heumann Pharma, 1996 and 1997)	

Salbutamol	Salbutamol sulfate lot 0902390 (Glaxo, older than 1995), Salbutamol sulfate lot 032 031 (3M Medica, older than 1999), Salbutamol sulfate lot 93A19 (Stada, older than 1995), Salbutamol sulfate lot 0170088 (Kettelhack Riker, 1988), Salbutamol sulfate lot 976968 (unknown manufacturer, older than 1999), Salbutamol Base lot AN1103 (KlingePharma, 1990)	
Scopolamine	Scopolamine hydrochloride lot 76H7825 (Sigma® Chemical Company, 1996), Scopolamine hydrobromide trihydrate SLBP0022V (Sigma® Chemical Company, 2015)	
Timolol	Timolol maleate lot #281834 (Hexal, older than 1999), Timolol maleate lot 001T059 (Merck, older than 1999), Timolol maleate no lot number (unknown manufacturer, older than 1999)	

b) Peak identification and quantitation of impurities

The impurities are named according to the IUPAC nomenclature. **Atenolol:** Impurity A, 2-(4-hydroxyphenyl)acetamide; Impurity B, 2-[4-[*(*2*RS)*-2,3-dihydroxypropoxy]phenyl]acetamide; Impurity I, 2-[4-[(2*RS*)-3-(ethylamino)-2-hydroxypropoxy]-phenyl]acetamide; Impurity J, 2-[4-[(2*RS*)-3-amino-2-hydroxypropoxy]phenyl]-acetamide. **Bisoprolol fumarate:** Impurity G, (2*RS*)-1-[4-[[(2-isopropoxyethoxy)methoxy]methyl]phenoxy]-3-isopropylaminopropan-2-ol. **Carteolol hydrochloride:** Impurity H, 5-[(2*RS*)-3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]quinolin-2(1*H*)-one. **Nadolol:** Impurity A, *cis*-5-[(2*RS*)-2,3-dihydroxypropoxy]-1,2,3,4-tetrahydronaphthalene-2,3-diol; Impurity D, 5,5´[[(1,1-dimethylethyl)imino]bis[(2-hydroxypropane-1,3-diyl)oxy]]bis(*cis*-1,2,3,4-tetrahydronaphthalene-2,3-diol). **Dobutamine hydrochloride:** Impurity C, (2*RS*)-*N*-[2-(3,4-dimethoxyphenyl)ethyl]-4-(4-methoxyphenyl)butan-2-amine. **Fenoterol hydrobromide:** Impurity A, 5-[(1*RS*)-2-[(1*SR*)-2-(4-hydroxyphenyl)-1-methylethyl]amino-1-hydroxyethyl]benzene-1,3-diol. **Salbutamol sulfate:** Impurity C, *(*1*RS)*-2-[(1,1-dimethylethyl)amino]-1-(4-hydroxy-3-methylphenyl)ethanol; Impurity D, 5-[(1*RS*)-2-[(1,1-dimethylethyl)amino]1-1-hydroxyethyl]-2-hydroxybenzaldehyde; Impurity F, 1,1´-[oxybis-[methylene(4-hydroxy-1,3-phenylene)]]bis[2-[(1,1-dimethylethyl)amino]ethanol], Impurity N, 2-[(1,1-dimethylethyl)amino]-1-[3-[[5-[2-[(1,1-dimethylethyl)amino]-1-hydroxyethyl]-2-hydroxyphenyl]methyl]-4-hydroxy-5-(hydroxymethyl)phenyl]-ethanol, Impurity O, unknown structure. **Atropine sulfate:** Impurity C, (2*RS*)-3-hydroxy-2-phenylpropanoic acid (tropic acid); Impurity E, (1*S,*3*R,*5*S,*6*RS*)-6-hydroxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl(2*S*)-3-hydroxy-2-phenyl-propanoate (7-hydroxyhyoscyamine); Impurity G, (1*R,*3*r,*5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl (2*S*)-3-hydroxy-2-phenylpropanoate (littorine). **Hyoscine hydrobromide:** Impurity D, (2*RS*)-3hydroxy-2-phenylpropanoic acid (tropic acid). **Aciclovir:** Impurity A, 2-[(2-amino-6-oxo-1,6-dihydro-9*H*-purin-9-yl)methoxy]ethyl acetate; Impurity B, 2-amino-1,7-dihydro-6*H*-purin-6-one (guanine); Impurity F, *N*-[9-[(2-hydroxyethoxy)methyl]-6-oxo-6,9-dihydro-1*H*-purin-2-yl]acetamide; Impurity N, unknown structure; Impurity O, unknown structure; Impurity P, 2-amino-9-(2-hydroxyethyl)-1,9-dihydro-6*H*-purin-6-one. **Ciprofloxacin:** Impurity C, 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid. **Ofloxacin:** Impurity C, (3*RS*)-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid; Impurity D, (3*RS*)-10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7*H*-pyrido[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid; Impurity E, (3*RS*)-9-fluoro-3-methyl-7-oxo-10-(piperazin-1-yl)-2,3-dihydro-7*H*-pyrido-[1,2,3-*de*]-1,4-benzoxazine-6-carboxylic acid. **Bufexamac:** Impurity C, butyl 2-(4-butoxy-phenyl)acetate. **Flurbiprofen:** Impurity A, (2*RS*)-2-(biphenyl-4-yl)propanoic acid. **Chlorprothixene hydrochloride:** Impurity F, (*E*)-3-(2-chloro-9*H*-thioxanthen-9-ylidene)-*N*,*N*-dimethylpropan-1-amine ((*E*)-isomer). **Perphenazine:** Impurity A, 2-[4-[3-(2-chloro-5-oxido-10*H*-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol; Impurity B, 2-[4-[3-

(10H-phenothiazin-10-yl)propyl]piperazin-1-yl]ethanol. **Dihydroergotamine mesilate:** Impurity B, (6a*R*,9*R*,10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-2-ethyl-10b-hydroxy-3,6-dioxo-octahydro-8*H*-oxazolo[3,2-*a*] pyrrolo[2,1-*c*]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octa-hydroindolo[4,3-*fg*]quinolone-9-carboxamide (9,10-dihydroergostine); Impurity C, (6a*R*,9*R*, 10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8*H*-oxazo lo[3,2-a]pyrrolo[2,1-c]pyrazin-2-yl]-9-hydroxy-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo [4,3-*fg*]quinolone-9-carboxamide (8-hydroxy-9,10-dihydroergotamine); Impurity D, (6a*R*,9*R*,10a*R*)-*N*-[(2*S*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-methyl-3,6-dioxooctahydro-8H-oxazolo[3,2-a]pyrrolo[2,1-c]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo [4,3-*fg*]quinolone-9-carboxamide (2´-*epi*-9,10-dihydroergotamine), impurity E, (6a*R*,9*R*, 10a*R*)-*N*-[(2*R*,5*S*,10a*S*,10b*S*)-5-benzyl-10b-hydroxy-2-(1-methylethyl)-3,6-dioxooctahydro-8*H*-oxazolo[3,2-a]pyrrolo[2,1-c]pyrazin-2-yl]-7-methyl-4,6,6a,7,8,9,10,10a-octahydroindolo [4,3-*fg*] quinolone-9-carboxamide (dihydroergo-cristine). **Dimenhydrinate:** impurity A, 1,3-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione (theo-phylline); Impurity F, 2-(diphenylmethoxy)-*N*-methylethanamine. **Diphenhydramine hydrochloride:** Impurity A, 2-(diphenylmethoxy)-*N*-methylethanamine

1.2. Supplementary data for chapter C 3.

Validation and impurity quantitation

Epinephrine: For preparing calibration solutions at five levels, five epinephrine references were weighted in equal intervals in the range from 48–72 mg, then dissolved in 5.0 ml 0.1 M HCL and diluted to 50.0 ml with solvent mixture B. 1.0 ml of each solution was further diluted to 100.0 ml with solvent mixture B to obtain five calibration solutions (9.6–14.4 µg/ml). For content determination, two additional calibration solutions were prepared for range extension. For this purpose, 33 mg and 36 mg of epinephrine were weighted and diluted according to the same procedure (extended: 6.6–14.4 µg/ml). Three levels of quality control (QC) samples were prepared according to the dilution procedure for calibration solution. Therefore, 48 mg, 60 mg, and 72 mg of epinephrine reference were weighted at 80%, 100% and 120% levels. For determination of precision, six times 0.5 ml of Suprarenin® reference ampoule (1 mg/ml) was diluted to 50.0 ml with solvent mixture B.

Etilefrine: For preparing calibration solutions at five levels, five etilefrine hydrochloride references were weighted in equal intervals in the range from 40–60 mg. The weighted portions were dissolved in 100.0 ml water. 10.0 ml of the primary stock solutions were further diluted to 100.0 ml with water. 10.0 ml of the secondary stock solutions were diluted to 50.0 ml with water (8.0–12.0 µg/ml). For preparing QC samples, 40 mg, 50 mg, and 60 mg of etilefrine reference were weighted, dissolved and diluted according to the dilution procedure for calibration solutions. For determination of precision, six times 0.5 ml of Effortil® drops (7.5 mg/ml) was diluted to 50.0 ml with water. 7.0 ml of each stock solution was diluted to 50.0 ml with water.

Synephrine: For preparing calibration solutions at five levels, five synephrine references were weighted in equal intervals in the range from 32–48 mg and dissolved in 100.0 ml mobile phase. 1.0 ml of each solution was diluted to 100.0 ml with mobile phase (3.2-4.8 µg/ml). For QC samples, 32 mg, 40 mg, and 48 mg of reference substance were weighted for 80%, 100% and 120% levels equal to the dilution procedure of calibration solutions. No suitable reference drug was available so a stock solution for substitution was prepared (0.4 mg/ml) to validate the precision. Six times 1.0 ml of this solution was diluted to 100.0 ml with mobile phase. Additionally, for suitability test of the method a test solution of synephrine (20 µg/ml) was prepared and spiked to each solution of octopamine (1 µg/ml), tyramine (1 µg/ml), and 1-(4-hydroxyphenyl)-2-(methylamino)ethanone (1 µg/ml) in a 1:1:1:1 ratio, respectively.

Caffeine/Procaine: For preparing calibration solutions at five levels, five caffeine references were weighted in the range of 28–42 mg and five procaine hydrochloride references were

weighted in the range of 40–60 mg, unified at each level and dissolved in 50.0 ml mobile phase. 1.0 ml of each stock solution was diluted to 100.0 ml with mobile phase to obtain five calibration levels (caffeine: 5.6–8.4 µg/ml; procaine: 8.0–12 µg/ml). For QC samples, 112 mg, 142 mg, and 170 mg of caffeine reference and 160 mg, 200 mg, and 240 mg of procaine reference were weighted. The weighted portions of caffeine and procaine at 80%, 100%, and 120% levels were unified and dissolved in 10.0 ml mobile phase. 0.5 ml of each primary stock solution was diluted to 100.0 ml with mobile phase. 1.0 ml of each secondary stock solution was further diluted to 10.0 ml with mobile phase. For validation of precision, procaine and caffeine were determined separately. Six times 0.5 ml of Procain 2.0% Steigerwald ampoule was diluted to 100.0 ml with mobile phase. 1.0 ml of each stock solution was diluted to 10.0 ml. For caffeine determination, a stock solution of caffeine needed to be prepared first. Two tablets (2 x 0.2 g) were dissolved in 30.0 ml mobile phase to obtain the concentration being equivalent to the ampoule. Six times 0.5 ml of the stock solution was diluted to 100.0 ml with mobile phase. 1.0 ml of each stock solution was further diluted to 10.0 ml.

Caffeine/Sodiumsalicylate: For preparing calibration solutions at five levels, five caffeine references were weighted in the range from 40 to 60 mg and also sodium salicylate references were weighted fivefold in the range of 40–60 mg. The weighted portions were unified at each level and dissolved in 50.0 ml mobile phase. 1.0 ml of each stock solution was diluted to 100.0 ml with mobile phase to obtain five calibration levels (caffeine: 8.0-12.0 µg/ml; sodiumsalicylate: 8.0–12 µg/ml).

Dipyridamole: For preparing calibration solutions at five levels, five dipyridamole references were weighted in equal intervals in the range from 40–60 mg. Each weighted portion was dissolved in 10.0 ml methanol. 0.5 ml of each primary stock solution was diluted to 50.0 ml with methanol (40.0–60.0 µg/ml). QC samples of three levels were prepared in accordance with the dilution procedure for calibration solutions. For this purpose, 40 mg, 50 mg, and 60 mg of dipyridamole reference were weighted and diluted for 80%, 100%, and 120% levels. For determination of precision, six times 0.5 ml of Persantin® reference ampoule (5 mg/ml) was diluted to 50.0 ml with methanol. For measurements of light instability, each of these solutions was stored under light protection and injected after seven days. Another solution was stored under daylight exposure and injected after seven days.

Furosemide: For preparing calibration solutions at five levels, five furosemide references were weighted in equal intervals in the range from 20–30 mg. Each weighted portion was dissolved in 100.0 ml mobile phase. 10.0 ml of each primary stock solution was diluted to 50.0 ml with mobile phase. 10.0 ml of each secondary stock solution was further diluted to 50.0 ml (8.0–12.0 µg/ml). Three levels of QC samples were prepared by dissolving weights

of 16 mg, 20 mg, and 24 mg in 100.0 ml mobile phase. 5.0 ml of each solution was diluted to 100.0 ml with mobile phase. For determination of precision six times 0.5 ml of Lasix® reference ampoule (10 mg/ml) was diluted to 50.0 ml with mobile phase. 5.0 ml of each stock solution was diluted to 50.0 ml with mobile phase.

Metamizole sodium: For preparing calibration solutions at five levels, five metamizole sodium references were weighted in the range from 40–60 mg. The weighted portions were dissolved in 100.0 ml methanol. 10.0 ml of primary stock solutions were diluted to 100.0 ml (40.0–60.0 µg/ml). QC samples were prepared at three levels. Therefore, 40 mg, 50 mg, and 60 mg of reference substance were weighted for 80%, 100%, and 120% levels and subsequently diluted according to the procedure for calibration solutions. For determination of precision six times 0.5 ml of Novalgin® was diluted to 50.0 ml with methanol. 1.0 ml of each stock solution was diluted to 100.0 ml with mobile phase.

Impurity assay: For impurity quantification calibration solutions, at five levels were prepared as follows: Adrenochrome in the range from 0.25–1.0 µg/ml, sulfonic acid derivative in the range from 1.0–5.0 µg/ml, norepinephrine in the range from 0.3–2.0 µg/ml, 4-aminobenzoic acid in the range from 0.5–1.0 µg/ml, and theobromine in the range from 0.8–1.2 µg/ml.

2. List of Publications and Documentation of Authorship

1 **A systematic review of the stability of finished pharmaceutical products and drug substances beyond their labeled expiry dates**

Zilker, M., Sörgel, F., Holzgrabe, U.

Journal of Pharmaceutical and Biomedical Analysis, Volume 166 (2019), Pages 222–235

doi:10.1016/j.jpba.2019.01.016

2 **A long-time stability study of 50 drug substances representing common drug classes of pharmaceutical use**

Zilker, M., Sörgel, F., Holzgrabe, U.

Revision submitted to *Journal of Drug Testing and Analysis,* (2019)

3 **A stability-study of expired ampoules manufactured more than 40 years ago**

Zilker, M., Sörgel, F., Holzgrabe, U.

Journal of Pharmaceutical and Biomedical Analysis, Volume 150 (2018), Pages 318–326

doi:10.1016/j.jpba.2017.12.019

This section contains a list of the individual contribution for each author to the publications reprinted in this thesis.

Zilker M., Sörgel F., Holzgrabe U., A systematic review of the stability of finished pharmaceutical products and drug substances beyond their labeled expiry dates. *Journal of Pharmaceutical and Biomedical Analysis* 166 (2019) 222–235			
Author	1	2	3
Manuscript planning	x	x	x
Manuscript writing	x		
Correction of manuscript	x	x	x
Supervision of Markus Zilker		x	x

Zilker M., Sörgel F., Holzgrabe U., A long-time stability study of 50 drug substances representing common drug classes of pharmaceutical use. Revision submitted to *Journal of Drug Testing and Analysis* (2019)			
Author	1	2	3
Study design and concept development	x	x	x
Experimental work	x		
Data analysis and interpretation	x	x	x
Manuscript planning	x	x	x
Manuscript writing	x		
Correction of manuscript	x	x	x
Supervision of Markus Zilker		x	x

Zilker M., Sörgel F., Holzgrabe U., A stability-study of expired ampoules manufactured more than 40 years ago. *Journal of Pharmaceutical and Biomedical* Analysis 150 (2018) 318-326			
Author	1	2	3
Study design and concept development	x	x	x
Experimental work	x		
Data analysis and interpretation	x	x	x
Manuscript planning	x	x	x
Manuscript writing	x		
Correction of manuscript	x	x	x
Supervision of Markus Zilker		x	x

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Prof. Dr. Ulrike Holzgrabe	_____	_____
	Ort, Datum	Unterschrift
Markus Zilker	_____	_____
	Ort, Datum	Unterschrift

3. Abbreviations

ACN	acetonitrile
API	active pharmaceutical ingredient
CEP	Certificate of suitability to the Monographs of the European Pharmacopoeia
CI	confidence interval
CYM	cymarose
DHE	dihydroergotamine mesilate
DIGIT	digitoxose
DoD	Department of Defense
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDOX	epidoxycycline
EDTA	ethylene diamine tetraacetic acid
EMA	European Medicines Agency
ESI	electrospray ionization
FDA	Food and Drug Administration
FPP	finished pharmaceutical product
GMP	Good Manufacturing Practice
GLU	glucose
HPLC	high performance liquid chromatography
IBMP	Institute for Biomedical and Pharmaceutical Research
ICH	International Conference on Harmonization
LOD	limit of detection
LOQ	limit of quantitation
MEOH	methanol
NSAID	non-steroidal anti-inflammatory drug
Ph. Eur.	European Pharmacopoeia
QC	quality control
SDS	sodium dodecyl sulfate
SHS	sodium heptanesulfonate
SHXS	sodium hexanesulfonate
SLEP	Shelf-Life Extension Program
SNS	Strategic National Stockpile
SOS	sodium octanesulfonate
SST	system suitability test
R^2	coefficient of determination
R_s	chromatographic resolution
RP	reversed-phase

RRT	relative retention time
RSD	relative standard deviation
TBAB	tetrabutylammonium bromide
TBAHS	tertrabutylammonium hydrogen sulfate
TEA	triethylamine
TLC	thin layer chromatography
USP	United States Pharmacopoeia
UV	ultra violet