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Stability study of losartan/hydrochlorothiazide tablets

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Abstract

The purpose of stability testing is to investigate how the quality of a drug product changes with time under the influence of environmental factors, to establish a shelf life for the product and to recommend storage conditions. Stability study of losartan/hydrochlorothiazide tablets is presented in this paper. Losartan (angiotensin II receptor antagonist) and hydrochlorothiazide (diuretic) are successfully used in association in the treatment of hypertension. Stability study of losartan/hydrochlorothiazide tablets consisted of three steps: stress test (forced degradation study), preliminary testing (selection of packaging) and formal stability testing. The results of stress test suggested that losartan/hydrochlorothiazide tablets are sensitive to moisture. It was demonstrated that the developed analytical methods are stability indicating. Additional preliminary testing was performed in order to select appropriate packaging for losartan/hydrochlorothiazide tablets. OPA/Al/PVC//Al blisters were found to provide adequate protection for the product. Based on the first 12 months of the formal stability study, a shelf life of 24 months was proposed. Losartan/hydrochlorothiazide tablets in OPA/Al/PVC//Al blisters are demonstrated to be chemically, physically and microbiologically stable.

Keywords: Losartan potassium; Hydrochlorothiazide; Tablets; Stability study; Packaging

1. Introduction

The quality of a drug product changes with time under the influence of environmental factors such as temperature, humidity and light. The purpose of stability testing is to investigate those changes, to establish a shelf life for the drug product and to recommend storage conditions, which will be applicable to all future batches of the tested drug product manufactured and packaged under similar circumstances. A well-designed stability study should include testing of those attributes that are susceptible to change during storage and are likely to influence quality, safety and efficacy (ICH Q1A(R2), 2003).

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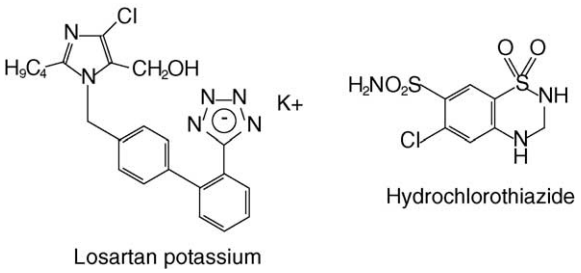

Fig. 1. Structures of losartan potassium and hydrochlorothiazide.

As part of the drug development program, stability of losartan/hydrochlorothiazide tablets has been tested. Losartan/hydrochlorothiazide tablets contain 50 mg losartan potassium and 12.5 mg hydrochlorothiazide per tablet.

Losartan, or 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole (Fig. 1) is a potent, highly specific angiotensin II type 1 (AT_1) receptor antagonist with antihypertensive activity (Chiu et al., 1990; Smith et al., 1992; Goldberg and Sweet, 1995; Timmermans et al., 1995). Losartan is given by mouth as the potassium salt.

Hydrochlorothiazide (HCTZ), or 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulphonamide-1,1-dioxide (Fig. 1), is a widely used thiazide diuretic. It increases urinary excretion of sodium and water by inhibiting sodium reabsorption in the renal tubules (Martindale, 1999).

Losartan and HCTZ are successfully used in association in the treatment of patients whose blood pressure is not adequately controlled with either substance alone. Clinical trials have shown that this combination therapy is effective and well tolerated (Goldberg and Sweet, 1995; Schoenberger, 1995; Soffer et al., 1995; Owens et al., 2000).

Losartan degradates were studied by Zhao et al. (1999). Three losartan degradates were identified in severely stressed losartan tablets (stored for 3 years at 40 °C/75% RH): the first one as aldehyde derivative of losartan (formed by oxidation of hydroxyl group in losartan) and the other two as dimeric degradates of losartan (formed by the condensation of two losartan molecules followed by the elimination of a water molecule). The reported analytical methods for determination of losartan in tablets employ techniques such as liquid chromatography, capillary electrophoresis, super-critical fluid chromatography (Williams et al., 1996) and spectrophotometry (Lastra et al., 2003). McCarthy et al. (1998) developed a stability indicating HPTLC method for determination of losartan and its dimeric degradates in losartan tablets.

In contrast to losartan, which is not yet official in European Pharmacopoeia (2001) and US Pharmacopoeia (2003), monographs on HCTZ are included in both of these pharmacopoeias as a monotherapy and in combination with other drugs. In aqueous solutions HCTZ degrades to 4-amino-6-chlorobenzene-1,3-disulphonamide and formaldehyde via hydrolysis. Mechanism of the reaction and its dependence on temperature and pH were studied by Mollica et al. (1969, 1971). Chlorothiazide and 4-amino-6-chlorobenzene-1,3-disulphonamide are well-known process impurities of HCTZ. Recently a new impurity, which is likely another by-product of the synthesis, has been identified as HCTZ–CH_2–HCTZ isomer (Fang et al., 2001). Deppeler (1981) reported that HCTZ in bulk could be stored at room temperature for 5 years without evidence of degradation. Daniels and Vanderwielen (1981) developed a stability indicating HPLC assay for hydrochlorothiazide in tablets and in the bulk form.

HPLC methods (Argekar and Sawant, 2000; Carlucci et al., 2000; Kanumula and Raman, 2000; Erk, 2001), spectrophotometric methods (Erk, 2001; Shah et al., 2001), HPTLC methods (Shah et al., 2001), and capillary electrophoretic and capillary electrochromatographic methods (Quaglia et al., 2002; Hillaert and Van der Bossche, 2003) have been reported for simultaneous determination of losartan and hydrochlorothiazide in tablets. Hertzog et al. (2002) developed HPLC methods for simultaneous quantitation of active components and their degradates and process impurities in losartan tablets and losartan/hydrochlorothiazide tablets.

Stability testing is an essential part of any drug development. The ultimate goal of this study was to achieve adequate quality of losartan/hydrochlorothiazide tablets throughout their shelf life. Stability study of losartan/hydrochlorothiazide tablets was performed sistematically, in three steps. Stress test was performed in order to validate stability indicating power of the developed analytical methods and to identify the key factors which will impact the stability of the drug product.

Additional preliminary testing enabled the selection of final packaging which would provide adequate protection for the product. Finally, formal stability study was performed in order to propose a shelf life and to recommend storage conditions.

Losartan/hydrochlorothiazide tablets in the selected immediate packaging are demonstrated to be chemically, physically and microbiologically stable.

2. Materials and methods

2.1. Materials

Losartan/hydrochlorothiazide tablets, containing 50 mg of losartan potassium and 12.5 mg of hydrochlorothiazide, were manufactured by Pliva (Croatia), as well as the hydrochlorothiazide active substance. Losartan potassium active substance was purchased from Dr. Reddy's (India). Losartan potassium, hydrochlorothiazide, chlorothiazide and 4-amino-6-chlorobenzene-1,3-disulphonamide working standards were obtained internally at Pliva. *Iso*-losartan and *o*-tolylbenzo-tetrazole working standards were obtained from Dr. Reddy's.

Polyvinylchloride 250 μm/polyethylene 25 μm/polyvinylidenchloride 60 g/m^2 foil (hereafter referred to as PVC/PE/PVdC foil) was purchased from ac-Folien (Germany). Neutral aluminium foil 20 μm (hereafter referred to as Al foil) was purchased from Aluflexpack (Croatia). Oriented polyamide 25 μm/aluminium foil 45 μm/polyvinylchloride 60 μm foil (hereafter referred to as OPA/Al/PVC foil) was purchased from Lawson Mardon Singen (Germany).

Acetonitrile, methanol and acetic acid (glacial) were obtained from Merck (Germany). Orthophosphoric acid and ammonium acetate were obtained from Kemika (Croatia). Triethylamine was obtained from Fluka (Switzerland). Acetonitrile and methanol were of chromatographic grade; acetic acid, orthophosphoric acid and ammonium acetate were of analytical grade.

Solvent, Apura (solvent for volumetric KF titration with two component reagents, contains methanol) and Titrant 5, Apura (titrant for volumetric KF titration with two component reagents, contains methanol), both purchased from Merck, were used for water determination.

The media used for microbiological quality testing were purchased from Difco (USA).

2.2. Stability study

Stability study was performed according to CPMP (2003a) Guideline on stability testing: Stability testing of existing active substances and related finished products. The samples were placed inside stability chambers, where they were exposed to a range of different storage conditions (Table 1). Physical, chemical and microbiological attributes were studied, including particular attributes of the dosage form (dissolution rate).

2.3. Losartan potassium and hydrochlorothiazide assay

To prepare the standard solution, 50 mg of losartan potassium working standard and 12.5 mg hydrochlorothiazide working standard was accurately

Table 1
Description of storage conditions and storage facilities

Test	Storage facility	Storage condition
Freezing test	Test chamber, Kottermann (Germany), type 2770	-20 ± 5 °C
Control samples	Refrigerator, LTH (Slovenia), type HO 1450 GKI	5 ± 3 °C
Long-term testing	Stability testing room equipped with: (1) Air handling unit, GEA (Germany), type Aerotherm 11.05 F (2) Split system air conditioner, Sanyo Gallenkamp (UK), model SAP-KR 128 EH (3) Humidifier, Hygromatik (Germany), type DB 6 (4) Dehumidifier, Cuoghi (Italy), type Nader Midi 4	25 ± 2 °C/60 ± 5% RH
Intermediate testing	Pharmaceutical stability chamber, Sanyo Gallenkamp, model PSC061.XHA.C	30 ± 2 °C/60 ± 5% RH
Accelerated testing	Pharmaceutical stability chamber, Sanyo Gallenkamp, model PSC061.XHA.C	40 ± 2 °C/75 ± 5% RH
Stress test	Test chamber, Heraeus (Germany), type VT 5050 EK	50 ± 2 °C
Stress test	Test chamber, Weiss Umwelttechnik (Germany), type SB 111/300	50 ± 2 °C/80 ± 5% RH

weighed into a 50 ml volumetric flask, dissolved in mobile phase and diluted to volume. Five milliliters of that solution was transferred into a 50 ml volumetric flask and diluted with mobile phase to volume.

To prepare the sample solution, five losartan/hydrochlorothiazide tablets were placed into a 500 ml volumetric flask and approximately 300 ml of mobile phase was added. After 20 min of sonication, the volumetric flask was filled up to volume with mobile phase. Solution was filtered through a 0.45 μm filter. Five milliliters of the filtrate was transferred into a 25 ml volumetric flask, diluted to volume with mobile phase and mixed.

Final concentration of losartan potassium in standard solution and in sample solution was approximately 0.1 mg/ml. Final concentration of hydrochlorothiazide in standard solution and in sample solution was approximately 0.025 mg/ml.

An HPLC system, which consisted of an Agilent 1100 Series instrument (Agilent Technologies, Germany), equipped with a diode array detector set at 220 nm, was used to perform assays. Mobile phase, a mixture of acetonitrile, water, orthophosphoric acid and triethylamine in the ratio 400:600:1:1 (v/v) was used, at flow rate 1 ml/min. HPLC analysis was performed using Hypersil ODS (C18) column (200 mm × 4.6 mm, particle size 5 μm), obtained from Agilent Technologies (USA). The column was operated at temperature 30 °C. Sample injection volume was 10 μl. Elution was isocratic and run time was 10 min.

2.4. Determination of impurities

Sample solution was prepared by placing five losartan/hydrochlorothiazide tablets into a 250 ml volumetric flask, adding approximately 150 ml of mobile phase and then sonicating for 20 min. The volumetric flask was filled up to volume with mobile phase. Solution was filtered through a 0.45 μm filter and injected immediately after preparation.

An HPLC system, which consisted of an Agilent 1100 Series instrument, equipped with a diode array detector set at 220 nm, was used to determine impurities. Mobile phase, a mixture of 700 ml ammonium acetate buffer solution, 250 ml acetonitrile, 50 ml methanol and 2 ml triethylamine (pH adjusted to 6.6 with acetic acid) was used, at flow rate 1 ml/min. HPLC analysis was performed using Inertsil C8 column (250 mm × 4.6 mm, particle size 5 μm), obtained from Varian (USA). The column was operated at 30 °C. Sample injection volume was 10 μl. Elution was isocratic and run time was 60 min.

Ammonium acetate buffer solution was prepared by transferring 385 mg of ammonium acetate into a 1000 ml volumetric flask, dissolving and diluting to volume with ultra pure water, 18.2 MΩ (Ultra Clear UV Plus water system, SG Wasseraufbereitung und Regenerierstation, Germany). The obtained buffer solution was filtered through a 0.45 μm filter prior to use.

2.5. Dissolution

The USP dissolution apparatus (Van Kel, USA) Type II (paddles) at rotation speed of 100 rpm was used for dissolution testing. The dissolution medium consisted of 900 ml of degassed water at 37.0 ± 0.5 °C. A single tablet was added to the dissolution medium in each vessel. After 45 min samples were withdrawn, filtered (filter pore size 0.45 μm) and assayed by HPLC (using an on-line dissolution-HPLC system).

HPLC system consisted of a TSP Spectra System (ThermoSeparation Products, USA) with a UV–vis detector set at 220 nm. Mobile phase, a mixture of acetonitrile, water, orthophosphoric acid and triethylamine in the ratio 400:600:1:1 (v/v) was used, at flow rate 1 ml/min. Analyses were performed using Hypersil ODS (C18) column (200 mm × 4.6 mm, particle size 5 μm), obtained from Agilent Technologies (USA). The column was operated at 30 °C. Sample injection volume was 20 μl. Elution was isocratic and run time was 10 min.

2.6. Disintegration, hardness, water determination and appearance

Disintegration time was determined on six tablets, using disintegration tester Erweka ZT 74 and demineralised water (37 ± 1 °C) as medium. Analyses were performed in accordance with Ph. Eur. method 2.9.1., Test A (European Pharmacopoeia, 2001).

Hardness was determined on 10 tablets using TBH-30 hardness tester purchased from Erweka (Germany). Analyses were performed in accordance with Ph. Eur. method 2.9.8.

Water was determined on 300 mg of tablet powder, by Karl Fischer titrimetic method, using Karl Fischer automatic titration instrument 758 KFD Titrino, purchased from Metrohm (Switzerland). Stirring time before titration was 6 min. Tablets were powdered just before measurement due to hygroscopicity of the tablet powder. Analyses were performed in accordance with Ph. Eur. method 2.2.19.

Appearance was determined organoleptically on the received sample.

2.7. Microbiological quality

Total viable aerobic count was determined according to Ph. Eur. method 2.6.12. (Microbiological examination of non-sterile products): sample preparation was performed as described under Non-fatty products insoluble in water and examined as described under Plate-count methods, Pour-plate method. Specified microorganisms were tested according to Ph. Eur. method 2.6.13. Shaker type MS2, purchased from IKA (Germany) and Laminar Flow HB 2472 purchased from Holten (Denmark) were used.

3. Results and discussion

3.1. Stress test (forced degradation study)

Within the stress test, samples of losartan/hydrochlorothiazide tablets (without the immediate pack) were exposed to severe storage conditions: $50 \pm 2\,^{\circ}C$ (hereafter referred to as $50\,^{\circ}C$) and $50 \pm 2\,^{\circ}C/80 \pm 5\%$ RH (hereafter referred to as $50\,^{\circ}C/80\%$ RH). Losartan potassium, hydrochlorothiazide and impurities were determined on samples of a single laboratory-scale batch. Following impurities were monitored (Fig. 2): *o*-tolylbenzo-tetrazole (impurity A), *iso*-losartan (impurity B), chlorothiazide (impurity C), 4-amino-6-chlorobenzene-1,3-disulphonamide (impurity D) and unknown impurities. Impurities are expressed as area % with reference to total area of all peaks excluding placebo and mobile phase peaks.

After 4 weeks of storage at $50\,^{\circ}C$ no significant change in quality was observed. After 4 weeks of storage at $50\,^{\circ}C/80\%$ RH there was no significant change in assay (all the results were within ±5% of the label claim and of the respective initial value). There was also

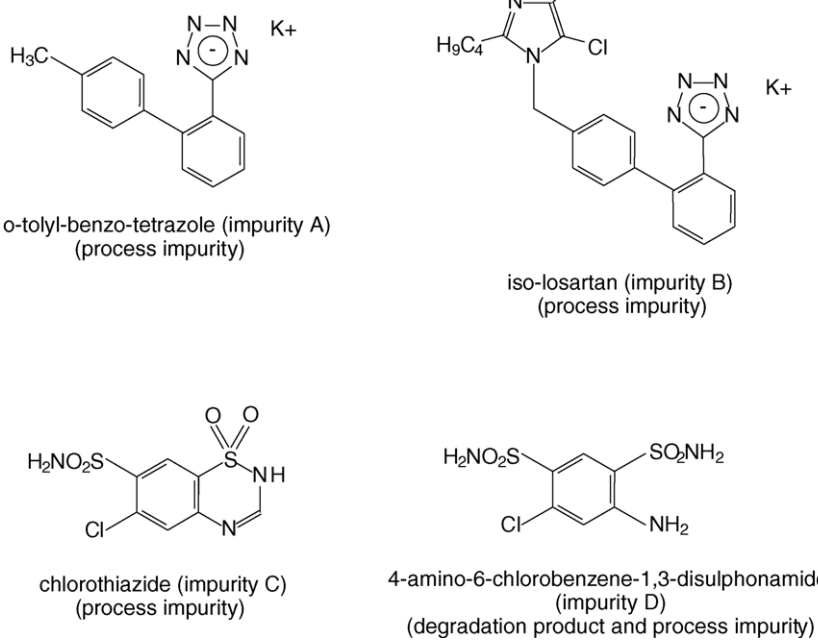

Fig. 2. Structures of the monitored degradation products and process impurities of losartan potassium (impurities A and B) and hydrochlorothiazide (impurities C and D) in losartan/hydrochlorothiazide tablets.

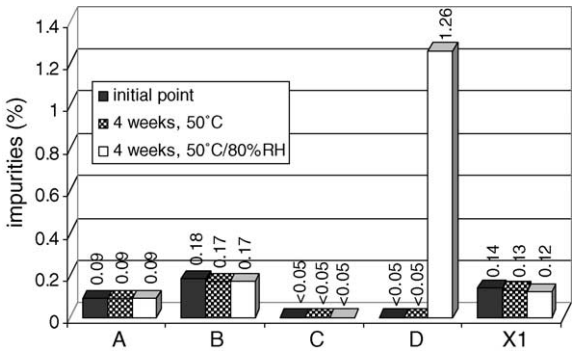

Fig. 3. Impurities at the initial point and after 4 weeks of storage at 50 °C and 50 °C/80% RH (area % with reference to total area of all peaks excluding placebo and mobile phase peaks): A: *o*-tolylbenzo-tetrazole; B: *iso*-losartan; C: chlorothiazide; D: 4-amino-6-chlorobenzene-1,3-disulphonamide; X1: unknown impurity. Note: The results for known and unknown impurities are presented taking into account the disregard limit of the analytical method (0.05%).

no significant change in impurities A–C and unknown impurity X1, but there was a significant increase in content of impurity D. It was concluded that the product is sensitive to moisture. The results for impurities obtained at the initial point and after 4 weeks of storage are presented in Fig. 3.

Additionally, selectivity of the analytical method for impurities was confirmed through stress studies in solution. Samples of hydrochlorothiazide substance, losartan potassium substance, losartan/hydrochlorothiazide tablets and placebo were treated with 0.1 mol/l HCl (60 min), 0.1 mol/l NaOH (60 min) and 3% H_2O_2 (10 min) and exposed to 70 °C (60 min).

It was shown that the proposed analytical method for determination of impurities is capable of separating losartan potassium, hydrochlorothiazide and their degradation products and is therefore suitable for use as a stability indicating method during stability studies. Selectivity of analytical methods for determination of active substances and dissolution was proven in a similar way.

Chromatograms (assay, impurities determination) obtained with non-treated samples and after 4 weeks at 50 °C/80% RH are considered representative for method selectivity and are presented in Figs. 4–7. (Chromatograms which prove the selectivity of the analytical method for dissolution are similar to chromatograms presented for assay.)

3.2. Preliminary testing (selection of packaging)

Many types of packaging for drug products are available on the market, offering different degrees of protection for the product. Depending on the sensitivity of the drug in question, as well as on marketing and regulatory requirements and available packaging facilities, adequate packaging is selected.

Stress test of losartan/hydrochlorothiazide tablets suggested that the product requires packaging which will protect it from moisture. However, it is well-known that changes observed during the forced degradation studies are likely to be much less expressed under normal storage conditions (some even may not be expressed at all). The extent of the observed change which may be expected during the shelf life of a product in its final packaging can only be revealed by testing at formal stability study conditions, which form the basis for establishing the shelf life and storage conditions.

In case of losartan/hydrochlorothiazide tablets, prior to final packaging selection it still had to be investigated whether partial protection against water vapour would be sufficient or an absolute barrier was necessary. Two packaging systems with barrier properties were chosen as candidates: PVC/PE/PVdC//Al blisters and OPA/Al/PVC//Al blisters. While PVC/PE/PVdC//Al blisters are an example of economical packaging with thermoforming properties, which protect the product partially from water vapour and gases, the cold-forming OPA/Al/PVC//Al blisters serve as an absolute barrier against gases, water vapour and light.

A 6-month preliminary testing was performed in order to compare the stability of losartan/hydrochlorothiazide tablets in these two types of packaging. Losartan/hydrochlorothiazide tablets are intended to be marketed in Europe. Therefore, testing was performed under a set of storage conditions recommended for climatic zones I and II (Grimm, 1998; ICH Q1A(R2), 2003): 25 ± 2 °C/60 ± 5% RH (hereafter referred to as 25 °C/60% RH) as the long-term condition and 40 ± 2 °C/75 ± 5% RH (hereafter referred to as 40 °C/75% RH) as the accelerated condition. Assay, impurities, disintegration and appearance were analysed on samples of a single laboratory-scale batch.

After 6 months of storage at 40 °C/75% RH significantly higher amount of impurity D was observed in samples in PVC/PE/PVdC//Al blisters in

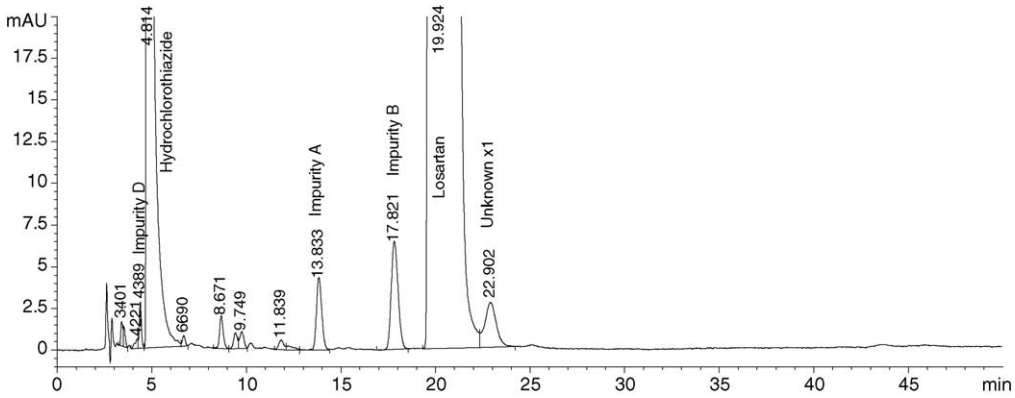

Fig. 4. Impurities determination, chromatogram of a non-treated sample.

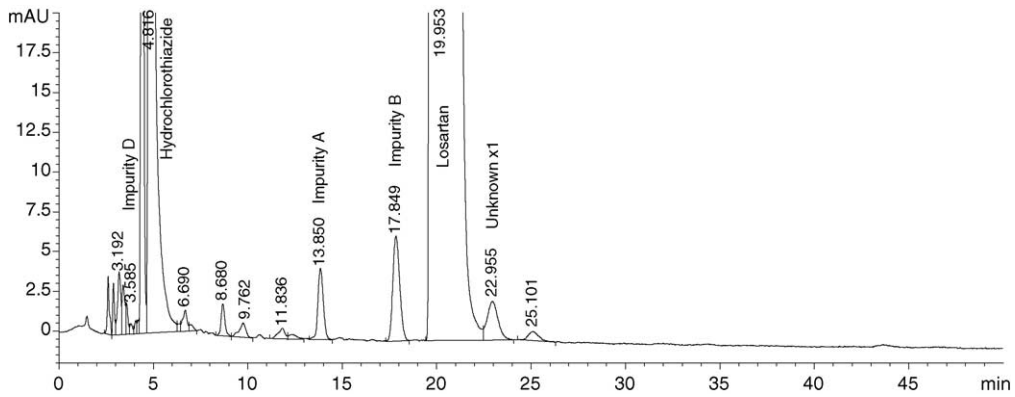

Fig. 5. Impurities determination, chromatogram of a sample stored for 4 weeks at 50 °C/80% RH.

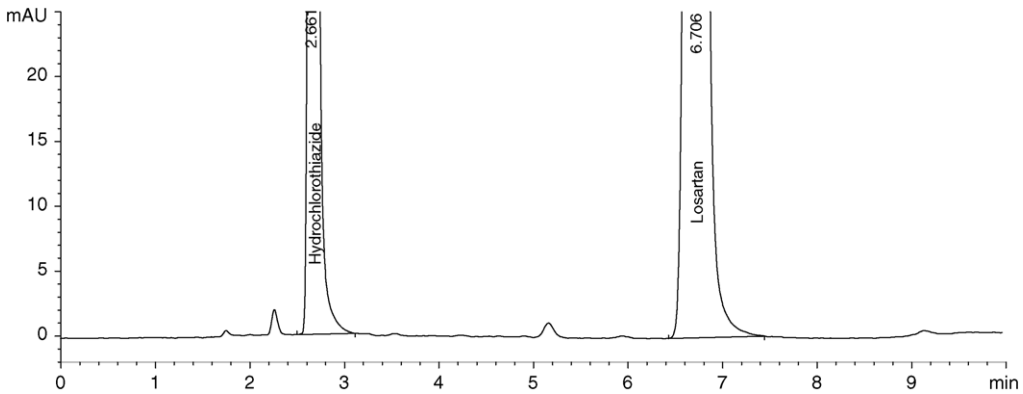

Fig. 6. Assay, chromatogram of a non-treated sample.

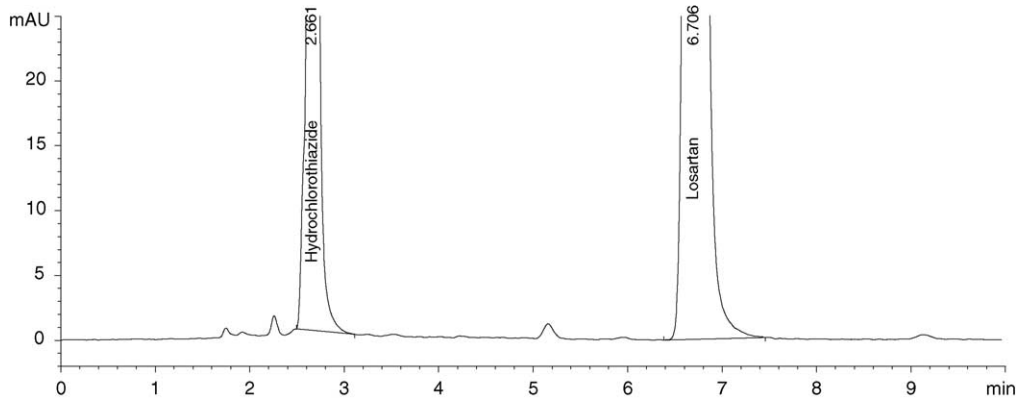

Fig. 7. Assay, chromatogram of a sample stored for 4 weeks at 50 °C/80% RH.

comparison with samples in OPA/Al/PVC//Al blisters (Fig. 8). Samples in PVC/PE/PVdC//Al blisters have also shown a drastic increase in disintegration time in comparison with samples in OPA/Al/PVC//Al blisters (52 min in PVC/PE/PVdC//Al blisters, 6.5 min in OPA/Al/PVC//Al blisters after 6 months at 40 °C/75% RH). No significant difference between losartan/hydrochlorothiazide tablets in two types of packaging was observed regarding other monitored characteristics (impurities A–C, unknown impurities, assay, appearance).

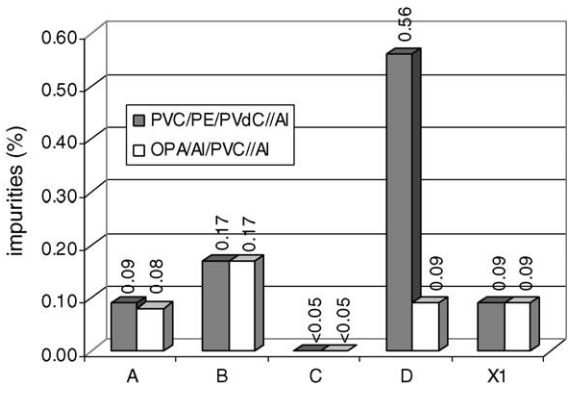

Fig. 8. Impurities in losartan/hydrochlorothiazide tablets, packaged in PVC/PE/PVdC//Al blisters and OPA/Al/PVC//Al blisters, after 6 months of storage at 40 °C/75% RH (area % with reference to total area of all peaks excluding placebo and mobile phase peaks): A: *o*-tolylbenzo-tetrazole; B: *iso*-losartan; C: chlorothiazide; D: 4-amino-6-chlorobenzene-1,3-disulphonamide; X1: unknown impurity. Note: The results for known and unknown impurities are presented taking into account the disregard limit of the analytical method (0.05%).

It was concluded that the protection against moisture offered by PVC/PE/PVdC//Al blisters is not sufficient and that losartan/hydrochlorothiazide tablets need to be packaged in OPA/Al/PVC//Al blisters.

3.3. Formal stability testing

Formal stability testing of losartan/hydrochlorothiazide tablets is performed using samples of two pilot-scale batches packaged in OPA/Al/PVC//Al blisters. Time points for analyses are defined in the stability testing protocol (Table 2). Following parameters are monitored: assay, impurities, dissolution, disintegration, hardness, water, appearance and microbiological quality. Validated analytical procedures are used. Up to now, 12 months of the formal stability study have been completed.

Losartan potassium assay and hydrochlorothiazide assay did not change significantly during 6 months of accelerated testing and 12 months of long-term testing. All of the obtained results are within ±5% of the label claim and of the respective initial value.

Based on chromatograms from preliminary testing of losartan/hydrochlorothiazide tablets and stress test of losartan potassium substance, HCTZ substance and losartan/hydrochlorothiazide tablets it was possible to divide impurities into those related to losartan potassium and those related to HCTZ and, consequently, to express impurities in relation to the active substance that they originate from. Since the declared content of two active substances in the

Table 2
Stability testing protocol

Storage conditions	Time of storage (months)							
	0	3	6	9	12	18	24	36
5 °C		(x)	(x)	(x)	(x)	(x)	(x)	(x)
25 °C/60% RH	x[a]	x	x	x	x	x	x[a]	x[a]
30 °C/60% RH		(x)	(x)	(x)	(x)			
40 °C/75% RH		x	x[a]					
−20 °C	x[b]							

x: time point; (x): the samples stored at 5 °C are control samples; the samples at 30 °C/60% RH do not need to be tested if there is no significant change at accelerated storage condition.

[a] Microbiological quality testing.

[b] After the freezing test (2 weeks at −20 °C and then 1 week at 25 °C/60% RH) only disintegration, hardness, water and appearance are analysed.

losartan/hydrochlorothiazide tablets is different (as well as their spectra), the new way of calculation would enhance the accuracy of the final results. It was therefore decided that the calculation of individual impurities be changed to area % with respect to the active substance that each impurity is related to. Impurities are presented as follows:

(1) Impurities related to losartan potassium:
- Impurity A;
- Impurity B;
- Unknown impurities related to losartan potassium;
- Total impurities related to losartan potassium.

(2) Impurities related to hydrochlorothiazide:
- Impurity C;
- Impurity D;
- Unknown impurities related to HCTZ;
- Total impurities related to HCTZ.

The obtained results show no significant change in impurities. The results for impurities in one of the tested batches are presented in Table 3. Although degradation products of losartan are described in literature, the presented stability study shows no degradation of losartan, neither at long term nor at accelerated storage condition. An increase in impurity D (up to 0.3%), and consequently in total impurities

Table 3
Results for impurities obtained during the first 12 months of the formal stability testing of batch B1 (area % with reference to the active substance that each impurity is related to)

Storage condition	Impurities related to losartan potassium (area %)				Impurities related to hydrochlorothiazide (area %)			
	A	B	Unknown X1	Total	C	D	Unknown X2	Total
Initial point	0.11	0.22	0.12	0.46	<0.05	0.07	0.07	0.13
3 months								
25 °C/60% RH	0.12	0.23	0.13	0.47	<0.05	0.09	0.08	0.17
40 °C/75% RH	0.11	0.22	0.13	0.47	<0.05	0.13	0.08	0.21
6 months								
25 °C/60% RH	0.12	0.23	0.15	0.49	<0.05	0.12	<0.05	0.12
40 °C/75% RH	0.11	0.22	0.16	0.50	<0.05	0.29	<0.05	0.29
9 months								
25 °C/60% RH	0.12	0.23	0.15	0.49	<0.05	<0.05	0.06	0.06
12 months								
25 °C/60% RH	0.11	0.22	0.12	0.45	<0.05	0.06	<0.05	0.06

A: *o*-tolylbenzo-tetrazole; B: *iso*-losartan; C: chlorothiazide; D: 4-amino-6-chlorobenzene-1,3-disulphonamide; X1, X2: unknown impurities. The results for known and unknown impurities are presented taking into account the disregard limit of the analytical method (0.05%).

related to HCTZ, was observed during 6 months of accelerated testing, but all the results are within the limits set in Ph. Eur. (2001) and USP (2003) monographs on hydrochlorothiazide substance.

No significant change was observed in dissolution rate during 6 months of storage at accelerated condition and 12 months at long-term condition. All results comply with the general requirement stated in British Pharmacopoeia (2002), Supplementary chapter IE, Dissolution Testing of Solid Oral Dosage Forms. No significant change in quality of losartan/hydrochlorothiazide tablets was observed regarding their disintegration, hardness, water content and appearance. Microbiological quality, tested at the initial point and after 6 months of storage at 40 °C/75% RH complies with the requirements described in Ph. Eur. 5.1.4., Category 3A.

4. Conclusions

Stress test suggested that non-packaged losartan/hydrochlorothiazide tablets (without the immediate pack) are sensitive to moisture. HPLC methods for assay and impurities were demonstrated to be stability indicating. Based on results of preliminary testing, OPA/Al/PVC//Al blisters were chosen as appropriate type of packaging for the product.

Up to now, 12 months of the formal stability study have been completed. The results obtained during 6 months of accelerated and 12 months of long-term stability testing have shown that losartan/hydrochlorothiazide tablets packaged in OPA/Al/PVC//Al blisters are chemically, physically and microbiologically stable. Based on the obtained results, a shelf life of 24 months is proposed.

A storage statement should be established for the labelling in accordance with relevant national/regional requirements. Since there is no significant change in quality either at long term (25 °C/60% RH) nor at accelerated (40 °C/75% RH) storage condition, no labelling statement regarding storage conditions is required in Europe (CPMP, 2003b). According to FDA guidance (1998), the recommended labelling statement would be: Store at 25 °C; excursions permitted to 15–30 °C (see USP Controlled Room Temperature).

The stability testing is being continued according to the stability protocol. The proposed shelf life and storage conditions need to be confirmed with long-term data.

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