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Drug-excipient compatibility testing—Identification and characterization of degradation products of phenylephrine in several pharmaceutical formulations against the common cold

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ABSTRACT

Different pharmaceutical preparations against the common cold containing phenylephrine (PHE) and saccharose were studied. New impurities were discovered in these preparations after exposure using isocratic ion-pair chromatography separation on a C18 column. LC–MS and NMR techniques were employed to identify and to fully characterize these new compounds. The products were identified as 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisochinolin-4,8-diol and 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisochinolin-4,6-diol. Identification of these degradation products allowed to understand and to confirm their formation mechanism. The developed HPLC method separates of all known impurities and impurities originated from PHE as well.

1. Introduction

Impurities in pharmaceutical products are often formed as a result of an interaction between the drug substance and species introduced by formulation [1]. Ideally, the excipients used in the formulations should not interact with the drug substance or introduce species capable of accelerating the formation of new impurities. The incompatibility between drugs and excipients in solid dosage form can alter the stability and bioavailability of drugs, thereby affecting its safety and efficacy. Although, the potential degradation products are in low concentrations, the stress studies of the drug substance-excipients mixture generate higher amounts of degradation products and can help to identify the degradation products using various analytical procedures [2].

The commercial pharmaceutical products containing PHE as a replacement for pseudoephedrine are marketed by various producers in sachet format that is supposed to be dissolved in hot water and administered as a hot drink. Chemically, PHE is [(*R*)-1-(3-hydroxyphenyl)-2-(methylamino)ethanol, $C_9H_{13}O_2N$] and is a sympathomimetic agent with direct effects on α-adrenoreceptors in certain areas of body [3,4]. PHE is known to undergo physical and chemical degradation. Degradation of PHE may be caused by a variety of factors including the presence of oxygen, moisture, reducing sugars, bases and high temperature. Degradation of PHE may be accompanied by a change in color, e.g. changing from a white or almost white into a darker, brownish color. Coloration is accelerated by light, but it occurs eventually even in light-protected media [5,6]. The photochemical oxidation of PHE was investigated and the product was identified as epinephrine [5,7]. Photochemical oxidation of hydroxyphenalkanolamines may well rather a general phenomenon. The studies establishing exact degradation mechanism of the PHE were carried out earlier [8,9].

PHE is used alone or in combination with other agents (e.g. paracetamol, ascorbic acid, acetaminophen) against a symptomatic relief of cold and flu symptoms, nasal congestion and sinusitis. The acetylated degradation products of PHE in tablet formulations containing acetylsalicylic acid were detected and described [10,11]. Various degradation products of PHE were observed by paper chromatography in drug products after stability study [12] but these degradation products were not identified. The combination of PHE with other active ingredients, namely, chlorpheniramine maleate and acetaminophen, was reported to produce a small amount of a degradant [13,14]. Cyclization of 3-hydroxyphenylethylamines with carbonyl compounds, namely formaldehyde or acetaldehyde, to form 1,2,3,4-tetrahydroisoquinolines (THQs) under mild conditions was found to be very selective reaction [15,16]. The reaction was named “phenolic cyclization” [17] and was observed during drug decomposition [18]. It has been applied also in analysis [19]. The formation of THQs is the most widely known degradation reaction of PHE.

The aim of the present work was to identify and characterize of unknown degradation products formed in various pharmaceutical formulations against the common cold containing PHE and paracetamol (PAR) as active compounds. Various analytical tools were

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employed, including HPLC separation and solute characterization by spectroscopic techniques coupled with off-line HPLC.

2. Experimental

2.1. Chemicals and materials

Acetonitrile and 2-propanol were of HPLC grade (J.T. Baker, USA). Water purified on Milli-Q system (Millipore, USA) was used. Other chemicals were of analytical grade and were purchased from Lach-Ner (Czech Republic, Neratovice). Impurities of PHE – (4*R*)-4,8-dihydroxy-*N*-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride monohydrate (4,8-THQ), (4*R*)-4,6-dihydroxy-*N*-methyl-1,2,3,4-tetrahydroisoquinoline hydrochloride (4,6-THQ), norphenylephrine and benzylphenylephrine were purchased from Epichem (Murdoch, Australia). Paracetamol, 4-aminophenol (4-AP) and 5-hydroxymethyl-2-furaldehyde (5-HMF) were purchased from Sigma–Aldrich (Czech Republic, Prague).

2.2. Instrumentation

All chromatographic experiments were carried out using a liquid chromatographic system consisting of Alliance 2695 separation module, fluorescence detector W2475 and PDA detector W2996 (all Waters, USA). The system was controlled by Empower software (Waters, USA). Impurities was identified by high-resolution MS technique using the LTQ Orbitrap Hybrid Mass Spectrometer (Fisher Scientific, Waltham, USA) with HPLC HTS PAL system (CTC analytics, Switzerland) and direct injection into APCI source in positive mode (vaporizer temperature 400 °C, capillary temperature 300 °C, discharge current 4 μA). Preparative HPLC separation and collection was carried out on an Waters Autopurification system (System fluidics organiser, 2545 binary gradient module, 2767 sample manager, 515 HPLC pump, 2487 dual-wavelength absorbance detector and SQ detector; Waters, USA). The collected fractions of samples were evaporated on RVO 400 vacuum evaporator (Ingos, Czech Republic).

2.3. HPLC, LC–MS and semipreparative LC conditions

The analytical separation was performed on a 100 mm × 4.6 mm, 2.7 μm Ascentis Express C18 Column (Supelco, USA). The mobile phase was a 74:18:8 (v/v/v) mixture aqueous solution of 10 mM sodium dodecyl sulfate adjusted with phosphoric acid to pH 2.4: acetonitrile: 2-propanol. The flow rate 0.8 ml/min was employed throughout the analysis. The analyses were performed at 20 °C and volume of solution injected onto the column was 5 μL. The analyte was monitored by fluorimeter with excitation wavelength at 275 nm and emission wavelength at 310 nm. The relative retention time (RRT) of all impurities was calculated relative to retention time of PHE.

The LC–MS investigation of unidentified impurities was carried out on an Obelisc R column, 250 mm × 4.6 mm, 5 μm (Sielc, USA). The mobile phase consisted of aqueous solutions of 10 mM ammonium formate adjusted with formic acid to pH 4.1: acetonitrile (95:5, v/v). The flow rate was 1.0 ml/min, and the injection volume was 5 μl. The optimized ion source settings were as follows: ionisation mode: ESI+; capillary voltage: 1 kV; cone voltage: 25 V; extractor voltage: 2 V; source temperature: 120 °C; desolvatation temperature: 300 °C; desolvatation gas (N_2) flow: 600 l/h; cone gas (N_2) flow: 50 l/h. The optimized MS data acquisition was as follows: data acquisition mode: full scan (m/z 100–500); scan duration: 0.2 s; inter-scan delay: 0.02 s. The proposed structures of impurities based on the LC–MS investigation were confirmed using the high-res MS experiment. APCI ion source was operated at vaporizer temperature of 400 °C and capillary temperature at 300 °C. Samples were analyzed in positive ion mode using discharge current 6.0 kV. Sheath gas flow and auxiliary gas flow was 35 and 5 (arbitrary units), respectively. MS^2 and MS^3 data were measured using normalized collision energy 35. The chromatographic conditions remained the same as described above.

The semipreparative Obelisc R column (100 mm × 22 mm, 5 μm; Sielc, USA) was used for preparative purposes using the mobile phase consisting of aqueous solution of 10 mM ammonium formate adjusted with formic acid to pH 4.1: acetonitrile (95:5, v/v). The flow rate 20 ml/min was employed throughout the preparation. The volume of solution injected onto the semipreparative column was 500 μL. In order to monitore UV and MS signal of the effluent from semipreparative column the effluent was splitted in ratio 1:1000 into the methanol flow from HPLC pump which was directed to detectors. The optimized MS conditions are described above. The fraction collection was triggered on minimal intensity threshold (MIT = 15000 μV) at UV 273 nm.

2.4. NMR conditions

Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker Avance 500 (Bruker Biospin GmbH) at 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C), respectively. All NMR experiments were performed in CD_3OD at 298 K therefore OH signals were not observed. COSY, HSQC, ^{1}H–^{13}C HMBC and selective 1D NOESY spectra were recorded using pulse programs from the Bruker NMR standard library. At 500 MHz, standard 5 mm TBI (triple-broadband inverse) probehead equipped with z-gradient coils was employed for all measurements.

2.5. Standard preparation

The standard of PHE hydrochloride (in house, Zentiva, Czech Republic; purity 100.0%) was dissolved in mobile phase at a concentration of 12 mg/l to obtain the standard stock solution. The methanol solutions of 4,8-THQ, 4,6-THQ, norphenylephrine and 4-AP were prepared to obtain concentration of 2 mg/l.

2.6. Stress studies

The investigated pharmaceutical formulation contains following excipients: starch, saccharose, tartaric acid, aspartame, citric acid, ascorbic acid; and paracetamol and PHE as drug in ratio 4:342:45:10:45:6:83:1. Stress studies were carried out under different temperature and time in air oven (Binder IP 20, Germany). The stressors, choice of their concentration, preparation of samples and stress conditions are described in Section 3. All mixtures prepared from dry powders were grounded together using a pestle and mortar. The final concentration of the PHE in all investigated solutions was about 12 mg/l. Impurities investigated through this study were not present in PHE standard solution.

3. Results and discussion

3.1. Development and optimization of the HPLC method

The separation of drug and their decomposition products was initially tested by varying proportions of acetonitrile and ion-pair buffer type (sodium 1-octanesulfonate and sodium 1-decanesuflonate) in the mobile phase. Acceptable resolution of all impurities was achieved with addition of 8% of 2-propanol as the organic modifier in isocratic mode using flow rate 0.8 ml/min. A gradient elution was not suitable due to long equilibration time and baseline drift. The separation of all degradation products is shown in Fig. 1A. Limit of quantitation (LOQ) was calculated based

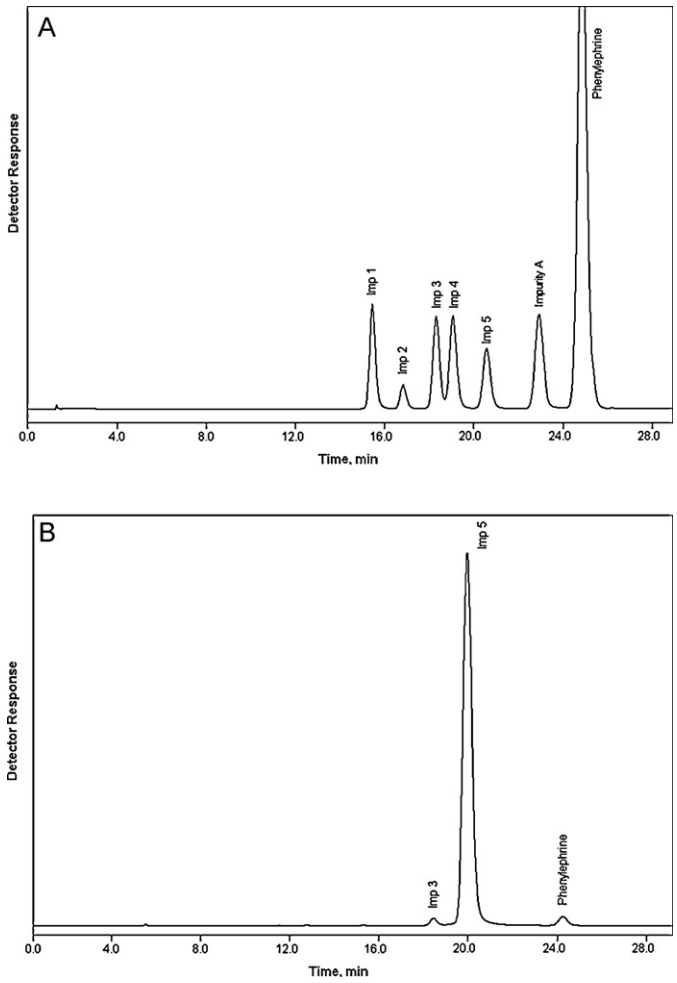

Fig. 1. (A) The ion-pair isocratic chromatographic separation of degradation products of PHE on Ascentis Express C18 Column using 10 mM sodium dodecyl sulfate adjusted with phosphoric acid to pH 2.4: acetonitrile: 2-propanol as the mobile phase. Flow rate 0.8 ml/min, separation temperature 20 °C, injected volume 5 μl. (B) The ion-pair isocratic chromatographic separation of Imp 3 and Imp 5 originated by cyclization of PHE and 5-HMF.

on detector signal-to-noise ratio and was found to be 0.01% relative to PHE.

3.2. Stress studies and characterization of degradation products

Unidentified degradation products with fluorescence behavior similar to that observed for PHE were detected in stability samples of the pharmaceutical formulation after 6 months in climatic chamber at 25 °C and 60% relative humidity. The impurity with relative retention time (RRT) 0.92 was identified as impurity A (norphenylephrine) described in European Pharmacopeia (*Ph.Eur.*) [20]. The other impurities described in current *Ph.Eur*. such as impurity D (benzylphenylephrine) were not found. Observed unidentified impurities are mentioned in Table 1.

3.2.1. Identification of degradation products Imp 1 and Imp 4

The impurities with RRT 0.62 (Imp 1) and RRT 0.77 (Imp 4) were detected. Using high-resolution LC–MS study protonated molecular ion $[M+H]^+$ at $m/z = 280.1019$ Da for both impurities was determined. The protonated molecular ion of PHE $[M+H]^+$ has $m/z = 268.1019$ Da and the observed increment of molecular mass (+12) corresponds to condensation of PHE with formaldehyde which was described in literature [15,16]. The reaction is known as "phenolic cyclization" and leads to formation of tetrahydroisoquinolines (THQs). The structure of THQs were confirmed by comparison with standards of 4,8-THQ and 4,6-THQ. The degradation product Imp 1 corresponded to 4,6-dihydroxy-*N*-methyl-1,2,3,4-tetrahydroisoquinoline and Imp 4 corresponded to 4,8-dihydroxy-*N*-methyl-1,2,3,4-tetrahydroisoquinoline (Fig. 2). The cyclization is very fast reaction within wide pH range which was demonstrated by reaction of 0.1% formaldehyde solution with stock solution of PHE at room temperature in 0.1 M citric acid or 0.1 M sodium citrate. Both mixtures were injected into the HPLC system immediately after mixing and the presumptive THQs eluted at RRT 0.62 and RRT 0.77 in high yield (>98% for both THQs). Formaldehyde is the simplest fragment of sugars. Its formation was confirmed in many studies concerning sugar degradation [21,22]. Formaldehyde was also suggested as a product of cleavage of C1–C2 bond of the acyclic adduct of hydroperoxide anion to ketoses [23,24].

3.2.2. Identification of degradation product Imp 2

Four sets of drug/excipient mixture (E1–E4) were prepared and stressed in closed glass vials at 90 °C for 30 min. After stress test, the samples were dissolved in 10 ml water, diluted in mobile phase to obtain analytical concentration and then analyzed by HPLC. The results are showed in Table 2. It is obvious, that the formation of Imp 2 depends on medium acidity and the formed amount increased with medium acidity (experiments E1, E2 and E4). The presence of PHE has no influence on formation of Imp 2 hence the degradation product could to be originated from PAR (experiment E3). The amount of Imp 2 in experiment E2 was higher than in experiment E3 (with the same concentration of PAR and HCl). The experiment E2 was repeated under the same conditions without presence of PAR. The formation of any impurity was not observed (<0.01%). Under the conditions of high temperature and pH, PAR undergoes hydrolysis forming 4-AP and acetic acid as degradation products [25,26]. 4-AP shows a fluorescence behavior (maximum excitation and emission wavelengths of 4-AP are 323 and 373 nm, respectively) [27] under detection conditions as well. The identification of Imp 2 was verified by comparison of retention time of Imp 2 with retention time of 4-AP reference standard. Further identification of Imp 2 was carried out by comparison of UV spectra of Imp 2 with reference UV spectra of 4-AP under method conditions using PDA detection. The presence of impurities (Imp 1 and Imp 4) in experiments E1–E4 can be explained by presence of native formaldehyde in samples.

3.2.3. Identification of degradation product Imp 3 and Imp 5

Six sets of drug/excipient mixture (E5–E10) were stressed in closed glass vials at 60 °C for 12 h. The composition of drug/excipient mixtures (E5–E10) is described in Table 3A. After stress tests, the samples were dissolved in 10 ml water, diluted in mobile phase to obtain analytical concentration and then analyzed by HPLC. It was observed, that the formation of Imp 5 depends strongly on several aspects including presence of saccharose and medium acidity. No formation of impurities was observed under described conditions (E6–E9) in case that citric and tartaric acid was not added (data not shown). Formation of Imp 3 and Imp 5 could be probably attributed to reaction of PHE with degradation products of saccharose. The formation of Imp 1 and Imp 4 from ascorbic acid was observed under described conditions which is in agreement with similar studies carried out by Harkrader [28].

The most common saccharides such as fructose, saccharose and glucose and organic acids may serve as precursors of furan derivatives. It is known that the primary sources of thermally produced furan and its derivatives (such as 5-HMF) are saccharides [21]. The highest potential to produce 5-HMF upon thermal treatment has fructose originating from saccharose hydrolysis. Similarly as in the reaction known as "phenolic cyclization", there is possibility of

Table 1
The normalization assay of identified impurities of different pharmaceutical formulations containing PHE.

Identified impurities (RRT)	Normalization relative to PHE (%)		
	Paralen hot drink[a]	Cold relief powders[b]	Coldrex[c]
Imp 1 (0.62)	0.19	0.07	0.19
Imp 2 (0.67)	0.40	0.71	0.06
Imp 3 (0.74)	0.03	0.02	0.61
Imp 4 (0.77)	0.02	0.14	0.05
Imp 5 (0.82)	0.31	0.17	0.46

[a] Producer—Zentiva, Czech Republic.
[b] Producer—Unichem, United Kingdom.
[c] Producer—GlaxoSmithKline, United Kingdom.

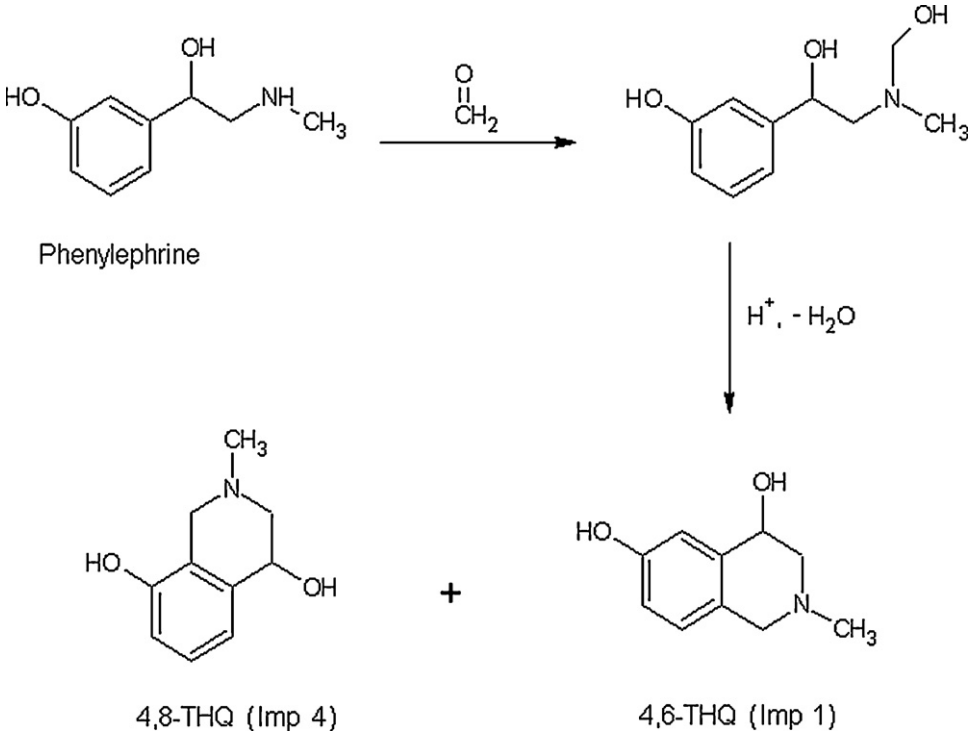

Fig. 2. Scheme for cyclization of PHE with formaldehyde to 4,6-THQ (Imp 1) and 4,8-THQ (Imp 4).

condensation of 5-HMF with PHE forming 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisochinolin-4,8-diol (4,8-THQ-HMF) and 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisochinolin-4,6-diol (4,6-THQ-HMF) (Fig. 3).

Formation of the proposed derivatives of THQs-HMF was verified by the following experiments. The drug/excipient mixtures (E11–E14) were prepared and stressed in closed glass vials at 60 °C for 12 h (see Table 3B). Fructose has the highest potential to form Imp 5 in acidic medium, while formation of Imp 3 was not observed. Formation of Imp 1 and Imp 4 from glucose and fructose (experiments E11 and E12) was observed under described conditions which is in agreement with conclusions described in Section 3.2.1. Reaction of PHE with 5-HMF in alkaline medium (experiment E14) provides Imp 3 and Imp 5 in high yield (>85% for Imp 5; Fig. 1B). Formation of Imp 1 in experiments E13 and E14 has not been clarified.

The chromatographic separation for LC–MS study was optimized on analytical Obelisc R column. It was observed, that Imp 5 provides two peaks (labeled as Imp 5/1 and Imp 5/2) under these conditions (Fig. 4). It is obvious that the pair of Imp 5/1 and Imp

Table 2
Stress tests for identification of Imp 2 (the experiments (E1–E4)).

Identified impurities (RRT)	Normalization relative to PHE (%)[a]			
	E1	E2	E3	E4
Imp 1 (0.62)	<0.01	0.01	<0.01	0.01
Imp 2 (0.67)	<0.01	9.90	7.49	0.32
Imp 3 (0.74)	<0.01	<0.01	<0.01	<0.01
Imp 4 (0.77)	<0.01	0.01	<0.01	0.01
Imp 5 (0.82)	<0.01	<0.01	<0.01	<0.01

E1: 1000 mg PAR/12 mg PHE; E2: 1000 mg PAR/12 mg PHE and addition of 1 ml 5 M hydrochloric acid; E3: 1000 mg PAR and addition of 1 ml 5 M hydrochloric acid; E4: 1000 mg PAR/12 mg PHE/536 mg tartaric acid.
[a] The normalization assay (%) was recalculated with regard to concentration of PHE corresponding to 12 mg.

Table 3A
Stress tests for identification of Imp 3 an Imp 5 (the experiments (E5–E10)).

Identified impurities (RRT)	Normalization relative to PHE (%)[a]					
	E5	E6	E7	E8	E9	E10
Imp 1 (0.62)	<0.01	0.02	2.21	0.50	0.01	<0.01
Imp 2 (0.67)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Imp 3 (0.74)	<0.01	<0.01	0.27	<0.01	0.01	<0.01
Imp 4 (0.77)	<0.01	<0.01	<0.01	0.15	0.01	<0.01
Imp 5 (0.82)	<0.01	<0.01	0.38	<0.01	0.01	<0.01

E5: placebo (45 mg starch/4100 mg saccharose/536 mg tartaric acid/115 mg aspartame/536 mg citric acid/77 mg ascorbic acid/100 mg water); E6: 45 mg starch/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E7: 4100 mg saccharose/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E8: 77 mg ascorbic acid/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E9: 115 mg aspartame/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E10: 536 mg citric acid/536 mg tartaric acid/100 mg water/12 mg PHE.

[a] The assay of Imp 2 (%) was recalculated with regard to concentration of PHE corresponding to 12 mg.

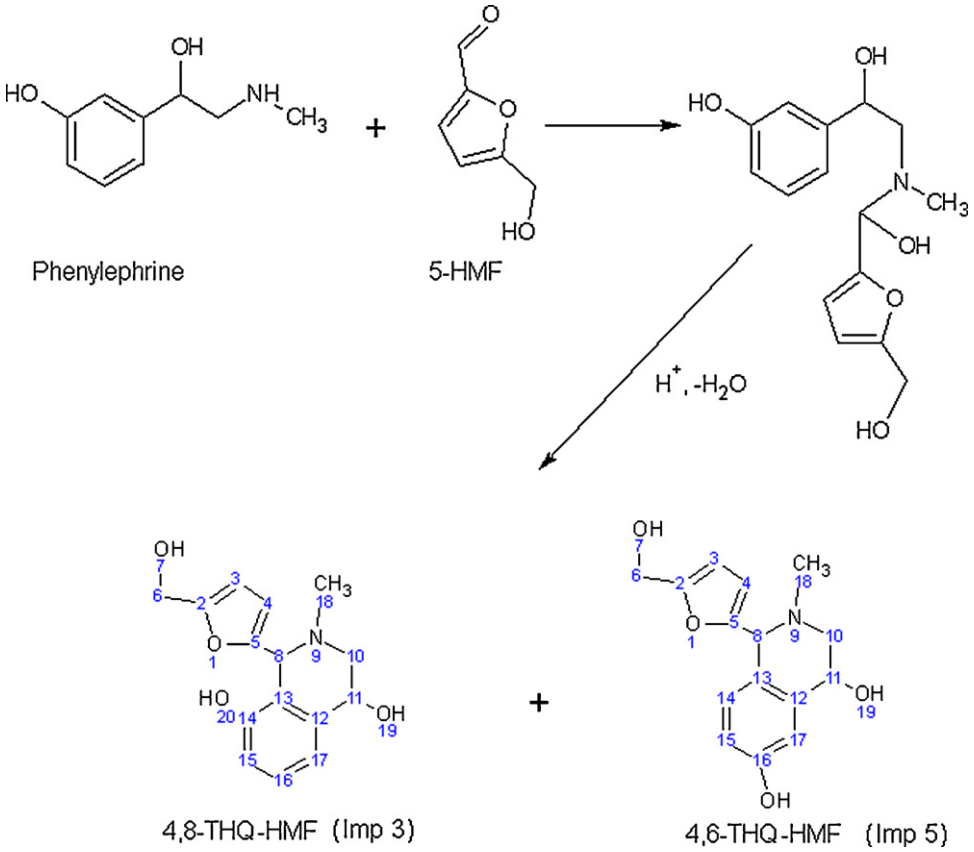

Fig. 3. Scheme for cyclization of PHE with 5-HMF to 4,8-THQ-HMF (Imp 3) and 4,6-THQ-HMF (Imp 5).

5/2 is coeluting as a single peak of Imp 5 under ion-pair isocratic chromatography conditions.

Consequently, the protonated molecular ions $[M+H]^+$ of Imp 3, Imp 5/1 and Imp 5/2 using LC–MS study were determined at $m/z = 276.1230$ Da. The molecular ion peak of m/z 276.1230 fragmented in MS2 experiment into ions of m/z 160.0755, 199.0751, 240.1017 and 258.1123 which is illustrated in Fig. 5. All detected ions were assigned a structure as demonstrated in Fig. 6. No significant difference was observed in intensity of fragmentation of studied impurities. The structure of ions of m/z 135.0438 and

Table 3B
Stress tests for identification of Imp 3 an Imp 5 (the experiments (E11–E14)).

Identified impurities (RRT)	Normalization relative to PHE (%)			
	E11	E12	E13	E14
Imp 1 (0.62)	1.51	3.27	0.04	0.04
Imp 2 (0.67)	<0.01	<0.01	<0.01	<0.01
Imp 3 (0.74)	<0.01	<0.01	<0.01	3.71
Imp 4 (0.77)	0.02	0.42	<0.01	6.98
Imp 5 (0.82)	<0.01	0.89	1.85	89.10

E11: 2160 mg glucose/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E12: 2160 mg fructose/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E13: 7.5 mg 5-HMF/536 mg tartaric acid/536 mg citric acid/100 mg water/12 mg PHE; E14: 7.5 mg 5-HMF/1070 mg sodium citrate/100 mg water/12 mg PHE.

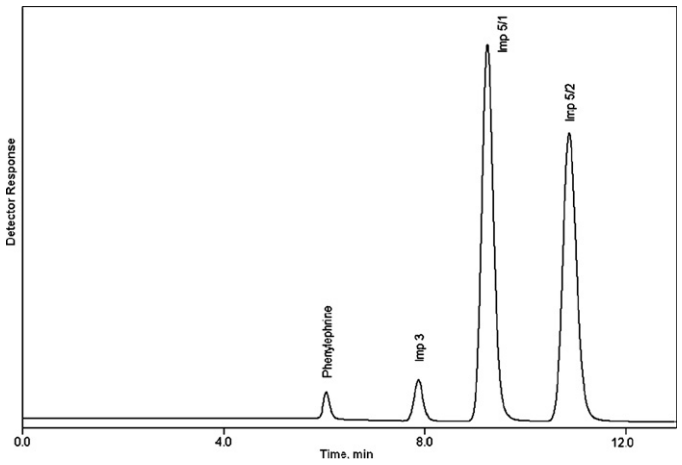

Fig. 4. The chromatographic separation of Imp 3 and Imp 5 originated by cyclization of PHE and 5-HMF on an Obelisc R 250 mm × 4.6 mm, 5 μm (Sielc, USA) using the mobile phase consisting of aqueous solutions of 10 mM ammonium formate adjusted with formic acid to pH 4.1: acetonitrile (95:5, v/v). Flow rate 1.0 ml/min, separation temperature 20 °C, injected volume 5 μl. Detection: fluorimetric with excitation at 275 nm and emission at 310 nm.

176.0704 was not studied due to their very low relatively intensity. The elementary structure $C_{15}H_{18}NO_4^+$ was generated by Elemental Composition Calculator and is in agreement with observed masses and structures of THQs-HMF.

The NMR studies were used for confirmation of the proposed structures of impurities. Imp 3 and Imp 5 were prepared by reaction of 5-HMF with PHE (experiment E15): 75 mg of 5-HMF and 120 mg of PHE was diluted in 10 ml water, 100 μl of 24% ammonia solution was added and the mixture was heated in closed glass vial at 60 °C for 12 h. After reaction, the sample was diluted in the mobile phase to obtain analytical concentration and then analyzed by HPLC. The reaction provides the sum of Imp 3 and Imp 5 in high yield (>97%). The semipreparative HPLC method was developed to obtain of pure impurities for NMR studies of Imp 3 and Imp 5. The sample obtained by experiment E15 was injected into preparative column Obelisc R and the fractions of Imp 3, Imp 5/1 and Imp 5/2 were repeatedly collected and combined. The combined fractions were evaporated under vacuum at 45 °C to dryness to obtain pure solid impurities for structural identification by NMR study.

The NMR data for the investigated impurities are listed in Table 4. The assignment of NMR signals was performed for all three impurities and the given structures were unambiguously confirmed.

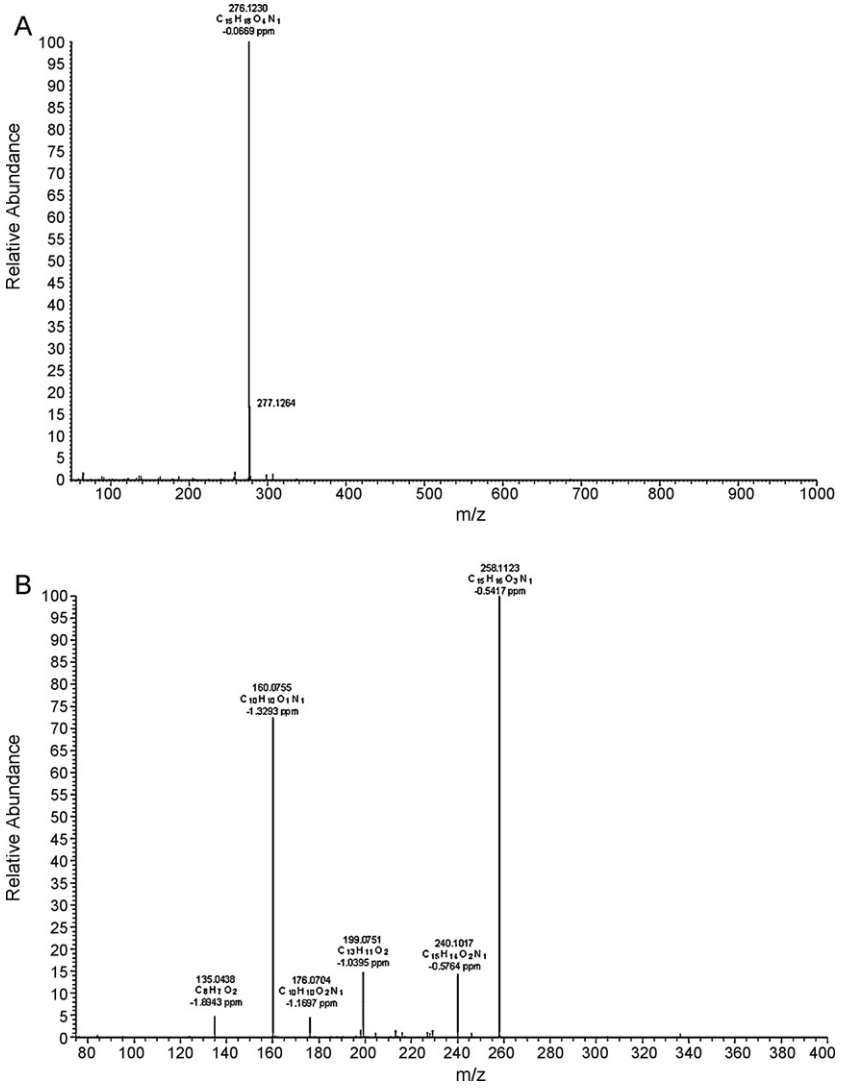

Fig. 5. Positive ion mode APCI full scan mass spectra of Imp 3 and Imp 5. (A) Mass spectrum of $m/z = 276$. (B) MS^2 spectrum of $m/z = 276$.

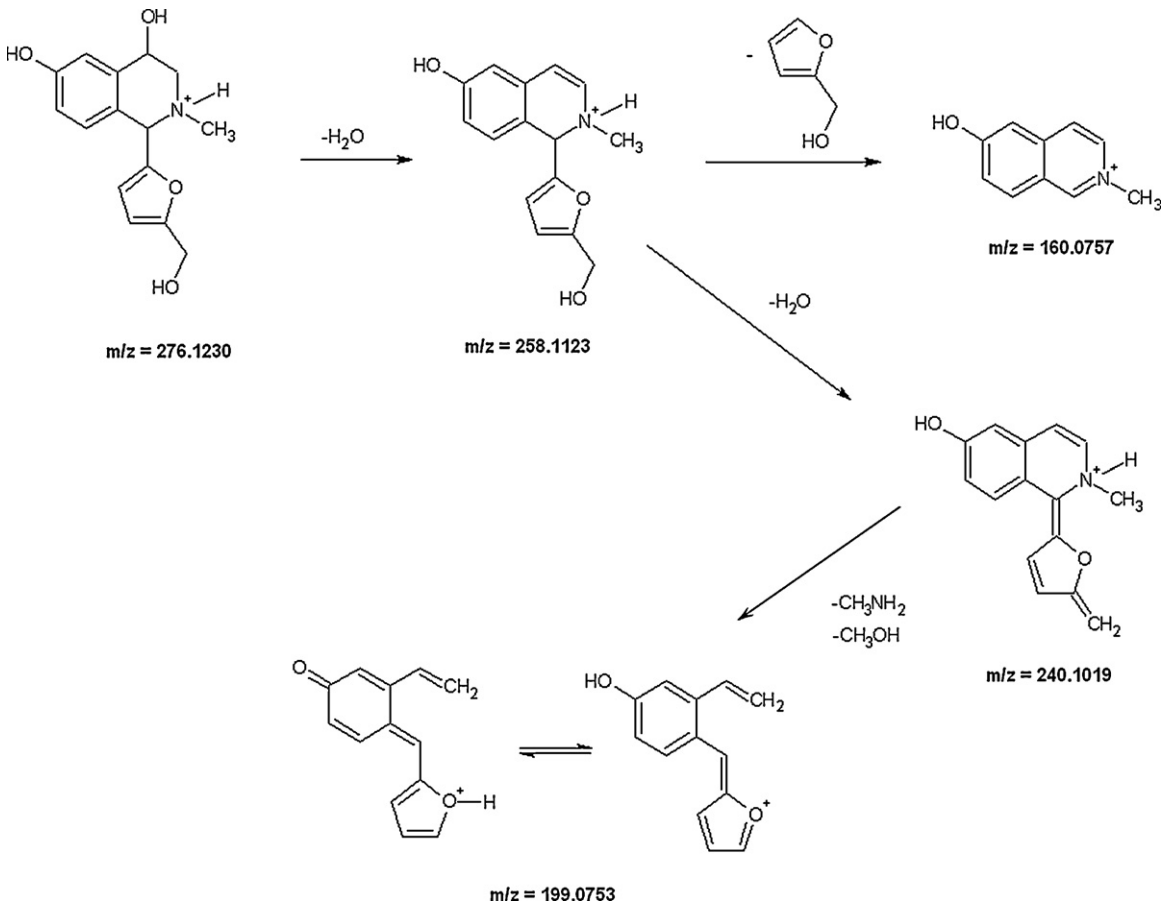

Fig. 6. The suggested MS fragmentation pathway of Imp 3 after multiple stage MS experiments in the positive mode.

In the NMR data of Imp 3, the assignment corresponds to 4,8-THQ-HMF. In the NMR data of Imp 5/1 and Imp 5/2, the assignment corresponds to 4,6-THQ-HMF.

The impurities Imp 5/1 and Imp 5/2 have very similar but not identical NMR spectra. Because of two chiral centers in the piperidine ring they correspond to the two of four possible diastereoisomers. It was not possible to determine the exact geometry of these two isomers only from the values of J_{H-H} interaction constants of protons H10 and H11 and from the NOE contact signals (Fig. 3). Ab initio calculations would be probably needed for this purpose. The NOE measurements excluded the spatial proximity of H8 and H11 protons in both isomers.

Table 4
The assignment of ^{1}H and ^{13}C NMR signals of impurities Imp 3 and Imp 5/1 and Imp 5/2.

No	Imp 3					Imp 5/1					Imp 5/2				
	$\delta_{(C)}$	$\delta_{(H)}$	M	Int	J	$\delta_{(C)}$	$\delta_{(H)}$	M	Int	J	$\delta_{(C)}$	$\delta_{(H)}$	M	Int	J
2	155.4	–				156.3	–				156.3	–			
3	109.0	6.21	d	1	3.2	109.0	6.29	d	1	3.1	109.0	6.28	d	1	3.1
4	112.2	5.90	d	1	3.2	113.1	6.25	d	1	3.1	112.5	6.25	d	1	3.1
5	153.5	–				154.0	–				154.4	–			
6	57.6	4.48	s	2		57.4	4.47	s	2		57.4	4.65	s	2	
8	57.1	5.14	s	1		63.2	4.72	s	1		64.2	4.63	s	1	
10	54.1	2.92	dd	1	11.8	57.7	3.08	dd	1	12.1	59.5	3.28	m	1	
					7.0					6.2					
		2.82	dd	1	11.8		2.92	dd	1	12.1		2.62	dd	1	11.7
					9.8					4.6					7.2
11	66.3	4.88	dd	1	9.8	66.2	4.75	t	1	5.4	66.7	4.84	t	1	5.1
12	140.0				7.0	139.0	–				139.6	–			
13	123.0	–				126.3	–				126.3	–			
14	155.0	–				129.6	6.72	d	1	8.5	129.4	6.67	d	1	8.5
15	114.4	6.64	d	1	7.8	116.5	6.64	dd	1	8.5	116.1	6.61	dd	1	8.5
										2.4					2.4
16	129.2	7.14	t	1	7.8	157.9	–				157.8	–			
17	118.8	7.10	d	1	7.8	115.3	6.96	d	1	2.4	114.5	6.97	d	1	2.4
18	43.1	2.40	s	3		43.5	2.38	s	3		43.8	2.37	s	3	

Note: Values of $\delta_{(C)}$ and $\delta_{(H)}$ in ppm, values of J in Hz, M means multiplicity (s – singlet, d –doublet, dd – doublet of doublets, t – triplet and m – unresolved multiplet), Int means integral intensity of proton signal.

4. Conclusion

The formulations containing both PHE and saccharose were found to be susceptible to loss of PHE activity. There is evidence that the degradation of PHE resulted from condensation reactions of PHE with aldehydes (such as formaldehyde or 5-HMF). HPLC analyses revealed formation of two new degradation products (Imp 3 and Imp 5), all of which were hitherto unknown. The new compounds have been isolated and identified by means of LC–MS and NMR studies. Imp 3 formed under acidic conditions was characterized as 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisochinolin-4,8-diol (4,8-THQ-HMF), whereas Imp 5 was identified as mixture of two possible diastereoisomers of 1-[5-(hydroxymethyl)-2-furyl]-2-methyl-1,2,3,4-tetrahydroisochinolin-4,6-diol (4,6-THQ-HMF). The results indicate that PHE is molecule which should be handled with special care during pharmaceutical processing to avoid decomposition and interactions. The new identified degradation products of PHE were found in pharmaceutical preparations against the common cold containing PHE and saccharose.

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