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Quantitative and structural determination of pseudoephedrine sulfate and its related compounds in pharmaceutical preparations using high-performance liquid chromatography

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Abstract

A simple isocratic reversed-phase high performance liquid chromatograghic method for the separation of pseudoephedrine and its related compounds in pharmaceutical formulations is described. The separation is achieved in less than 35 min on a C-18 column (4.6 mm I.D. × 25 cm length, 5-μm particle size) using a mobile phase consisting of a mixture of ammonium acetate and methanol. The results described in this report demonstrate that the method is sensitive and selective. Structural elucidation of two new pseudoephedrine degradation products is described. On the basis of structural analysis by liquid chromatography-mass spectrometry (LC-MS) and liquid chromatography-nuclear magnetic resonance spectroscopy (LC-NMR), the two newly elucidated degradation products were identified to be 2-(carboxyamino)propiophenone (molecular ion of $m/z = 194$) and 2-formyl-2-(methylamino)-acetophenone (molecular ion of $m/z = 178$).

Keywords: Pseudoephedrine sulfate; 2-(Methylamino)propiophenone; 2-(Carboxyamino)propiophenone; 2-Formyl-2-(methylamino)-acetophenone; High performance liquid chromatography; Nuclear magnetic resonance spectroscopy; Mass spectrometry; Electrospray ionization

1. Introduction

Pseudoephedrine (PSE) has long been known to be a stable compound [1] and a variety of methods have been used to analyze this active ingredient in pharmaceutical products [2–8]. The only known degradation product of pseudoephedrine is 2-(methylamino)propiophenone (MAPP). Two new compounds, 2-(carboxyamino)-propiophenone (CAPP) and 2-formyl-2-(methylamino)-acetophenone (FMAAP) have just been identified from the degradation of pseudoephedrine in a dosage form.

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To the best of our knowledge, these two new compounds, CAPP and FMAAP, have never been reported. These compounds may not have been detected or isolated in previous studies [1] due to the low concentration (submicrogram) of CAPP and the high retention time of FMAAP.

In this paper, the identification of CAPP and FMAAP were achieved by the combined techniques of LC-NMR and LC-MS. The reversed-phase HPLC method described in this paper is sensitive and selective.

2. Experimental

2.1. Materials

Pseudoephedrine sulfate drug substance was from Knoll AG (Ludwigshafen Am Rhein, Germany) and the dosage forms were from Schering–Plough Research Institute (Kenilworth, NJ). 2-(Methylamino)propiophenone (purity > 99%) was from Sigma Scientific (St. Louis, MO). Methanol (HPLC grade) and all other chemicals (analytical grade) were purchased from Fisher Scientific (Fair Lawn, NJ). All solutions were prepared in doubly deionized water and filtered through a 0.45-μm membrane.

2.2. High-performance liquid chromatography (HPLC)

The HPLC analysis system consisted of a Hewlett–Packard (Palo Alto, CA) 1100 series quaternary pump and a diode array detector set at 254 nm. The HPLC analytical column was an Inertsil ODS-3 (G. L. Sciences, Tokyo, Japan), 25 cm × 4.6 mm I.D, 5-μm particle size. The column temperature was set at 40 °C.

Chromatograms were obtained using the data software (ChemStation) supplied with the instrument on a Hewlett–Packard computer or processed using a Perkin–Elmer (Norwalk, CT) software (Turbochrom). The mobile phase containing four volumes of 1% ammonium acetate and one volume of HPLC grade methanol was filtered through a 0.45-μm membrane and degassed before use. The flow rate was set at 1.5 ml/min and sample injections were typically 20 μl.

2.3. LC-mass spectrometry (MS)

LC-MS and LC-MS/MS experiments were performed on a Micromass Quattro LC triple–quadrupole mass spectrometer (Manchester, UK) with a Waters Alliance 2690 LC system (Milford, MA). Similar chromatographic conditions were used as described in the HPLC section except that an Inertsil ODS-3 column of 3-μm particle size (15 cm × 4.6 mm I.D.) was used instead of an Inertsil ODS-3 column of 5-μm particle size (25 cm × 4.6 mm I.D.) For the analysis of CAPP, the column was kept at ambient temperature and the mobile phase was a 9: 1 (v/v) mixture of 1% aqueous ammonium acetate and acetonitrile. The mobile phase for the analysis of FMAAP contained 65% water and 35% acetonitrile. The flow rate was set at 1 ml/min. A 19:1 post-column split fed the larger volume to the UV detector set at 254 nm and the smaller volume into the mass spectrometer. An electrospray interface (ESI) was used with nitrogen as the desolvation gas. The ESI voltage supplied to a stainless-steel needle was 3750 V and cone voltage was set at 30 V. The temperature of the desolvation chamber was set at 350 °C and the source block was set at 100 °C. The first quadrupole mass analyzer was used for the LC-MS experiment. In order to optimize the fragmentation of the selected parent ions for MS/MS experiments, the collision gas (Ar) cell pressure was adjusted to 1.4×10^{-3} torr. The selected parent ions from the first quadrupole mass analyzer was collided with Ar in the second quadrupole with a collision energy of 20 V and the resulting product ions were scanned by the third quadrupole mass analyzer.

2.4. LC-nuclear magnetic resonance (NMR)

LC-NMR was performed on a Varian (Palo Alto, CA) LC-NMR instrument using a Varian model 9012 pump system with a diode array detector and a Varian Inova 500 MHz or UNITYplus 600 MHz spectrometer. A microflow LC-NMR probe with ^{1}H channel and pulsed-field

gradient along the z-axis was used. The cell volume of the NMR probe is approximately 60 μl and the transfer time from the UV cell to the probe was calibrated to be 11 s at a flow rate of 1 ml/min. The chromatographic conditions were the same as described in LC-MS section except that OmniSolv acetonitrile (EM Science, NY), ^2H_2O (99.9%, Cambridge Isotope Laboratory, MA) and ammonium acetate-d_3 (98%, Isotech, Inc., OH) were used in the mobile phases. Proton NMR experiments were performed in 'stop-flow' mode, where the HPLC flow was halted after the sample elution fraction was transferred to the NMR probe. All NMR data were collected at 20 °C. Pulse sequences WET [9,10] and WETTOCSY [10] from the Standard Varian Pulse Sequence Library were used. Double solvent suppression was applied on the proton resonance of water and acetonitrile. One-dimensional (1D) proton NMR spectra were recorded into 32,000 data points with a spectral width of 9500 Hz and acquisition time of 1.7 s. A total of 2000 transients were collected in approximately 1.5 h for each 1D proton spectrum. Two dimensional (2D) proton-proton TOCSY spectra were acquired using 96 scans per increment and 256 hypercomplex data points in t_1 for a total collection time of 21 h. The values for the spectral width in both F1 and F2 dimensions were set to be 11,000 Hz, and the spin-lock time was 30 ms.

2.5. Sample preparation

The concentration of pseudoephedrine sulfate in the reference standard and sample solutions was 0.6 mg/ml in the mobile phase. The tablets were extracted with methanol first and then diluted with the mobile phase. PSE was quantitated against an external reference standard. PSE-related degradation products were generated by stressing the tablet or PSE drug substance. CAPP was formed by heating the tablets in a moisturized chamber at 75 °C for 72 h; FMAAP was formed by exposure of pseudoephedrine to hydrogen peroxide for 24–48 h at ambient temperature. The degradation products were quantitated by their HPLC peak areas relative to that of PSE in the chromatogram of the sample solution.

For LC-MS and LC-NMR analyses, the individual fractions of the degradation products were collected on a FRC-10A fraction collector (Shimadzu, Kyoto, Japan) using an Inertsil ODS-3 column of 10-μm particle size (15 cm × 10 mm I.D.). A spin-dry or freeze-dry system was used for concentrating the collected fractions.

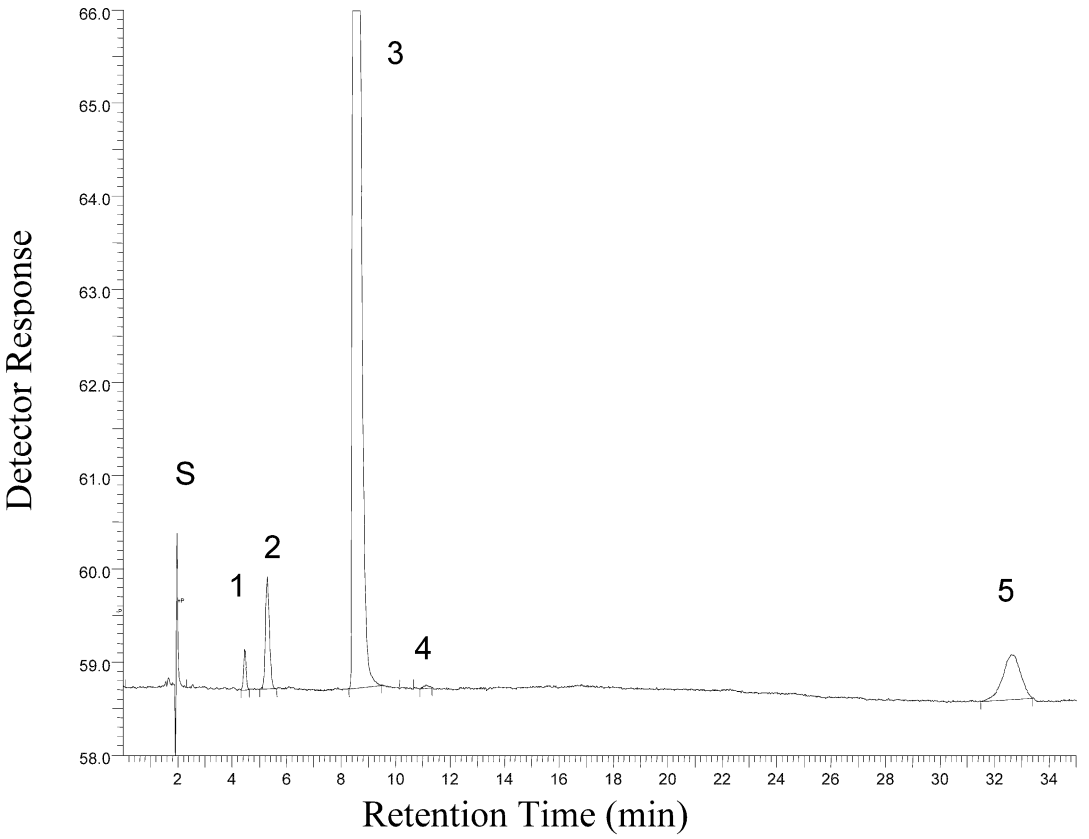
3. Results and discussion

A typical chromatogram of a sample solution of the tablets is presented in Fig. 1 showing separation of PSE from its potential degradation products. PSE is a very polar compound. Reducing peak tailing while maintaining peak resolution is critical for the separation. The Inertsil ODS-3 column gave the most satisfactory results among all columns tested. Optimal conditions for separating PSE from its potential degradation products were obtained with a mobile phase containing an 8:2 (v/v) mixture of 1% aqueous ammonium acetate solution and methanol. Baseline resolution (>2.5) was obtained between the critical pair, CAPP and an excipient peak. Under optimal conditions, the tailing factor was less than 1.6 and the theoretical plate number was greater than 4900 for the PSE peak, the tailing factor was less than 1.3 and the theoretical plate number was greater than 12,000 for the FMAAP peak, respectively.

3.1. Structural elucidation of CAPP and FMAAP

3.1.1. CAPP

The HPLC fraction containing the PSE earlier-eluting degradation product was isolated from the sustained-release layer of heat-stressed tablets. The HPLC-UV chromatogram and the HPLC-ESI-MS total ion chromatogram of the isolated fraction are presented in Fig. 2(a) and (b), respectively. The PSE degradation product eluted at approximately 4.1 min in the chromatogram presented in Fig. 2(a) and is labeled as peak 1 in the chromatogram shown in Fig. 1. Its mass spectrum is presented in Fig. 3. The protonated molecular ions $[M+H]^+$ at m/z 194 observed in the mass spectrum supports the molecular weight assignment of 193 Da.



Peak Identification:

S	Solvent-Related Peaks
1	CAPP
2	Excipient Peak
3	PSE
4	MAPP
5	FMAAP

The molecular structures of 1, 3, 4, 5 are presented in Scheme 4.

Fig. 1. Chromatogram of tablets stored for 2 months at 50 °C.

Structural analysis was further performed by LC-NMR and the proposed molecular structure is shown in Scheme 1.

NMR chemical shift assignments are listed in Table 1. The NMR spectrum is presented in Fig. 4. The aromatic proton pattern is consistent with an aromatic ketone instead of an alcohol at position 1 and the methyl protons at 1.16 ppm (position 3) appear as a doublet, split by its vicinal proton (position 2). This vicinal proton should be a quartet located between 3 and 4 ppm. Due to trace impurities in this elution fraction, a few weak

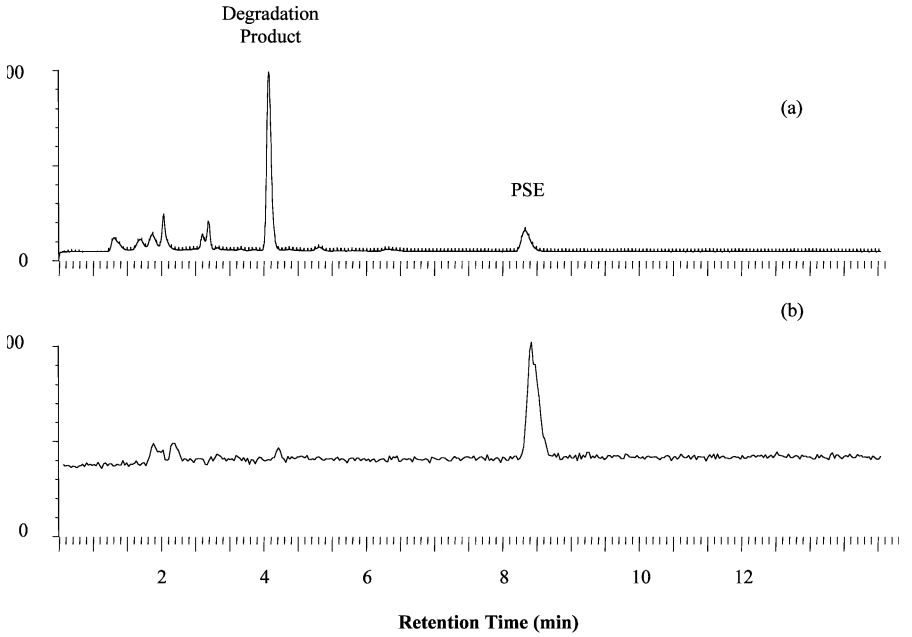


Fig. 2. Chromatograms of the earlier-eluting peak of PSE degradation product (CAPP): (a) UV at 254 nm; (b) total ion chromatogram of the sample solution.

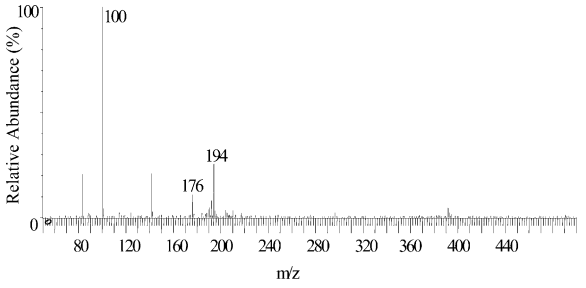
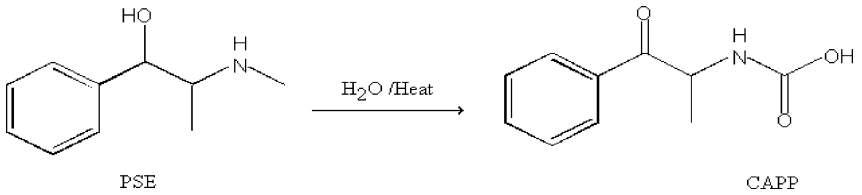


Fig. 3. Mass spectrum of CAPP, the earlier-eluting PSE degradation product labeled 'Degradation Product' in Fig. 2.

multiplets are observed in this region. Therefore, the NMR assignment for the proton at position 2 is ambiguous. Nonetheless, the overall proton structure of the degradation product is quite clear from the NMR spectrum and is consistent with the proposed structure. Its molecular weight of 193 agrees well with the NMR results.

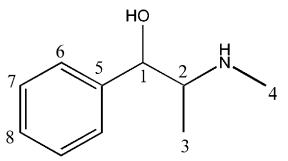
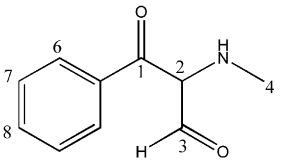
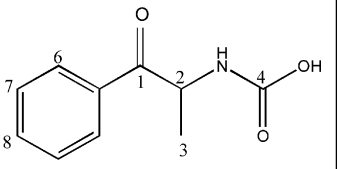
3.1.2. FMAAP

The second degradation product of PSE eluted at a relative retention time of approximately 3.8 and is labeled as peak 5 in Fig. 1. It was first examined by photodiode array detection (Fig. 5(b)). Significant amounts ($>150\%$ based on PSE peak area) of this degradation product were



Scheme 1. Proposed structure of CAPP.

Table 1
The proton NMR chemical shifts for PSE and its oxidative products at 20 °C.

Chemical Structures	PSE 	FMAAP 	CAPP 
Proton positions	δ (ppm) [a]	δ (ppm) [b]	δ (ppm) [c]
1	4.58, d [d], 9.2 Hz	--	--
2	3.43, m [e]	--	--
3	1.12, d, 6.7 Hz	9.92, s [f]	1.16, d, 6.1 Hz
4	2.67, s	3.26, s	--
6	7.39, d, 7.6 Hz	7.92, d, 7.4 Hz	7.89, d, 8.1 Hz
7	7.36, t [g]	7.59, t	7.49, t
8	7.36, t	7.72, t	7.56, t

[a] The proton chemical shifts are referenced at 2.0 ppm for acetonitrile proton resonance. The solvent for SCH 483 is 35% acetonitrile in deuterium oxide. [b] The solvent for FMAAP is 35% acetonitrile in deuterium oxide. [c] The solvent for CAPP is 12% acetonitrile and 0.44% ammonium acetate-d_3 in deuterium oxide. [d]Doublet. [e]Multiplet. [f]Singlet. [g]Triplet.

found when PSE was subjected to oxidative-stress by exposure to hydrogen peroxide (H_2O_2). The spectra of the related compounds of PSE presented in Fig. 5 exhibited of UV maxima shifts to longer wavelengths relative to PSE as a consequence of the extended conjugation afforded by the ketone.

1D NMR data were collected on the chromatographic fractions containing PSE (Fig. 6) and the later-eluting degradation product (Fig. 7). In the NMR spectrum of the degradation product (Fig. 7), the proton resonances arising from trace residual PSE were identified, as they have the same cross-peaks in the 2D TOCSY spectrum as those of PSE. The singlet peak at 9.96 ppm is a strong evidence for an aldehyde proton. In contrast to the clustered phenol protons in the NMR spectrum of PSE, the aromatic protons in the spectrum of the degradation product are more differentiated and suggests the presence of a phenone [11]. The singlet at 3.30 ppm is assigned to the methyl protons at position four. The methine proton at position two is not observed in the degradation product. Possible explanations are its overlap with the water resonance or its

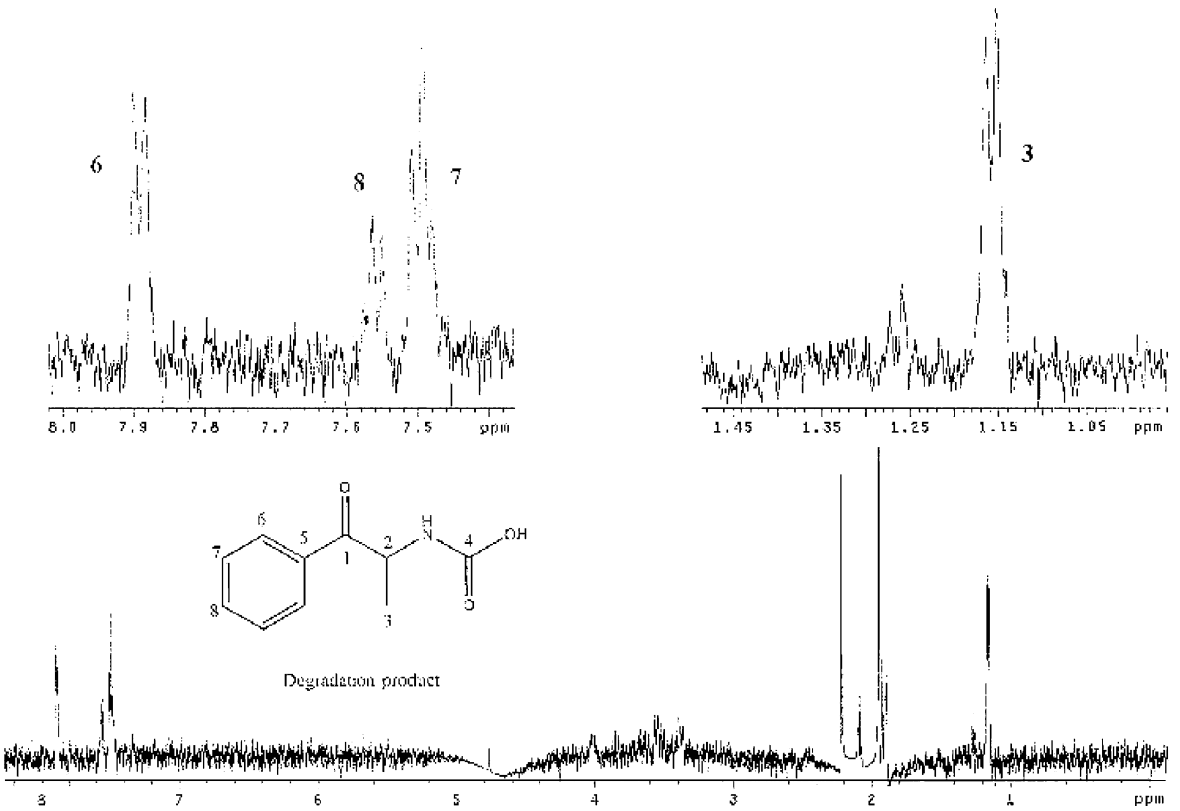


Fig. 4. Proton NMR spectra and chemical shift assignments for CAPP, the earlier-eluting PSE degradation product labeled in Fig. 2. On the top panel, the expansion spectra of the relevant regions are plotted and labeled with the corresponding proton assignments.

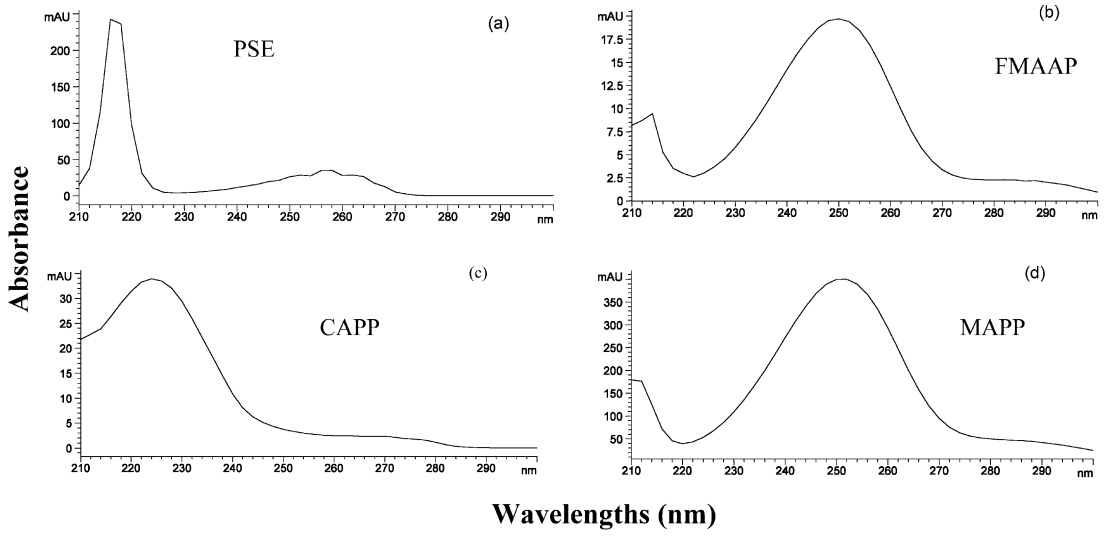


Fig. 5. Photodiode array UV spectra of (a) PSE, (b) FMAAP, (c) CAPP, (d) MAPP.

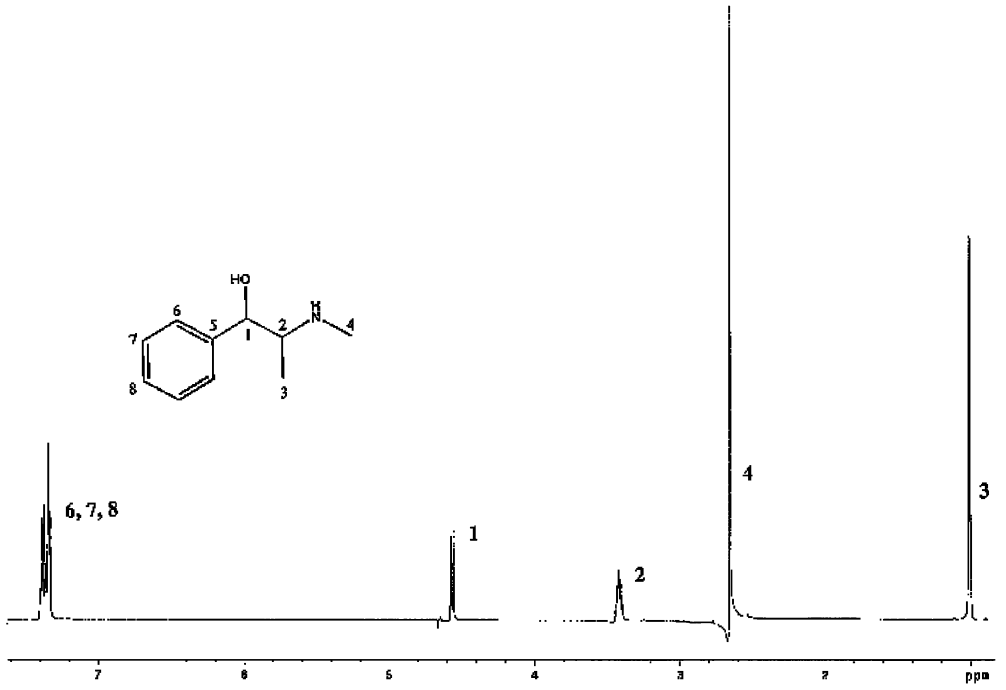


Fig. 6. NMR spectrum of PSE drug substance.

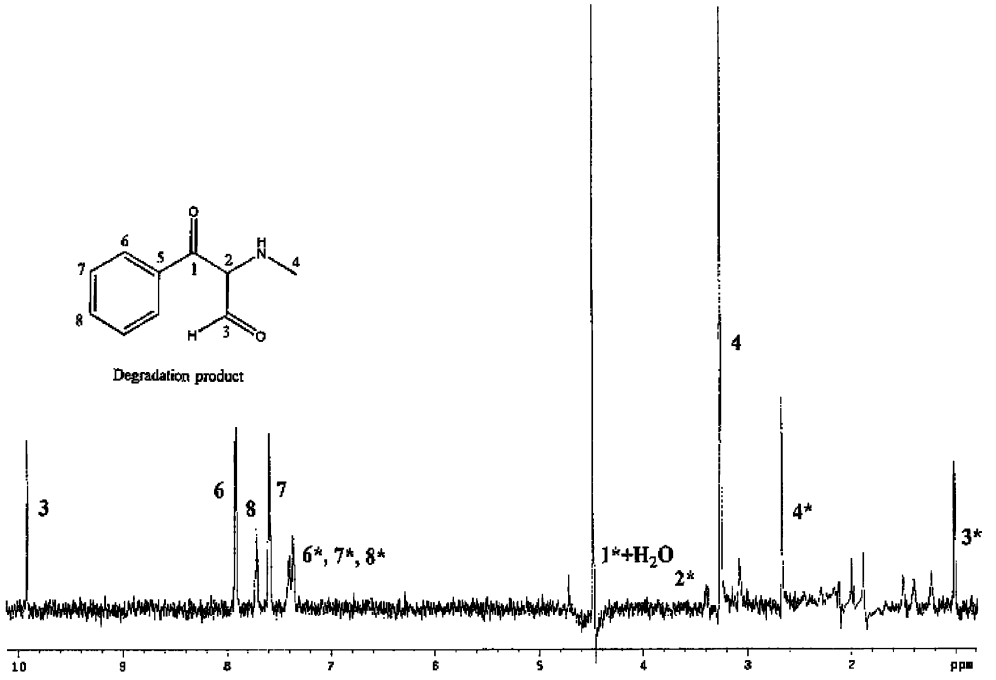
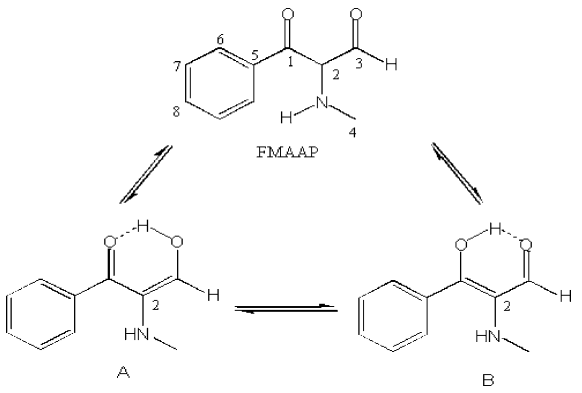


Fig. 7. NMR spectrum of FMAAP, the later-eluting degradation product of PSE, '*' indicates the protons assigned to PSE.



Scheme 2. Proposed intra-molecular hydrogen bonding and proposed proton–deuterium exchange in FMAAP.

involvement in the following equilibrium with A and B in solution (Scheme 2): the formation of hydrogen bond on A or B results in a six-membered conjugated ring and exchange of the proton at position 2 with deuterium of the NMR solvent.

The LC-MS total ion chromatogram and LC-UV chromatogram of the later-eluting fraction are presented in Fig. 8(a) and (b), respectively. The peak eluting with a retention time of 8.9 min corresponds to the oxidative degradation product, and its selected ion chromatogram ($m/z = 178$) is shown in Fig. 8(c). The corresponding mass spectrum for this peak is displayed in Fig. 9. The protonated molecular ion $[M+H]^+$ at m/z 178 observed in this mass spectrum supports the molecular weight of 177 Da. This is further confirmed by the appearance of the acetonitrile adduct ion at m/z 219 $[M+CH_3CN+H]^+$.

In order to obtain structural information for this product, HPLC-ESI-MS/MS experiments were conducted on the protonated molecular ion (m/z 178). The fragmentation pattern observed from the MS/MS spectrum (Fig. 10) is rationalized in Scheme 3 and is consistent with the proposed structure of FMAAP.

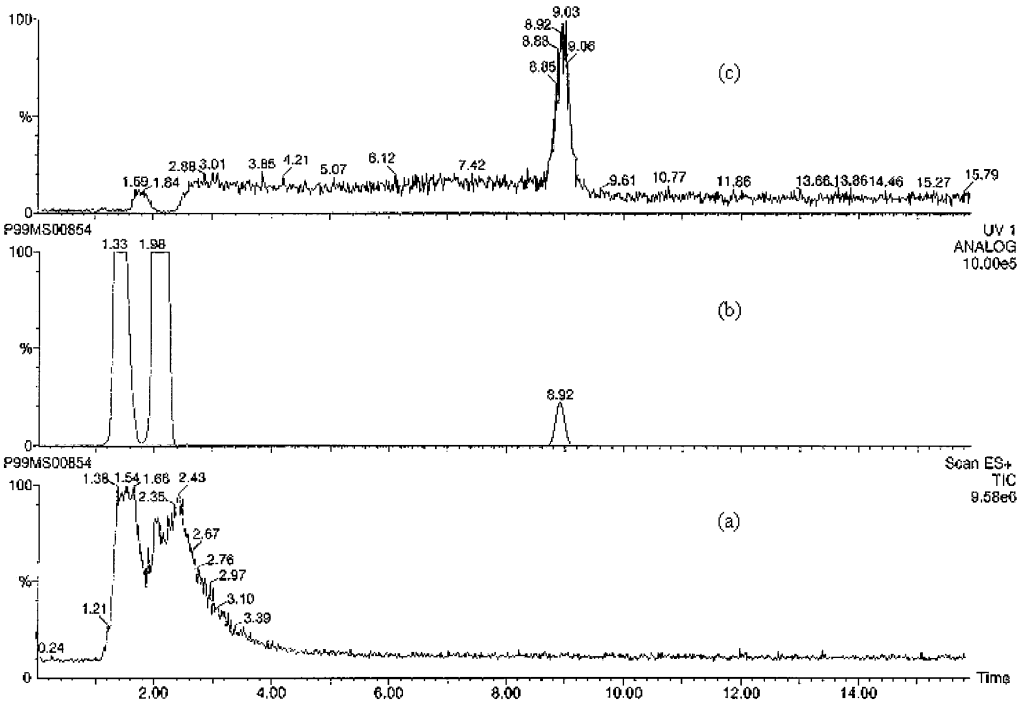


Fig. 8. Chromatograms of FMAAP, the later-eluting degradation product of PSE: (a) total ion chromatogram; (b) UV at 254 nm; (c) selected ionchromatogram of FMAAP.

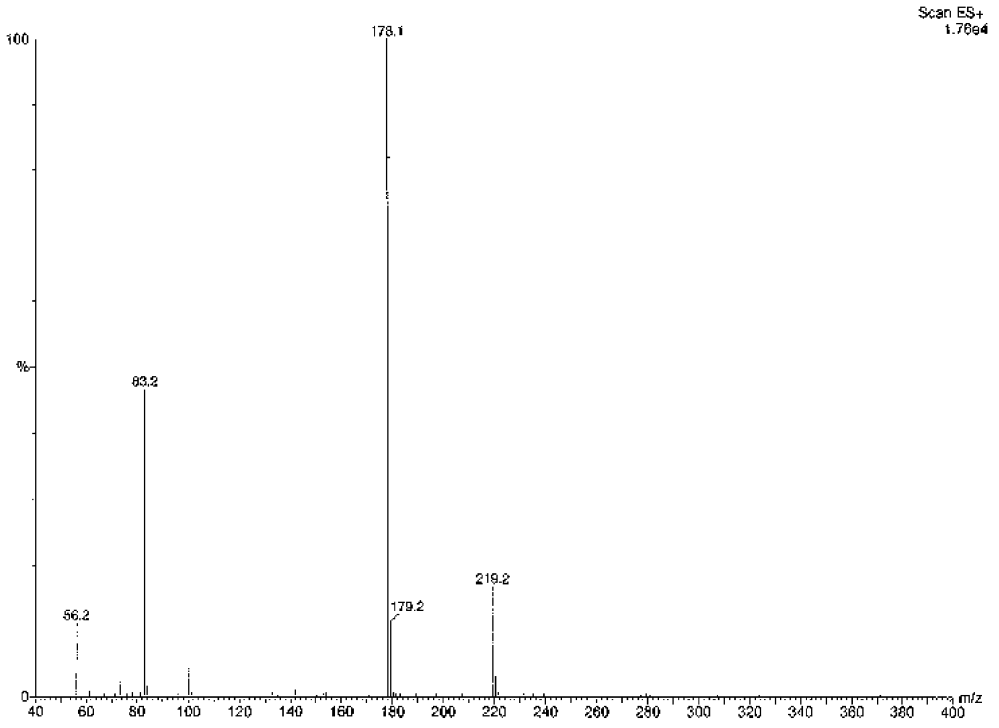


Fig. 9. Mass spectrum of the selected peak (FMAAP) shown in Fig. 8(c).

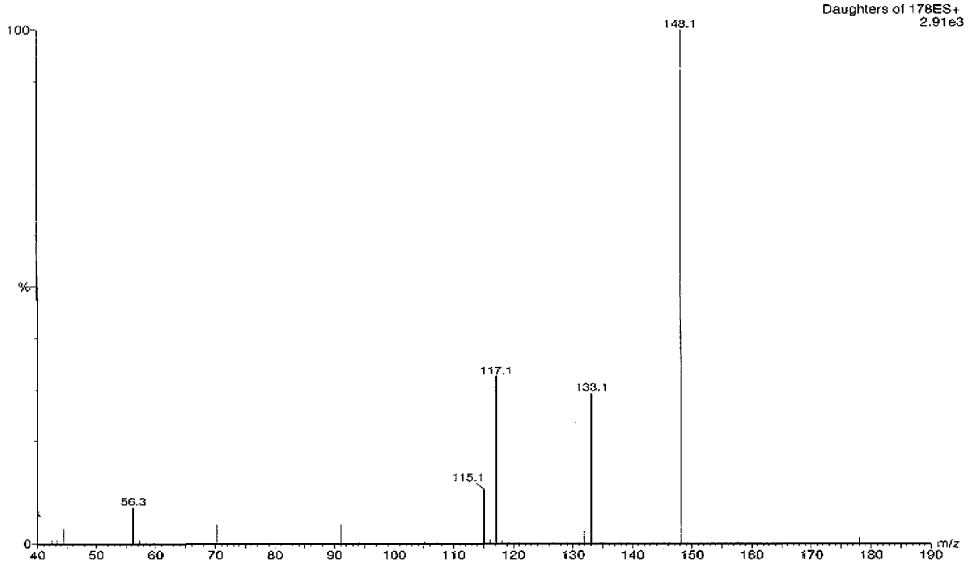
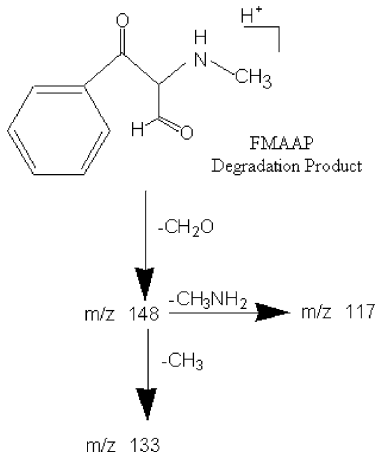


Fig. 10. MS/MS spectrum of the product ion at $m/z = 178$ (see Fig. 9).



Scheme 3. Interpretation of LCMS/MS fragmentation pattern.

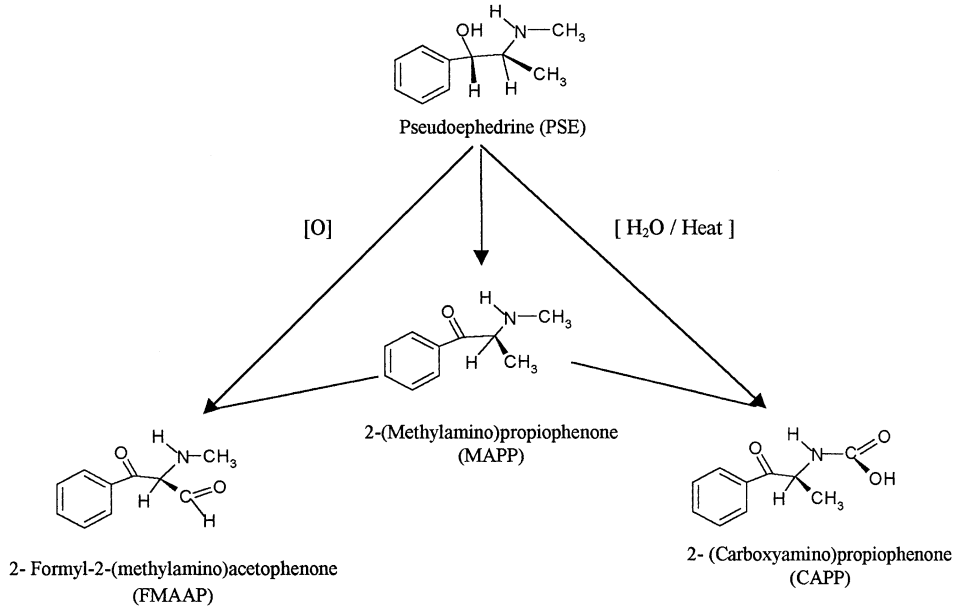
3.2. Relative response factor of degradation products

The relative response factor is defined here as the response factor of PSE divided by the response factor of the degradation product and is approximately 0.01 for MAPP. The relative response factor (RRF) of the degradation products of PSE was determined and then confirmed by several experiments. The molecular structures of PSE and its known degradation products, CAPP, MAPP and FMAAP, are shown in Scheme 4. Highly pure (> 99%) 2-(methylamino)propiophenone (MAPP), a known degradation product of PSE, is commercially available and was used for these experiments. The UV response of MAPP, determined by LC-UV, is approximately 90 times that of PSE.

Authentic materials of CAPP and FMAAP are not available. However, because of their structural similarity to MAPP, the UV response of each of these compounds is expected to be similar to that of MAPP. Their RRFs were further supported experimentally as described below. The following equation derived from the Lambert–Beer Law establishes the relationship between absorbance, A and concentration of the solute, c:

$$A = \varepsilon bc$$

where b is the path length through the sample and the extinction coefficient, ε is proportional to the ratio of A and c.



Scheme 4. Proposed degradation pathway of PSE.

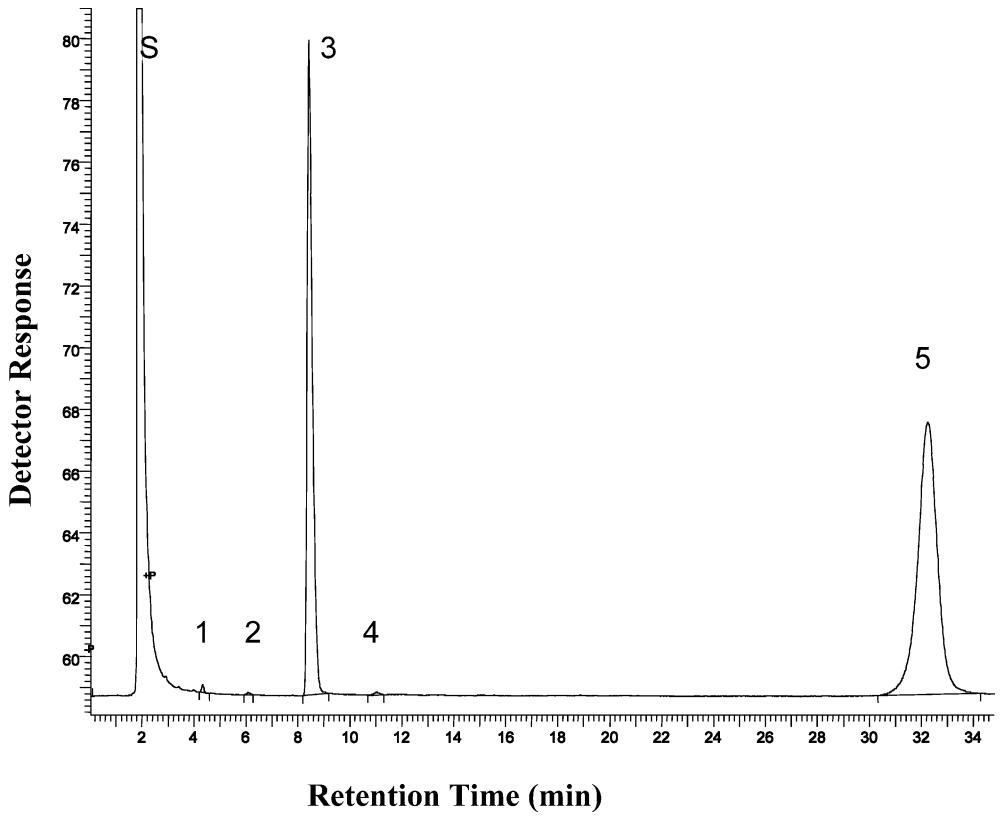


Fig. 11. Chromatogram of PSE drug substance subjected to oxidative condition (6% hydrogen peroxide (v/v) for 40 h). For CAPP, MAPP and unknown peak: 0.3–0.6% of HPLC peak area corresponds to 0.02% w/w after response factor correction. For FMAAP, 155% of HPLC peak area corresponds to 3.09% w/w after response factor correction. See text for details.

$$\varepsilon = \frac{A}{bc}$$

The relative concentrations of PSE and CAPP or FMAAP can be approximated by their total ion counts from mass spectrometry. Thus, RRF was calculated by the following equation:

$$\mathrm{RRF} = \frac{\varepsilon_{\mathrm{PSE}}}{\varepsilon_{\mathrm{deg}}} = \frac{A_{\mathrm{PSE}} C_{\mathrm{deg}}}{A_{\mathrm{deg}} C_{\mathrm{PSE}}}$$

where A is the UV absorbance of PSE or degradation product and C is the total ion counts of PSE or degradation product from mass spectrometry.

From the above equation, the RRFs of CAPP and FMAAP were estimated to be 0.02 and 0.002, respectively. However, based on the similarities of their structures with that of MAPP, the RRF of all three degradation products was set at 0.02, which is the most conservative of the three RRFs, 0.01, 0.02 and 0.002, obtained for MAPP, CAPP and FMAAP, respectively.

To confirm the accuracy of the RRF assignment, a sample of pseudoephedrine sulfate drug substance was exposed to 6% hydrogen peroxide. The chromatogram in Fig. 11 shows that FMAAP is the most significant degradation product. The peak area of FMAAP in the resulting chromatogram is 155% of the PSE peak. The peak areas of CAPP and MAPP in the same chromatogram were 0.6 and 0.5%, respectively. These peak areas correspond to 3.09% for FMAAP and less than 0.02% for CAPP and MAPP when the response factor correction of 0.02 was applied to each. The PSE assay was 96.65% and the total recovery (PSE + FMAAP) was 99.74%, respectively. The results showed that using a relative response factor of 0.02 for MAPP, CAPP and FMAAP achieves mass balance and is appropriate.

Stability studies of the PSE drug product under high temperature and high humidity storage conditions reveal that while MAPP decreases with time, CAPP and FMAAP increase (Fig. 1). Scheme 4 is the proposed degradation pathway of PSE. The primary degradation pathway of PSE is thus proposed to be primarily oxidative.

4. Conclusion

A simple isocratic reversed-phase HPLC method has been developed that provides excellent separation of PSE from its potential degradation products. Structural elucidation of two previously unknown degradation products of pseudoephedrine has been achieved.

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