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The use of HPLC/MS, GC/MS, NMR, UV and IR to identify a degradation product of eperisone hydrochloride in the tablets

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Abstract

Understanding drug degradation in the pharmaceutical dosage forms is critical as it has significant impacts on drug efficacy, safety profile and storage conditions. In this study, analytical techniques, such as high-performance liquid chromatograph mass spectrometry (HPLC/MS), gas chromatograph mass spectrometry (GC/MS) and nuclear magnetic resonance (NMR) had been applied to the identification of a drug degradation product which grew over time in the stability study of eperisone hydrochloride tablets. The target unknown degradation product is ionizable by atmospheric pressure chemical ionization (APCI) in a positive mode to determine its relative molecular weight. GC/MS with electron impact ionization (EI) was employed to determine the fragmentation pattern of this unknown compound. Structure elucidation of eperisone and its unknown degradation product by spectral data had been discussed in detail. The isolated unknown was analyzed by NMR, UV and IR, from which the structure of the degradation product was further confirmed as 1-(4-ethylphenyl)-2-methyl-2-propen-1-one.

Keywords: Eperisone hydrochloride; 1-(4-Ethylphenyl)-2-methyl-2-propen-1-one; Drug degradation; HPLC/MS; GC/MS; NMR; UV; IR

1. Introduction

Eperisone hydrochloride (Fig. 1A), 4′-ethyl-2-methyl-3-piperidinopropiophenone (EMPP) hydrochloride is an antispastic agent, a centrally acting muscle relaxant [1–13]. It has a relatively low incidence of central depression when compared with other anti-spasmodic drugs, which makes it widely used for the therapeutic treatment of spastic patients to relieve skeletal muscle stiffness and back pain [4]. Several HPLC [7], HPLC/MS [8–11] and GC/MS [12,13] methods for the determination of EMPP in the pharmaceutical dosage forms and the human plasma samples have been reported. The degradation profile of EMPP has not been reported. Recently, an unknown degradation product (the unknown) has been observed in the EMPP tablets, and it grows over time during the storage period of the tablets under the conditions of ambient temperature. To identify the unknown, a forced degradation study of the EMPP in the tablets was carried out. The test results showed that the unknown could be obtained in the forced degradation when the EMPP tablets were heated or dissolved in sodium bicarbonate solution. The focus in this paper was to identify the structure of the unknown in the Myonal tablet (the commercial tablet formulation of eperisone hydrochloride). Hyphenated analytical techniques such as HPLC/MS with APCI, GC/MS with EI had been employed to characterize the unknown. After isolation and enrichment, the molecular structure of the unknown was further confirmed by NMR, UV and IR.

2. Experimental

2.1. Materials and reagents

The test drug was eperisone hydrochloride tablet containing 50 mg of eperisone hydrochloride per tablet, which was obtained from Eisai (China) Pharmaceutical Co. Ltd. (Suzhou, China). Methanol was of HPLC grade (Merck KGaA). Acetic acid, ammonium acetate, diethyl ether, hydrochloric acid and sodium bicarbonate were analytical grade purity and purchased from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China).

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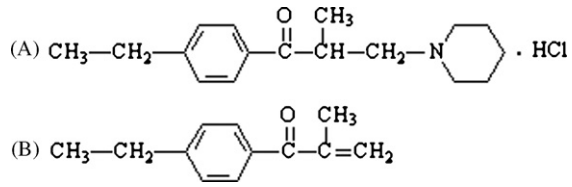

Fig. 1. Chemical structure of eperisone hydrochloride (A) and 1-(4-ethylphenyl)-2-methyl-2-propen-l-one (B).

2.2. High-performance liquid chromatograph/mass spectrometry

2.2.1. High-performance liquid chromatograph

The HPLC measurements were carried out on a chromatographic separation system consisting of an Agilent 1100 G1313A autosampler, Agilent 1100 G1312A binary pump, and Agilent 1100 G1314A VWD detector. The analytical column used was a Hanbon Lichrospher C_{18} column, 5 μm, 250 mm × 4.60 mm i.d. (Jiangsu Hanbon Science & Technology Co. Ltd., China). The mobile phase was 40 mM ammonium acetate buffer solution containing 0.05% acetic acid (pH 5.6)–methanol (30:70, v/v) at a flow rate of 1.0 ml/min. The column temperature was maintained at 25 °C. The peaks of the unknown and EMPP were detected by UV absorbance at 254 nm.

2.2.2. Mass spectrometry

All HPLC/MS mass spectral data were acquired using an Agilent Technologies Series 1100 LC/MSD VL system (Agilent Technologies, Palo Alto, CA). A quadruple mass spectrometer equipped with an APCI source was employed. In order to ionize the unknown compound, the positive (+) APCI mode was investigated. The range of *m/z* acquired was from 50 to 400. The mass conditions were set as capillary temperature 350 °C, capillary voltage 4 kV (positive mode), drying gas (N_2) flow of 4.0 l/min, nebulizer pressure of 30 psi, drying gas temperature of 300 °C and the fragmentor voltage of 70 V.

2.3. GC/MS

GC/MS analysis was performed using a quadruple mass spectrometer (GC-MS-QP2010, Shimadzu) equipped with an EI source. The capillary column used was a J&W DB-5MS, 30 m × 0.25 mm i.d. × 0.25 μm (Agilent Technologies, Wilmington, DE, USA). The carrier gas was high purity helium (99.999%) under a constant column flow rate of 1.0 ml/min. The column temperature was initiated at 180 °C, held for 4 min, raised to 250 °C by 25 °C/min. The temperature of the GC/MS interface and the injector were both 280 °C. The injection was operated in the split mode (50:1), and the MS data acquisition was set at 2.0 min. The scan mode was chosen and the range of *m/z* was from 50 to 400 during GC/MS analyses with an ion source temperature at 220 °C and a detector voltage of 1.1 kV.

2.4. Sample preparation

After removed the sugar coating, an EMPP tablet was dissolved with 35 ml distilled water. Following filtration, the solution was added 15 ml saturated sodium bicarbonate solution and stored at the ambient temperature to obtain sufficient amount of the unknown. Two weeks later, a 2.0-ml aliquot of the solution was extracted with 5.0-ml diethyl ether and the organic phase was separated and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 300 μl methanol, and a 10.0 and 1.0 μl aliquot was injected into HPLC/MS system and GC/MS system, respectively, for analysis.

2.5. Isolation and enrichment

In order to generate sufficient amount of the unknown, ten EMPP tablets were removed the sugar coating and dissolved into 350 ml distilled water. Following filtration, the filtrate was added 150 ml saturated sodium bicarbonate solution. The mixture solution was stored at the ambient temperature for 1 month and extracted with equal volume diethyl ether for twice, and the organic phase was separated and washed with 0.1 M hydrochloric acid to get rid of EMPP. The resulting organic portion was dried to 0.5 ml under a nitrogen stream and then was dried in the vacuum oven at 30 °C. The purity of the final isolated unknown was 99.3 %, as determined by HPLC.

2.6. NMR

The ^1H and ^{13}C NMR spectra were recorded at 303 K on Bruker AC300 spectrometer (300 MHz) equipped with a 5 mm probe, using $CDCl_3$ as solvent and TMS as internal standard.

2.7. UV and IR

The UV spectra of EMPP and the unknown were recorded on Shimadzu UV-2401 PC spectrophotometer using methanol as the solvent, and the range of wavelength acquired was from 200 to 400 nm. The IR spectrum of the unknown was recorded on Nicolet Impact 410 Fourier transform infrared spectrometer using tetrachloromethane as the solvent, and the range of wavenumber acquired was from 400 to 4000 cm^{-1}.

3. Results and discussion

3.1. HPLC/MS spectrometry

Fig. 2A shows HPLC and HPLC/MS chromatograms of a sample of the EMPP tablet. EMPP was eluted at 5.4 min while the unknown was eluted at 16.6 min. Protonated molecular ion was observed for the unknown peak using APCI (+) mode. Fig. 2B shows the typical full-scan APCI mass spectra of EMPP and the unknown. The HPLC/MS results showed that the protonated molecular ion of EMPP and the unknown was at *m/z* 260.4 and 175.3, respectively. The relative molecular weight of the unknown was determined as 174 Da.

3.2. GC/MS spectrometry

Because EMPP drug substance could be detected by GC/MS [12,13], the unknown might be amenable to this technique as

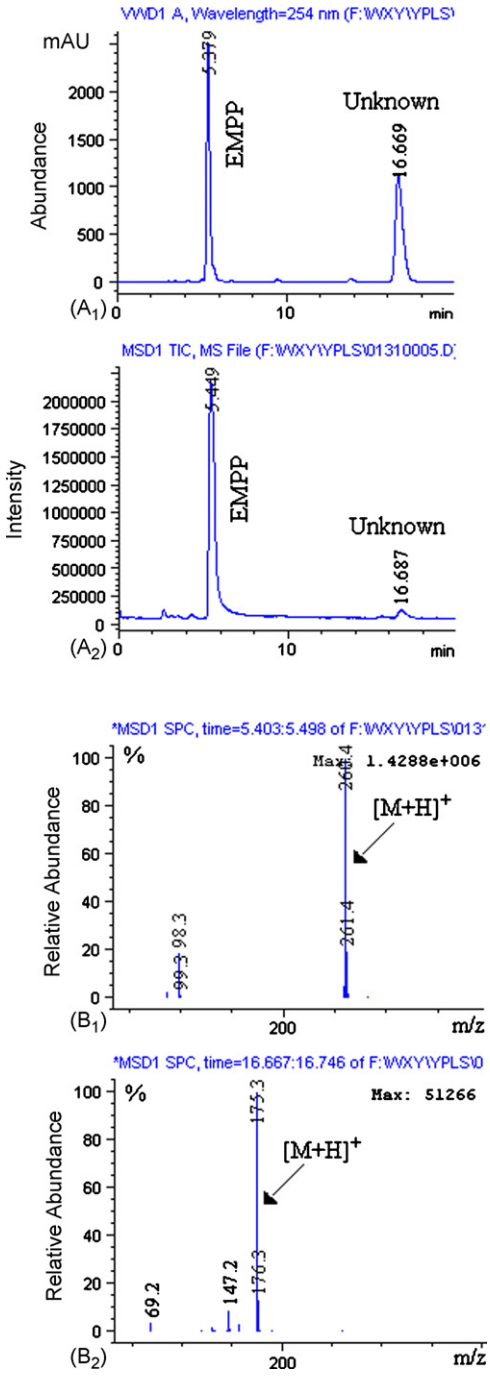

Fig. 2. HPLC (A_1), HPLC/MS (A_2) chromatograms of eperisone hydrochloride tablet sample and the mass spectra of the positive ions of EMPP (B_1), the unknown (B_2) at 70 V fragmentor voltage.

it was a degradation compound of the drug substance. EMPP and the unknown were analyzed by EI source installed in the mass spectrometer, and they could be unambiguously be identified, respectively, in the EI-total ion chromatogram (Fig. 3) based on their relative molecular weight. The peak of EMPP was eluted at 7.4 min and the peak eluted at 2.4 min was presumed to be the unknown. The EI-mass spectrum of EMPP was shown in Fig. 4A, while the unknown was shown in Fig. 4B.

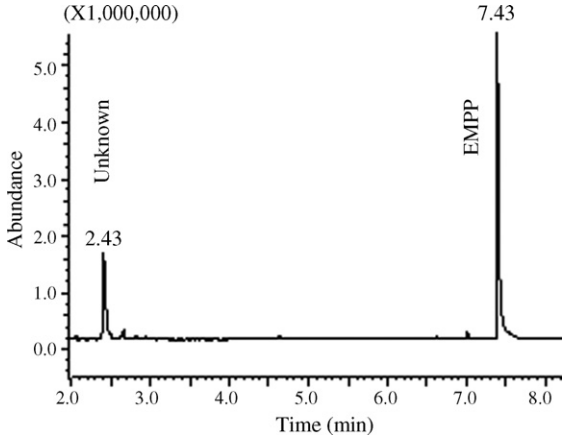

Fig. 3. GC/MS EI-total ion chomatogram of EMPP and the unknown.

In general, the degradation product has the similar structure to its precursor compound, and mimics the fragment pathways of its precursor compound in its mass spectrum. So, the fragment profile of EMPP was studied. The fragment pathways of the main ions of EMPP were shown in Fig. 5. The most significant characteristic ion in the EI-MS spectrum of EMPP was produced by the α cleavage of piperidyl to yield $[M-161]^+$ ion, corresponding to the base peak ion of *m/z* 98. By rupture of the benzyl bond in the branched alkyl side chain and the loss of the methyl, fragmentation ion of *m/z* 244 was produced. The characteristic ion of *m/z* 133 corresponded to the loss of $C_8H_{16}N$ from the carbonyl side of EMPP. Further loss of the CO from the ion at *m/z* 133 led to the ion of *m/z* 105, and the further cleavage of

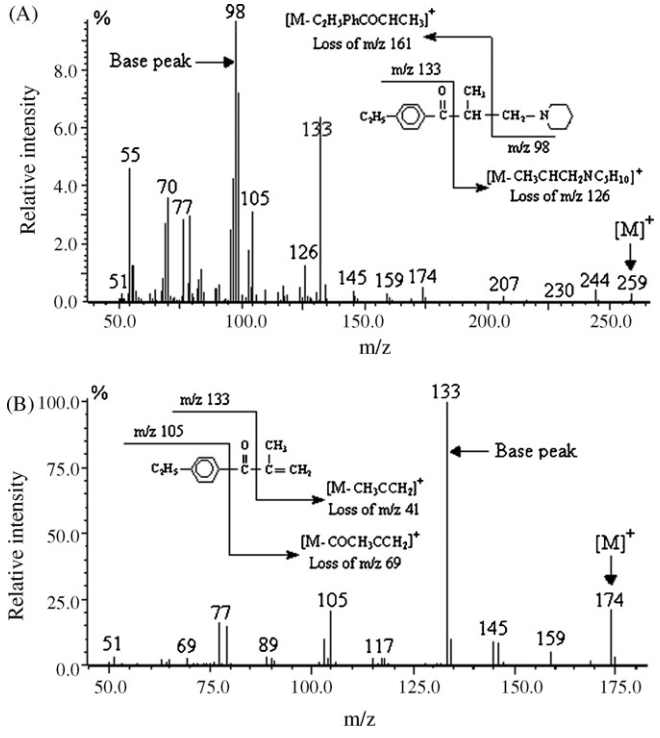

Fig. 4. The EI-mass spectra of EMPP (A) and the unknown (B).

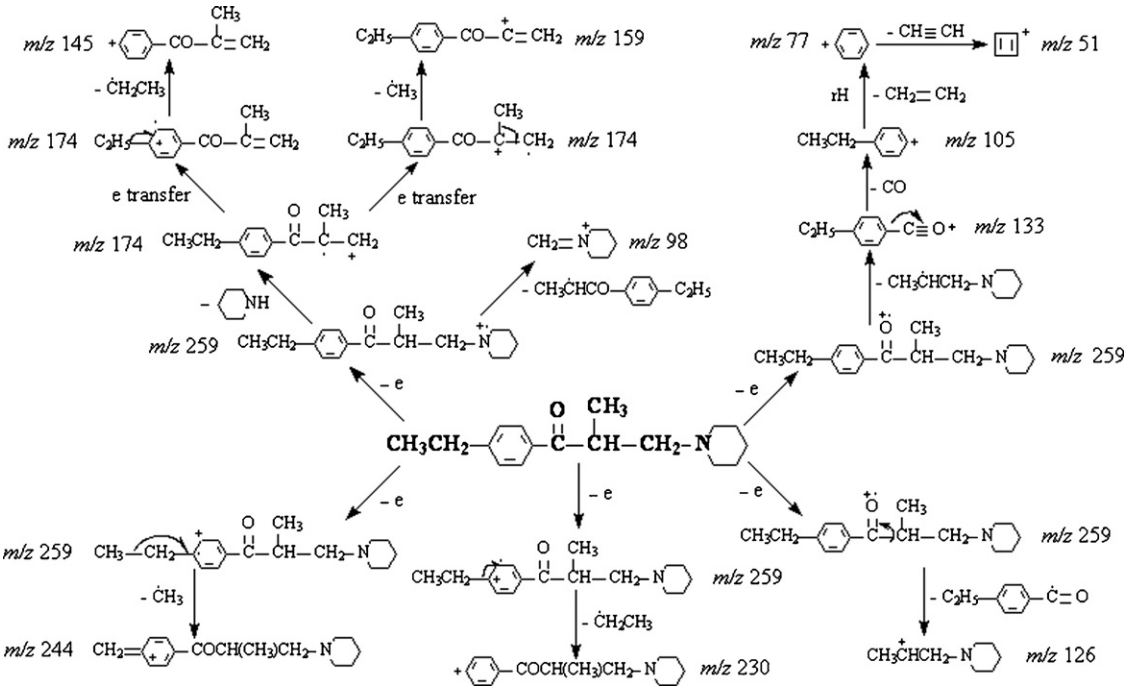

Fig. 5. Fragment pathways of main ions of EMPP.

the ion at *m/z* 105 led to the phenyl ion at *m/z* 77 and ion at *m/z* 51. These secondary decompositions were specific for EMPP producing ion of *m/z* 133, and they were also recognized for the unknown product. Following the loss of the neutral piperidine fragment, the remaining was rearranged to the ion at *m/z* 174. By the electron transfer and α cleavage, the fragment ion at *m/z* 174 produced two secondary ions at *m/z* 159 and 145 which were also observed in the EI-mass spectrum of the unknown product. Additionally, the low abundances of the fragment ions at *m/z* 126 ($[M - C_2H_5PhCO]^+$) and *m/z* 230 ($[M - C_2H_5]^+$) corresponded to i cleavage of carbonyl side and α cleavage of phenyl side, respectively.

Compared with the EI-mass spectrum of EMPP, many similar fragment ions were observed in the mass spectrum of the unknown, such as ions of *m/z* 159, 145, 133, 105, 77, 51. All of these ions could be produced from the parent ion of *m/z* 174 shown in both Fig. 5A and B, and the results of HPLC/MS also revealed that the relative molecular weight of the unknown was 174 Da. The above information suggests that the unknown might correspond to the loss of piperidine from EMPP and its structure was 1-(4-ethylphenyl)-2-methyl-2-propen-1-one shown in Fig. 1B. The fragment pathways of the unknown are shown in Fig. 6. The proposed structure of the unknown is consistent with the previous observation that it gives a base peak of $[M + H]^+$ at *m/z* 175.3 in the HPLC/MS in positive ionization mode.

3.3. NMR

In order to confirm the identification of the unknown, ^{1}H and ^{13}C NMR spectra of the unknown were recorded.

The ^{1}H NMR spectra (Fig. 7A) could be divided into five main different domains.

- Three methyl protons in α position of a methylene group (triplet, 3 H, 1.26 ppm),
- three methyl protons in α position of a vinyl group (multiplet, 3 H, 2.06 ppm),
- methylene protons (quartet, 2 H, 2.71 ppm),
- vinyl prontons, respectively (multiplet, 1 H, 5.59 ppm; multiplet, 1 H, 5.85 ppm),
- phenyl prontons (doublet, 2 H, 7.25 ppm; doublet, 2 H, 7.68 ppm).

The ^{13}C NMR method was complementary to the ^{1}H NMR, and the ^{13}C NMR spectra (Fig. 7B) could be divided into six main different domains.

- Methyl carbon in α position of a methylene group (15.1 ppm),
- methyl carbon in α position of a vinyl group (18.8 ppm),
- methylene carbon in α position of a phenyl group (28.9 ppm),
- phenyl carbons (127.6, 129.7, 135.2, 148.9 ppm),
- vinyl carbons (125.7, 144.0 ppm),
- carbonyl carbon (198.0 ppm).

The ^{1}H NMR and ^{13}C NMR spectra of the unknown match the compound of 1-(4-ethylphenyl)-2-methyl-2-propen-1-one.

3.4. UV and IR spectra

The UV spectra of EMPP and the unknown are shown in Fig. 8. Compared to EMPP (see Fig. 8A), the wavelength at maximum absorbance of the unknown was shifted to a longer wavelength (from 255 to 259 nm). This "red shift" indicates that the molecular structure of the unknown has a higher conjugation than EMPP.

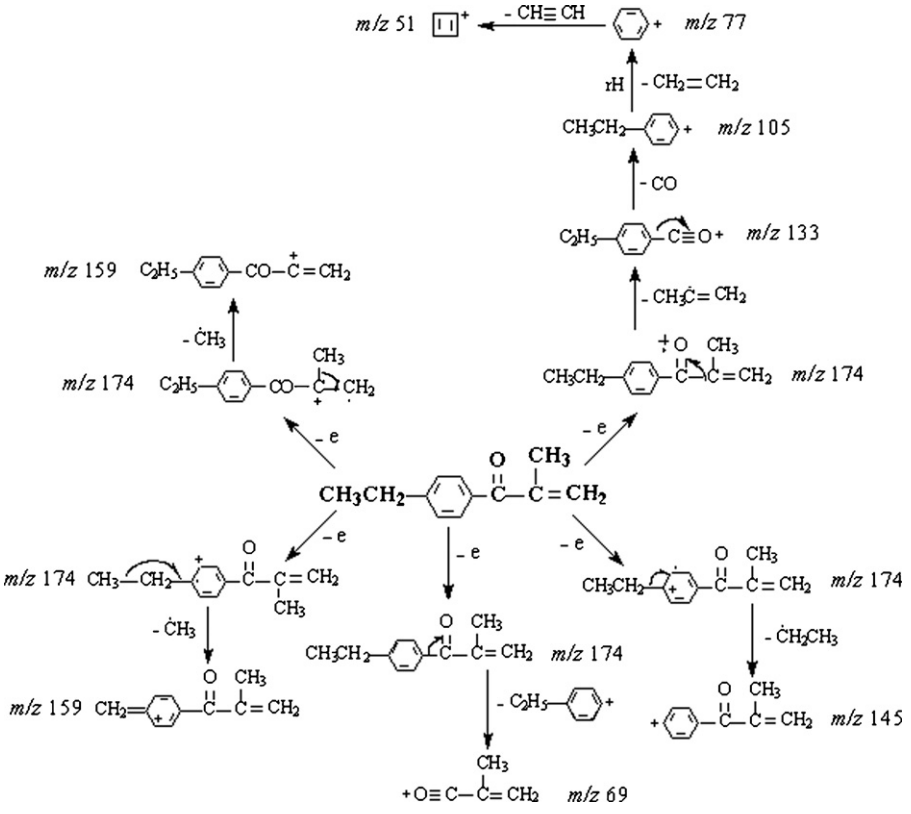

Fig. 6. Fragment pathways of main ions of the unknown.

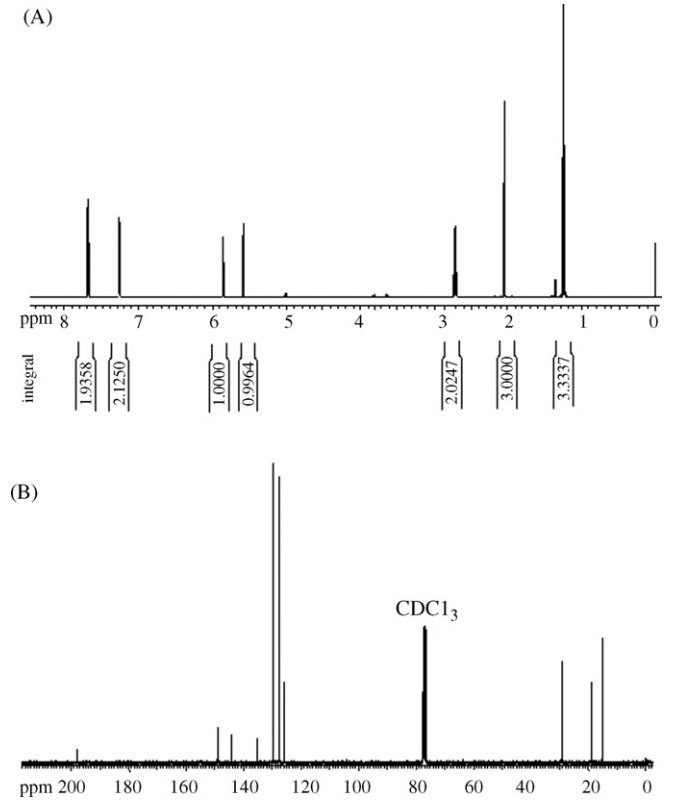

Fig. 7. ^{1}H NMR spectrum (A) and ^{13}C NMR spectrum (B) of the isolated unknown.

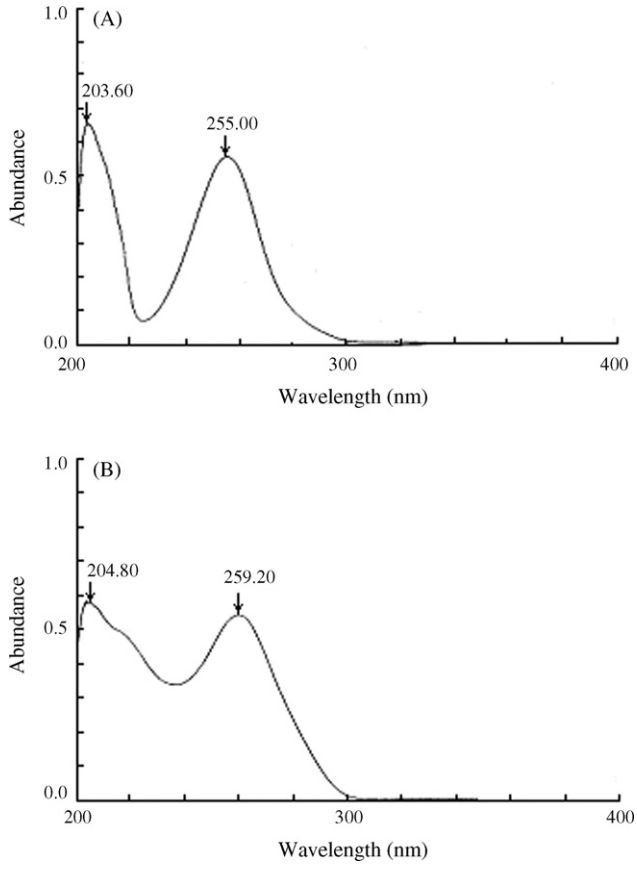

Fig. 8. UV spectra of EMPP (A) and the isolated unknown (B).

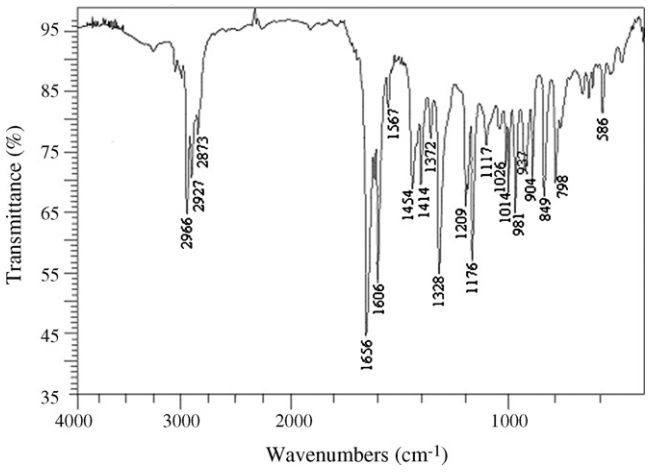

Fig. 9. IR spectrum of the isolated unknown.

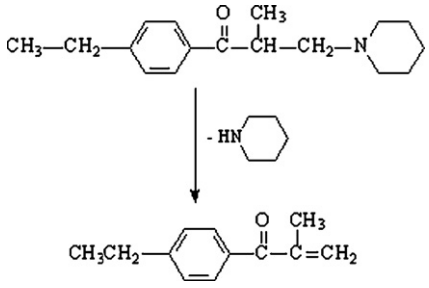

Fig. 10. The proposed degradation pathway of EMPP.

The IR spectrum of the unknown is shown in Fig. 9. The infrared absorption spectrum is the unique characteristic of functional groups. The most important features of infrared spectra of the unknown are the very strong conjugation carbonyl group. The sharp absorption band of the conjugation carbonyl group appears around 1656 (C=O) and 1606 cm^{-1} (C=C). And the absorption bands of 2966/2927/2873 cm^{-1} (ν_{C-H}), 1626/1567/1454 cm^{-1} ($\nu_{Ph-C=C}$) and 849 cm^{-1} (OOP_{Ph-C-H}) suggest the existing of the 1, 4-phenyl structure. All of the above results identify the unknown degradation product is the compound of 1-(4-ethylphenyl)-2-methyl-2-propen-1-one.

4. Conclusions

This study combined various hyphenated techniques to identify an unknown degradation product in the EMPP tablets. HPLC/MS was used to determine the relative molecular weight of this unknown. Using GC/MS, the fragmentation ions were determined under EI-MS. The mass data characterized the detailed structure of this compound, and the unknown degradation product in the EMPP tablets had been further confirmed by the NMR, UV and IR results as the neutral compound of 1-(4-ethylphenyl)- 2-methyl-2-propen-1-one. The degradation pathway for EMPP was proposed as shown in Fig. 10.

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