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Characterization of an unknown component in Noscapine using liquid chromatography–mass spectrometry and proton nuclear magnetic resonance spectroscopy

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Abstract

Analytical and semipreparative LC methods were used to quantitate and isolate an unknown component (Impurity A) found in samples of bulk Noscapine. This component was also examined by LC–ESI-MS and ^{1}H-NMR. It was concluded that the structure of Impurity A only differed from Noscapine in that it possessed a hydroxyl group at position 21 of the isobenzofuranone moiety. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Nuclear magnetic resonance spectroscopy; Noscapine

1. Introduction

Noscapine, 1-α-2-methyl-8-methoxy-6,7-methylenedioxy - 1 - (6,7-dimethoxy-3-phthalidyl)-1,2,3,4-tetrahydroisoquinoline, (see Fig. 1 for structure) is a naturally occurring alkaloid, which may be extracted from the opium poppy (*Papaver somniferum*) [1]. It is a centrally acting cough suppressant and has recently been credited with dramatically decreasing the size of solid lymphoid tumors in mice [2,3].

As with all pharmaceuticals, analytical testing is required to demonstrate product integrity. Currently, the USP and BP manuals specify only titration methods for the assay analysis of Noscapine [4,5]. These methods are not specific for Noscapine, in that process impurities and degradants are not individually separated and quantified. The vast majority of

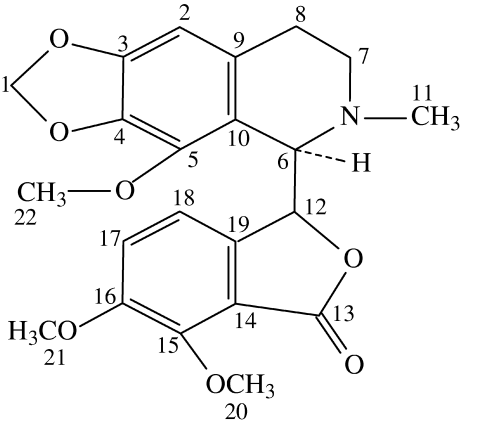

Fig. 1. Structure of Noscapine, with positional assignments indicated numerically.

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pharmaceutical assay analyses overcome these limitations of titration, by employing analytical chromatography [6–10]. ICH guidelines recommend that organic impurities present at an apparent level of ≥0.1%, should be characterized in drug substances (i.e. bulk drugs) with a maximum daily dose of 2 g/day [11].

In this work, a novel LC method was developed for the analysis of Noscapine. This allowed for the separation and quantitative determination of an unknown component (Impurity A), which was observed at a level of >0.1% by area in several samples of Noscapine bulk. A semipreparative LC method was then developed to enable Impurity A to be isolated so that its structure could be elucidated using LC–ESI-MS and ^{1}H-NMR.

2. Experimental

2.1. Reagents

All solvents and reagents were of LC grade and were purchased from Burdick and Jackson (Muskegon, MI, USA). Noscapine bulk was supplied by Mallinckrodt (St. Louis, MO, USA), Noscapine USP standard was obtained from the USP (Rockville, MD, USA) and $^{2}H_{4}$-methanol was obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

2.2. Stressing experiments

Samples of bulk noscapine were stressed as follows and then prepared in mobile phase A. Oxygen–heat: 100°C for 2 h, under a constant flow of oxygen; acid: 1 h at 90°C in a solution of 0.1 *M* HCl; Base: 1 h at 90°C in a solution of 0.1 *M* NaOH. These solutions were then neutralized with either 0.1 *M* NaOH or 0.1 *M* HCl and diluted with mobile phase A. Light: a sample was exposed to UV light for a minimum of 200 Wh/m^2 and to visible light for a minimum of approximately 540 (Wh/m^2).

2.3. Analytical LC

The LC system was an HP1100 equipped with a variable wavelength UV detector and a photodiode array (PDA) detection system (Hewlett-Packard)). The column was a Prodigy ODS-2, 4.6×150 mm, 5 μm (Phenomenex), operated at 1 ml/min and 30°C. UV detection was at 254 nm, the injection volume was 10 μl (of a 0.5 mg/ml stock solution), mobile phase A was water–0.1% trifluoroacetic acid (TFA) and mobile phase B was MeCN–0.1% TFA. The gradient was: 0–20 min (0–50% B), 20.1 min (0% B), 25 min (0% B). Samples were prepared in mobile phase A and data were captured using ACCESSCHROM software (PE Nelson).

2.4. Semipreparative LC

This LC system had two LC-8A pumps and an SPD-10A variable wavelength UV detector operated at 254 nm (Shimadzu). The column was an Inertsil ODS-3, 5 μm, 20×250 mm (Metachem) operated at 20 ml/min at room temperature with a 1-ml injection volume (of an 18 mg/ml stock solution). The gradient was: 0–20 min (0–40% B), 25.0 min (40% B), 25.1 min (0% B), 30 min (0% B). All other conditions were as for the analytical LC. Fractions were collected using a manual switching valve (Alltech) and evaporated to dryness with a rotary evaporator (Buchs) maintained at 45°C.

2.5. LC–MS and LC–MS–MS

The mass spectrometer was a Finnigan MAT triple stage quadrupole 7000 equipped with an electrospray ionization (ESI) source, linked to a 1050 LC system (Hewlett-Packard). Data was collected using XCALIBUR, version 1.0 SR1. The LC column was an Inertsil ODS-3 (150 mm×2.0 mm) 5 μm (MetaChem) operated at 0.2 ml/min, with a 40-μl injection volume. All other conditions were as used in the analytical LC work. Data was collected in the Centroid mode with positive ion detection. MS operating parameters are shown in Table 1.

2.6. ^{1}H-Nuclear magnetic resonance

Data was acquired using an AMX-300 NMR spectrometer (Bruker) operating at 300.13 MHz for ^{1}H. Acquisitions were collected into 32 K data points with a spectral width of 6024 Hz giving a digital

Table 1
LC–MS and LC–MS–MS experimental operating conditions

Experimental parameters	LC–MS	LC–MS–MS
Sheath gas pressure	50 p.s.i.[a]	50 p.s.i.
Heated capillary temperature	200°C	200°C
Octapole offset voltage (CID)	−10 V	−10 V
Collision gas and pressure	–	Argon, 3.0 mTorr
Q2 offset voltage	–	−35 V (m/z 400)
Acquisition quadrupole	Q1	Q3
Acquisition scan range	100–850 m/z	10–450 m/z (m/z 400)
Acquisition scan time	2.0 s	2.0 s
Electron multiplier voltage	1000 V	1000 V

[a] 1 p.s.i. = 6894.76 Pa.

resolution of 0.18 Hz/pt. Spectra were obtained at 30°C with either a 2.5- or 5.0-mm multinuclear probe and the residual protonated water signal (HOD) was suppressed by presaturation. All samples were dissolved in $^{2}H_4$-methanol.

3. Results

3.1. Analytical LC

When using the specified linear gradient of water and MeCN (containing 0.1% TFA), it proved possible to separate Noscapine from the other unknown materials present in the bulk samples. The TFA level of 0.1% ensured that the pH of the aqueous portion of the mobile phase was ≈2.1. Noscapine is a very weak base with a pK_a of 7.8 [12]. Therefore, it would predominately exist in the ionized state under these mobile phase conditions. This would help to create a sharp chromatographic peak shape and, more importantly, subsequently aid in the MS analysis, by ensuring that the main analyte and hopefully any other related substances would exist in solution as ions.

Fig. 2 is a chromatogram of Noscapine bulk material showing only the time region of interest. Noscapine elutes at ~14.6 min and Impurity A elutes at ~12.8 min. Impurity A was subsequently found in six different lots of Noscapine bulk, at 0.07–0.11% by area. Several other peaks were also present, however they were at or below 0.04% by area and so did not require identification [11].

The results of a PDA peak purity measurement indicated that no other significant sample components were co-eluting with the main analyte peak. Furthermore, systematic changes to the gradient did not reveal the presence of any additional unknown

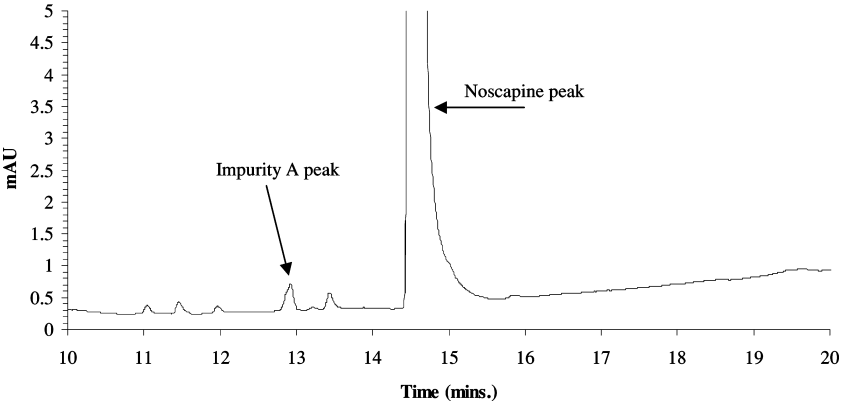


Fig. 2. Chromatogram of Noscapine bulk material, using analytical LC method (see Section 2 for details).

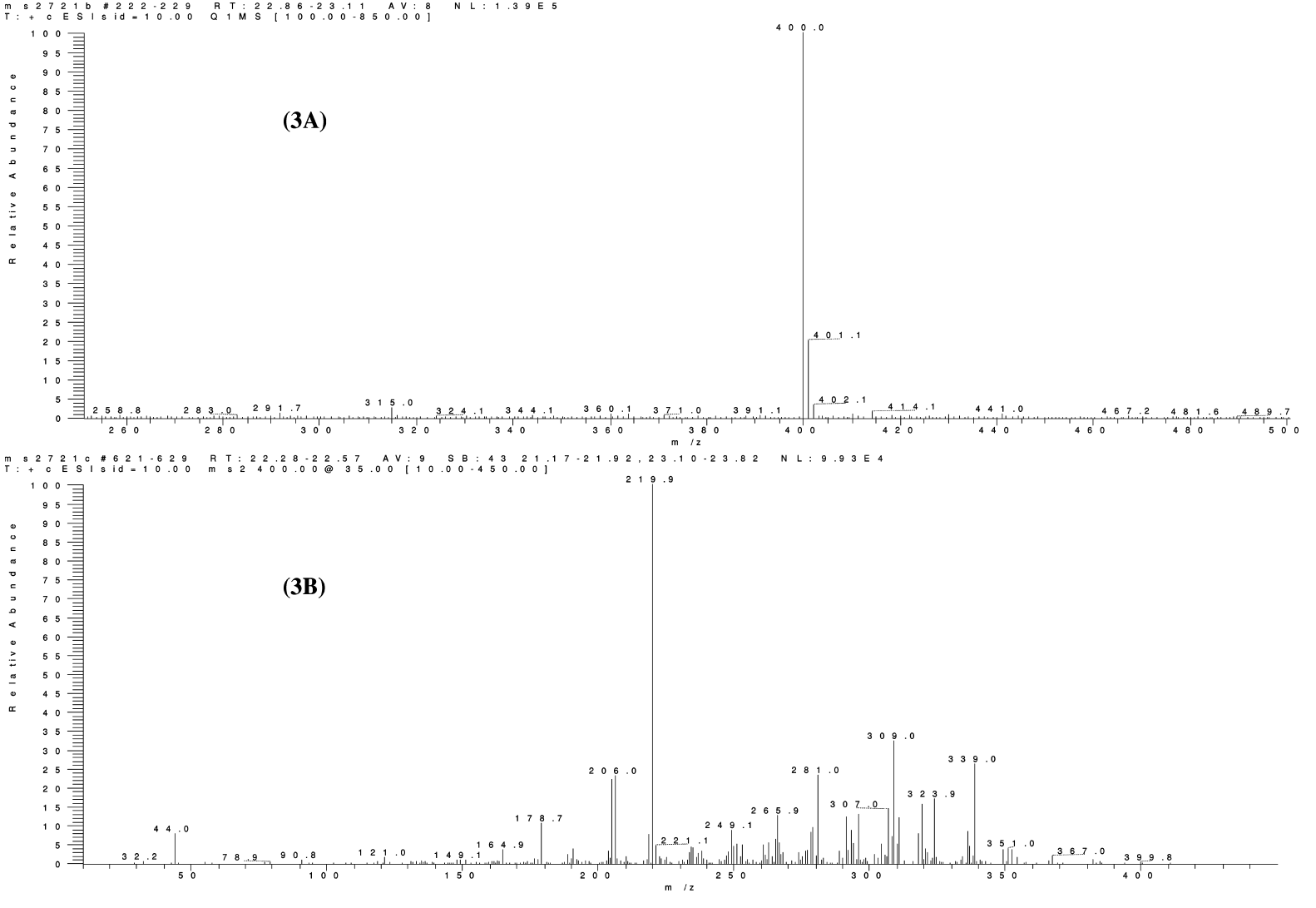


Fig. 3. (A) Mass spectrum of Impurity A, showing the parent m/z 400 and (B) MS–MS fragmentation of Impurity A. See Section 2.5 for details.

peaks. In an effort to increase the level of Impurity A, samples of bulk Noscapine were exposed to various stress conditions — acid, base, heat/oxidation and light stressing (ICH guidelines [13]). However, the Impurity A peak did not increase in size under any of these conditions, which suggested that it is a process impurity of Noscapine and not a degradant.

3.2. Semipreparative LC

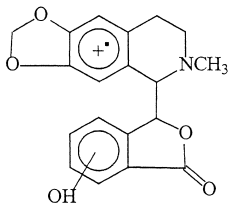
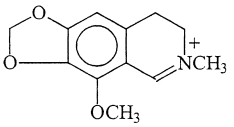
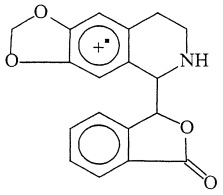
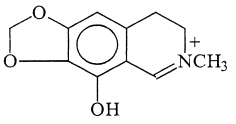
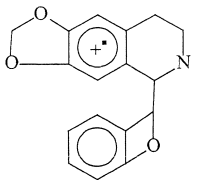
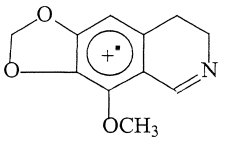
A semipreparative LC method was developed to obtain enough Impurity A material for structural analysis. Successive fractions corresponding to the Impurity A peak were collected from replicate injections of a Noscapine stock solution. The combined eluent fractions were then evaporated/concentrated to approximately 5 ml. Confirmation that Impurity A had been correctly isolated, was obtained by injecting a diluted aliquot of this solution onto the analytical LC system. However, Noscapine was also present (~20% by area), probably arising as a result of the relatively imprecise means of collecting the peak fractions i.e. using a manual switching device.

3.3. LC–MS and LC–MS–MS

With the modified LC–MS method, Noscapine eluted at approximately 24 min and Impurity A at 23.0 min. The parent m/z for Impurity A was 400 $[M+H]^+$, which is 14 lower than that of Noscapine ($M_w = 413$, $m/z = 414$ $[M+H]^+$), see Fig. 3A. This mass difference is explained by the replacement of one of the Noscapine methoxy groups at positions 20, 21 or 22, with a hydroxyl group. Alternatively, the methyl group at position 11 of Noscapine may have been a secondary amine in Impurity A.

Fig. 3B shows the MS–MS fragmentation data for Impurity A, which produced the following significant daughter ions: m/z 339, m/z 309, m/z 281, m/z 220 (most abundant ion), m/z 206 (not labeled) and m/z 205. Predicted structures for these ions are shown in Table 2. The structure of the m/z 220 ion agrees with other published work on Noscapine [14]; it possesses a methyl group on the nitrogen and a

Table 2
Proposed structures for daughter ions of impurity A peak

m/z	Proposed structures	m/z	Proposed structures
339		220	
309		206	
281		205	

methoxy at position 5 on the isoquinoline group. So the proposed structural difference cannot have occurred at either of these two positions. Additionally, the structure for the daughter ion at m/z 339 is consistent with an intact methyl on the nitrogen and with one hydroxyl on the isobenzofuranone moiety. This leaves the two methoxy groups at positions 20 and 21 as the only possible sites for a $-CH_2$ substitution. All the other fragment ions in Table 2 are consistent with this proposal.

3.4. ^{1}H-NMR

The ^{1}H-NMR signal for one of the methoxy groups normally seen in Noscapine, should be absent in Impurity A, if it possessed a hydroxyl group in its place. The ^{1}H-NMR for Impurity A and the USP Noscapine standard were then compared. The only region of the two spectra showing a significant difference, lay between 4.5 and 3.5 ppm, where the USP Noscapine standard showed a singlet at 3.859 ppm. This was assigned to the methoxy group at position 21. Further downfield were singlets at 3.971 and 3.982 ppm, which were non-specifically assigned to the other methoxy groups in positions 20 and 22.

There was only a very weak signal at position 21 in Impurity A, where the signal for a methoxy group had been readily apparent in the USP Noscapine standard. This may have been caused by the presence of residual levels of Noscapine. Therefore, the ^{1}H-NMR data showed that Impurity A did not possess a methoxy group in position 21 of the isobenzofuranone moiety. Otherwise, its structure was determined to be the same as that of Noscapine. In conjunction with the results obtained from the LC and LC–MS experiments, the final proposed structure for Impurity A, shown in Fig. 4, was determined to be a 16-hydroxy Noscapine derivative.

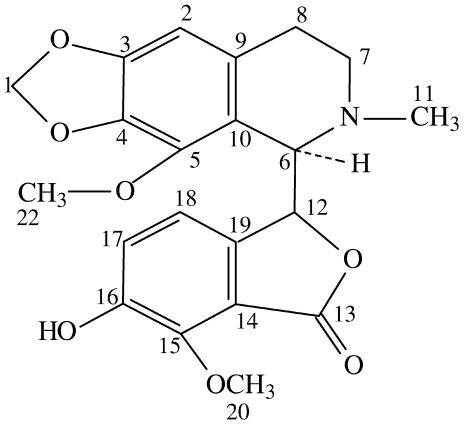


Fig. 4. Proposed structure of Impurity A, with positional assignments indicated numerically.

4. Conclusions

The results from the different analytical disciplines allowed for the structural determination of a minor impurity component (Impurity A) in bulk Noscapine. It was concluded that Impurity A only differed from Noscapine in that it possessed a hydroxyl group (now denoted at position 16) in place of the original methoxy group at position 21 of the isobenzofuranone moiety. Impurity A may therefore be described as a 16-hydroxy derivative of Noscapine.

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