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Determination of noscapine in human plasma using solid-phase extraction and high-performance liquid chromatography

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Abstract

A simple, reliable high-performance liquid chromatographic (HPLC) method has been developed and validated for the determination of noscapine in human plasma. Noscapine and the internal standard, papaverine were quantitatively extracted from plasma onto disposable extraction columns by means of an automated off-line solid-phase extraction system and were separated onto a reversed-phase column. The method was found to be precise and accurate within the range 7.2–270 ng/ml. No endogenous compounds interfered with the assay. © 1997 Elsevier Science B.V.

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1. Introduction

The antitussive drug noscapine (narcotine) is an opium alkaloid isolated from the plant *Papaver somniferum*. Its clinical evaluation as antitussive has been reviewed [1]. Noscapine is known to undergo a pH-dependent, reversible hydrolytic dissociation of its lactone moiety into the hydroxyl carboxylate form, noscapine acid. The equilibrium between the two forms has been studied [2,3]. The relative amounts of noscapine to noscapine acid were close to unity at physiological pH in buffer solutions. However, in blood and plasma, no transformation of noscapine into noscapine acid occurred. The metabolism of noscapine has not been extensively investigated [4–6]. Only a few metabolites have been identified in urine [4,5]. Following single oral doses of 150 [6,7] and 196 mg noscapine embonate, mean plasma peak concentrations of 200 ng/ml ($n=1$) [6], 270 ng/ml ($n=5$) [7] and 150 ng/ml ($n=1$) [8] were attained in 1.5, 0.5–2.5 and 0.5 h, respectively. No data have been reported after rectal administration.

Noscapine plasma levels have been determined by either normal-phase or reversed-phase high-performance liquid chromatography (HPLC) with UV detection [6,8]. These methods required tedious sample preparations involving a sample clean-up onto glass columns packed with silica, the evaporation of dichloromethane amounts as large as 20 ml per sample followed by either a single or a double liquid–liquid extraction. A multidimensional HPLC system combining four columns, three pumps and several six-port valves has been also developed for the determination of noscapine in the supernatant of plasma samples chemically deproteinized using a

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mixture of acetonitrile, phosphate buffer and perchloric acid [2].

This paper presents a new, simple HPLC method suitable for the determination of noscapine for phase I pharmacokinetic studies. Noscapine and the internal standard, papaverine were extracted from plasma by solid-phase extraction onto disposable extraction C_{18} columns (DECs) by means of a Gilson ASPEC automate.

2. Experimental

2.1. Chemicals

Noscapine hydrochloride and the internal standard, papaverine hydrochloride were from Sigma Chemicals (St. Louis, MO, USA). They were used without further purification. Methanol and acetonitrile were of HPLC grade from Rathburn Chemicals (Walkerburn, Scotland). Water was purified by means of a Milli-Q device from Millipore (Millipore SA, Le Mont, Switzerland). All other reagents were of analytical grade from E. Merck (Darmstadt, Germany). Blank heparinized plasma samples were obtained from volunteers and blood donors. The plasma were stored frozen at less than −20°C.

2.2. Solid-phase extraction system

An ASPEC XLi (Automatic Sample Preparation with Extraction Columns) system from Gilson (Omnilab SA, Chavannes de Bogis, Switzerland) was used. The system has been previously described [9]. It combined a stroke vertical arm, a Model 401 dilutor/pipettor and a set of racks as well as accessories necessary for handling DECs and solvents. All were controlled by a sample controller keypad. Bakerbond spe C_{18} DECs (1-ml) were used. DECs were supplied by Stehelin AG (Basel, Switzerland). The extracts were evaporated under nitrogen using a TurboVap LV evaporator from Zymark (Brechbühler SA, Plan-les-Ouates, Switzerland).

2.3. Chromatographic equipment and conditions

The HPLC system consisted of a Model 616 pump, a Model 600S controller, an autosampler Model 717plus equipped with temperature-controlled rack set at 6°C, a Model 486 UV detector and a Maxima 825 data station, all from Waters Ass. (Milford, MA, USA). The eluents were degassed by means of a GasTorr Model GT-104 degassing unit (Omnilab SA). HPLC separation was achieved using a Nucleosil 120-5-C_{18} reversed-phase column, 125× 4.0 mm I.D., d_p=5 μm (Macherey Nagel AG, Oensingen, Switzerland). The mobile phase was 0.025 *M* (Na^+) phosphate buffer (pH 4.5)–acetonitrile 60:40 (v/v). The flow-rate of the mobile phase was 1.0 ml/min. The detection wavelength was set at 211 nm. The injected volume was 50 μl.

2.4. Sample preparation

The thawed plasma samples were quickly centrifuged. A volume of 1000 μl was combined with 40 μl of a working solution of internal standard (903 ng/ml papaverine free base) and 2000 μl of 0.05 *M* (Na^+) phosphate buffer, pH 7.4, in a glass tube. The tube was vortexed and placed on the rack of the ASPEC system.

All the following steps were performed automatically by the ASPEC system. In all instances, the needle was rinsed with 1 ml of water before pipetting the liquid to be transferred.

2.4.1. DEC conditioning

DECs were conditioned by passing successively 2 ml of methanol, 2 ml of water and 1 ml of 0.05 *M* (Na^+) phosphate buffer, pH 7.4 (flow-rate, 1.5 ml/min; air volume, 70 μl).

2.4.2. Sample extraction

A volume of 2750 μl of diluted plasma was loaded at a rate of 0.36 ml/min (air volume, 150 μl). The DEC was then washed successively with 1 ml of 0.05 *M* (Na^+) phosphate buffer, pH 7.4, and 2 ml of water (air volume, 150 μl). The DECs were dried by passing 5 ml of air (flow-rate, 5 ml/min).

2.4.3. Elution

Noscapine and the internal standard were eluted with 1.5 ml of acetonitrile and the next sample was processed.

The eluates were evaporated under nitrogen (30°C) to dryness. They were reconstituted with 200

μl of acetonitrile. This solution was transferred to the autosampler vials.

2.5. Standard solutions

The stock and the working solutions of both noscapine and papaverine were prepared in methanol. The concentration of the noscapine stock solution was 90.1 μg/ml noscapine free base. The working solutions 9.01 and 0.901 μg/ml were prepared by successive dilution. Papaverine stock solution (90.3 μg/ml papaverine free base) was diluted 1/100 to obtain a working solution of 0.903 μg/ml. All solutions were found to be stable at 2°C for up to 1 week (deviation ±5%).

2.6. Calibration curves

A typical calibration curve was constructed with seven blank plasma spiked with various amounts of the appropriately diluted working solutions. The calibration range was 0–270 ng/ml of noscapine free base. The standard samples were prepared according to the procedure as unknown samples. The calibration curves were obtained by plotting the peak area ratio of noscapine to internal standard versus the nominal concentrations expressed in ng/ml noscapine free base. The equations were calculated by the least-squares method using linear regression.

2.7. Method validation

Low-, medium- and high-level plasma samples were prepared for quality control and were aliquoted in glass tubes. Their theoretical concentrations were 22.0, 53.7 and 133.2 ng/ml, respectively. The extraction recoveries were calculated by comparing the peak area obtained for low-, medium- and high-level controls ($n=8$ for each level for noscapine, $n=39$ for papaverine) and those resulting from the direct injection ($n=3$, working solutions) of the theoretical amount of either noscapine or internal standard (=100% recovery). The specificity of the assay against endogenous compounds was investigated using seven plasma samples from different volunteers. The precision and accuracy of the method were evaluated by repetitive analysis of quality control plasma samples. Intra-day precision and accuracy data were obtained by the analysis of these samples on 1 day by the same operator ($n=8$ for each level). Inter-day precision and accuracy were obtained assaying these three levels ($n=6$) on different days by different operators. The precision and accuracy were defined as the relative standard deviation and as the deviation from the theoretical nominal concentration, respectively. The linearity data were obtained by means of calibration curves ($n=6$). The limit of quantification was defined as the lowest amount quantifiable with a precision of less than 15% ($n=3$) and an accuracy of ±15% ($n=3$). Investigations were performed with plasma samples spiked with 0.9, 3.6 and 7.2 ng/ml noscapine free base. The limit of detection was defined as the lowest amount detectable with a signal-to-noise ratio of about 2. Noscapine has been found to be stable in plasma at 37°C for 24 h [1] and at −20°C for 8 months [4]. The stability of extracts under experimental conditions, i.e. in the autosampler rack, was investigated using controls and working solutions.

3. Results and discussion

3.1. Solid-phase extraction

The application of off-line solid-phase extraction on C_{18} DECs to the extraction of noscapine and its internal standard from human plasma gave satisfactory results. Extraction recoveries of 106.5±10.3% ($n=8$), 89.5±6.8% ($n=8$) and 91.0±2.7% ($n=8$) were obtained for low, medium and high noscapine levels, respectively. The overall recoveries were found to be 94.5±8.8 ($n=24$) and 99.9±5.0% ($n=39$) for noscapine and the internal standard, respectively. No noticeable deviation from DEC to DEC was observed. The extraction steps were performed automatically by the ASPEC system. The plasma samples were diluted prior to extraction in order to improve the extraction process. Because of the sensitivity required, the procedure involved the evaporation of the final eluates followed by the reconstitution of the extract in a small volume. The use of acidic and alkaline aqueous media were avoided in order to not displace the equilibrium between the lactone and the carboxylate forms of noscapine. Noscapine and papaverine were found to

be stable under the experimental conditions of the solid-phase extraction for up to 15 h (deviation <5%).

3.2. Chromatography

Typical chromatograms obtained for a blank plasma, for spiked plasma samples and a sample collected from a healthy volunteer after rectal administration of 60 mg of noscapine hydrochloride are shown in Fig. 1. No endogenous compounds were found to interfere with the assay. The retention times were found to be stable. The mean values measured were 3.98±0.03 (R.S.D.=0.7%, n=39) and 4.99±0.02 min (R.S.D.=0.5%, n=39) for the internal standard and noscapine, respectively. Noscapine acid was not retained under the experimental conditions. Preliminary investigations performed with noscapine in methanol, in acetonitrile, in acidic medium, in plasma as well as with noscapine acid in alkaline medium, showed no transformation of noscapine into noscapine acid under the experimental conditions of the whole procedure.

3.3. Method validation

The method was validated according to the EEC note for guidance [10]. Intra-day and inter-day precision and accuracy data are shown in Table 1. The results obtained for intra-day variability ranged from 1.9 to 8.2% and from −4.9 to 4.4%, respectively. These values were 9.0–9.9 and 4.0–11.2% for the inter-day variability, respectively. The linearity range was 7.2–270 ng/ml. A mean correlation coefficient of 0.9997±0.0002 was calculated (n=6). Mean slope and intercept (n=6) were found to be 3.54±0.35

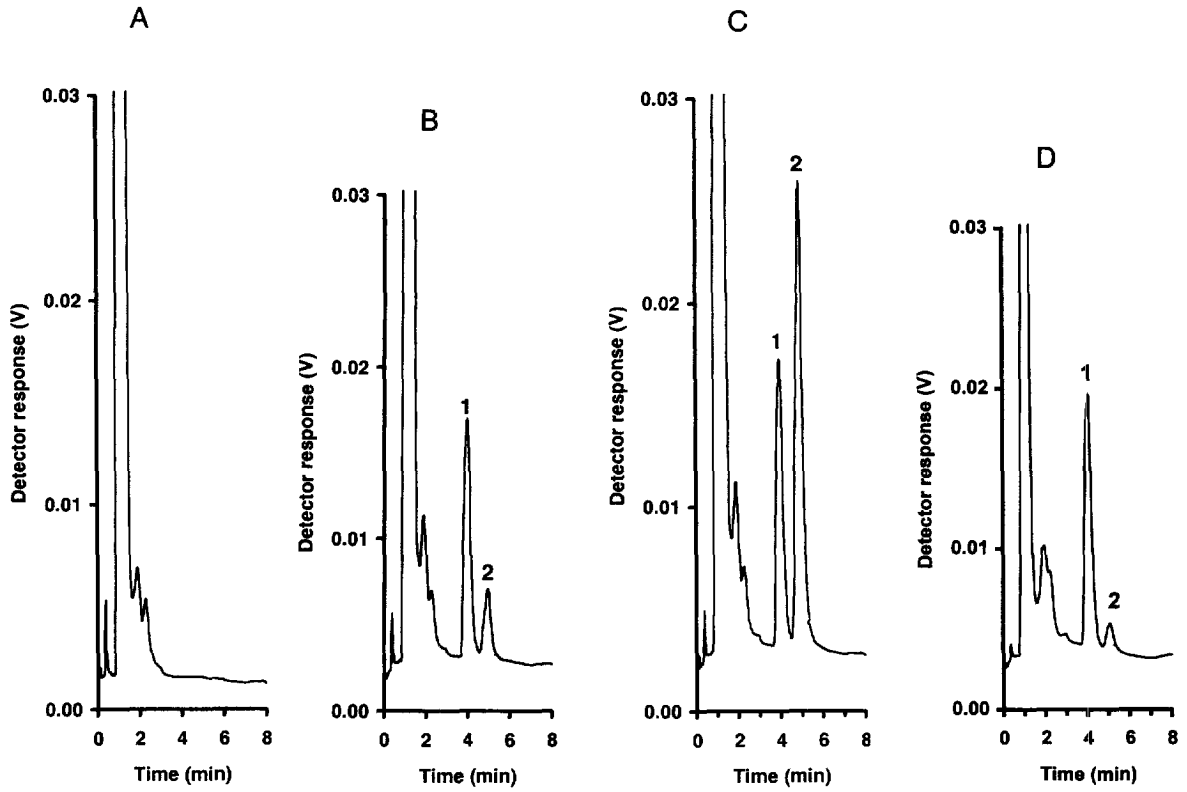

Fig. 1. Typical chromatograms obtained for a blank plasma (A), a plasma sample spiked with 45.1 ng/ml noscapine free base (B), 273.0 ng/ml noscapine free base (C) and a plasma sample (18.7 ng/ml) collected from a healthy volunteer after rectal administration of a single dose of 60 mg noscapine hydrochloride (D). The plasma samples were assayed according to the recommended procedure. 1, papaverine; 2, noscapine.

Table 1
Precision, accuracy and limit of quantification of noscapine assay

Nominal concentration (ng/ml)	Concentration found mean±S.D. (ng/ml)	R.S.D. (%)	Confidence interval of the mean value (P=95%) (ng/ml)	Deviation (%)
Intra-assay variability (n=8)				
22.0	23.0±1.9	8.2	23.0±1.7	4.4
53.7	53.7±3.3	6.2	53.7±3.0	−0.1
132.2	236.7±2.4	1.9	126.7±2.1	−4.9
Inter-assay variability (n=6)				
22.0	24.4±2.2	2.2	24.4±2.5	11.2
53.7	58.7±5.0	8.5	58.7±5.7	9.3
133.2	138.5±13.6	9.9	138.5±15.5	4.0
Limit of quantification (n=3)				
7.2	7.7±0.4	5.5	7.7±1.0	6.3
3.6	6.3±2.5	39.3	6.3±6.2	75.5
0.9	2.8±0.1	3.9	2.8±0.3	210.3

Controls and spiked plasma samples were assayed according to the recommended procedure. R.S.D., relative standard deviation. The concentrations are expressed as noscapine free base.

(R.S.D.=9.9%) and 2.7±2.6, respectively. The calibration curves were neither forced through origin nor weighted. As shown in Table 1, the limit of quantification was found to be 7.2 ng/ml. The limit of detection was found to be in the order of 0.9 ng/ml. Both working solutions and controls samples extracts were found to be stable (deviation ±5%) in the autosampler rack for up to 15 h.

4. Conclusion

The developed method is simple and suitable for the determination of plasma noscapine for phase I pharmacokinetic studies. The solid-phase extraction system described allows the reproducible automated extraction of diluted plasma samples. The validation data demonstrate the reliability of the method.

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