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Note

New sensitive method to determine noscapine in serum by reversed-phase liquid chromatography

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Noscapine is widely used in pharmaceutical preparations for its antitussive effect. Several methods have been described for the analysis of this isoquinoline alkaloid in biological fluids, including fluorometric [1, 2], gas and thin-layer chromatographic [3, 4] and liquid chromatographic [5, 6] techniques. The liquid chromatographic method of Johansson et al. [5] which has a detection limit of 5 ng/ml of plasma is a straight-phase system combined with ultraviolet (UV) detection at 313 nm. It involves several extraction and purification steps and is therefore time-consuming. The reversed-phase system of Jensen [6] is highly dependent on the chromatographic conditions because no internal standard is used. The detection limit at 230 nm is 10 ng/ml of serum.

This paper presents a new, simple and sensitive reversed-phase high-performance liquid chromatographic (HPLC) method for pharmacokinetic study of noscapine in serum with a detection limit of 1 ng/ml. The method is also suitable for analysis of noscapine in serum after oral administration of noscapine embonate.

EXPERIMENTAL

Chemicals

Noscapine hydrochloride and papaverine hydrochloride were of Ph. Eur. grade. Both compounds were dried at 105°C before use. Methanol was of HPLC grade (Rathburn Chemicals). Noscapine embonate (1:1) was synthesized at the analytical laboratory of the University Pharmacy, and had a noscapine content of 45.1% and a particle size of 250 μm. All the other chemicals were of analytical grade.

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Extraction

The extractions were performed on glass columns (20 mm I.D.) packed with Extrelut® (E. Merck).

Chromatographic procedure

The HPLC system consisted of a Pye Unicam PU 4010 pump, LC-XP gradient programmer, PU 4020 UV detector and a Rheodyne Model 7125 injection valve with a sample loop of 50 μl. The chromatograms were recorded on a PU 8251 single-pen recorder. The column was a Hibar LiChrosorb CN (12.5 cm × 4 mm), particle size 5 μm.

The UV detector was set at 220 nm wavelength and 0.005—0.02 a.u.f.s. detector sensitivity range. The flow-rate was 1 ml/min. Peak heights were measured manually.

The mobile phase consisted of 25% methanol and 75% 0.02 *M* disodium hydrogen phosphate and 0.1 *M* sodium perchlorate adjusted to pH 3.8 with 2 *M* phosphoric acid. The time needed to stabilize the column was about 2 h.

Sample preparation

To the serum sample (2.00 ml) were added 400 ng of papaverine hydrochloride (internal standard) dissolved in 1.00 ml of water and 5.0 ml of phosphate buffer pH 4.0 (I = 1.0). The mixture was poured into the column packed with 4.0 g of Extrelut and allowed to adsorb over the supporting matrix for 10—15 min. Noscapine and papaverine were eluted with 25 ml of dichloromethane—butanol (19:1). The eluate was collected in 15-ml conical tubes siliconized before use with Aquasil® and then evaporated to about 2 ml under a stream of warm air (30—40°C), the tubes being in a water bath maintained at 38°C. The residue was evaporated to about 200 μl in the same way but with the bath at 50°C. Then 200 μl of *n*-heptane and 200 μl of 0.33 *M* sodium hydrogen phosphate solution adjusted to pH 2.0 with phosphoric acid were added and the tubes shaken gently by hand for 1 min. After centrifugation for 5 min at 2000 *g* an aliquot of 40—50 μl of the lower phase was injected into the chromatographic system.

The standard curve was prepared by analysis of standard samples containing 2.00 ml of blank serum, 400 ng of internal standard and spiked with noscapine hydrochloride in a concentration range corresponding to 2.9—230 ng of noscapine per ml of serum. The curve was constructed by plotting the peak height ratio of noscapine to the internal standard.

RESULTS AND DISCUSSION

Extraction and purification

Column extraction by dichloromethane—butanol has earlier been used for opiate analyses in biological samples [5, 7]. Johansson et al. [5] reported that the absolute recovery from plasma samples spiked with 92 ng of noscapine per ml was 81%. The method includes a complicated purification step from the crude extract.

In the present method noscapine and the internal standard are back-extracted after partial evaporation of the organic phase used in the column

extraction into an aqueous phase which is then directly chromatographed without any further purification. The absolute recovery of noscapine is about 83% and a little more for papaverine.

Chromatography

Typical HPLC chromatograms obtained from blank (A), spiked (B) and volunteers' (C, D) serum are shown in Fig. 1. The eluted peaks are clean, sharp and symmetrical and the blank serum does not show any interfering peaks at all near the elution volumes for drug or internal standard.

The pH and the electrolyte of the mobile phase (sodium perchlorate) have a significant effect on the retention times and symmetry of the peaks. With the conditions used, noscapine and papaverine are almost completely in their ionized forms, and ion-pair formation probably occurs during elution.

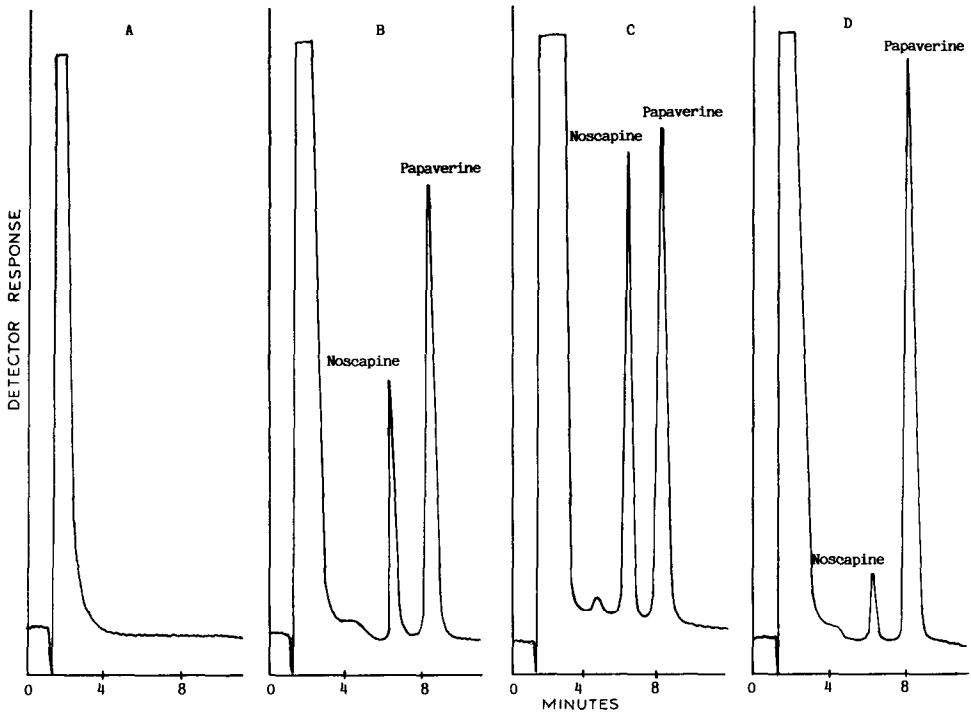

Fig. 1. Chromatograms of noscapine in human serum. (A) Control serum; (B) serum, spiked with 48 ng/ml noscapine with internal standard added; (C) serum, 90 min after an oral dose of 196 mg of noscapine embonate with internal standard added; (D) serum, 360 min after an oral dose of 196 mg of noscapine embonate with internal standard added. Sample size 40—50 μl. Detector sensitivity 0.01 a.u.f.s. See text for other conditions.

Linearity

A straight line was obtained by plotting ng/ml noscapine in serum against the peak height ratio of noscapine to internal standard. The relationship was linear in the concentration range 2.9—230 ng/ml of serum (higher concentrations were not tested). The standard curve had an equation of $y = (1.160 \cdot 10^{-2}x) + (9.85 \cdot 10^{-3})$ and a correlation coefficient of 0.999 ($n = 52$).

Precision

The precision of the method is represented by the relative standard deviation of the mean of replicate assays of the same sample. Because of the simple method used for sample preparation and the high selectivity and sensitivity, precision is good even at low concentrations, as shown in Table I.

TABLE I

PRECISION OF THE METHOD AT DIFFERENT SERUM LEVELS

Drug level (ng/ml)	Number of determinations (n)	Relative standard deviation (%)
184	7	2.2
46	7	3.1
11.5	7	3.3
5.7	5	5.7
2.9	4	9.3

Sensitivity

Using mobile phase as the solvent, noscapine has two absorption maxima, one at 310 nm and the other at 213 nm. The latter is a very strong one with a molar absorptivity of about $6 \cdot 10^4$. To obtain greater baseline stability, detection was performed at 220 nm with a sensitivity 24% below that for the maximum at 213 nm. Based on a signal-to-noise ratio of 3:1, 1 ng/ml was detected with an injection volume of 50 μl and the highest detector sensitivity (× 0.005 a.u.f.s.).

Stability of noscapine in serum

According to earlier reports [5, 6] no transformation of noscapine to noscapic acid occurs within 24 h at 37°C in blood or plasma samples.

In order to investigate the stability of noscapine in frozen serum, five serum samples with different noscapine concentrations (110—223 ng/ml) were stored at −20°C for two weeks. No deterioration of the samples occurred during this time. The average finding was 99.9%.

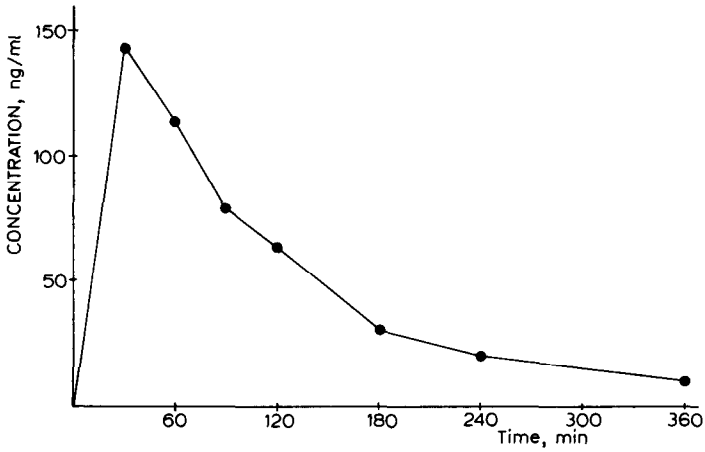

Fig. 2. Serum concentrations of noscapine following an oral dose of 196 mg of noscapine embonate corresponding to 88 mg of noscapine.

Application to biological samples

Serum concentrations of noscapine following an oral dose of 196 mg of nocapine embonate corresponding to 88 mg of nosapine (suspended in 75 ml of water) were monitored in a young healthy volunteer over a 6-h period (Fig. 2). The maximum concentration of noscapine in serum was reached within 30 min.

CONCLUSION

The procedure described in this paper provides a sensitive and reliable method for the determination of noscapine in serum. The preparation of the sample is easy and the rapid chromatographic analysis allows for determination of at least 35 samples in two normal working days.

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