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Note

Determination of noscapine in serum by high-performance liquid chromatography

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Noscapine [1-α-methyl-8-methoxy-6,7-methylenedioxy-1-(6,7-dimethoxy-3-phthalidyl)-1,2,3,4-tetrahydroisoquinoline] (Fig. 1), which is one of the alkaloids in opium, has been known for more than 100 years, and several methods for its quantitative determination are therefore available. In recent years gas chromatographic methods [1, 2] and high-performance liquid chromatographic methods (HPLC) [3—7] have been applied in separating and determining the opium alkaloids.

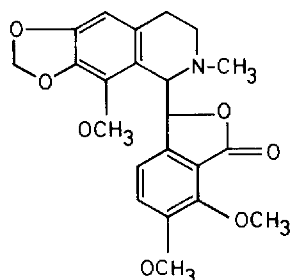

Fig. 1. Formula of noscapine.

Pharmaceutical preparations containing noscapine for its antitussive effect, have been analysed for noscapine by thin-layer chromatography with in situ scanning [8], or by HPLC [9]. In forensic laboratories HPLC is now the method most commonly used for identification of noscapine [10, 11].

In biological fluids noscapine has been determined by a fluorimetric method [12, 13] or by gas and thin-layer chromatography [14, 15]; and, finally, an HPLC method has been described for the determination of noscapine in serum [16].

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This study describes a simple HPLC method which is sensitive enough for the determination of noscapine concentrations in serum following therapeutic doses of the compound.

EXPERIMENTAL

Material and reagents

Hexane and dichloromethane were specially purified (nanograde) from Mallinckrodt (St. Louis, MO, U.S.A.). Acetonitrile (E. Merck, Darmstadt, G.F.R.) was special HPLC grade. All other chemicals were analytical grade. The tablets used contained ion-exchange resin bound noscapine (Longatin®) 25 mg, and were produced by Dumex Ltd.

HPLC conditions

The apparatus consisted of a solvent delivery system, Model 6000A (Waters Assoc., Milford, MA, U.S.A.), a loop injection system U6K (Waters Assoc.) a UV detector Model 440 with fixed wavelength of 254 nm (Waters Assoc.), and a variable-wavelength detector, Model 450, of wavelength 230 nm (Waters Assoc.). The column was μBondapak C_{18} (30 cm × 3.9 mm, particle size 10 μm). The temperature was 25°C.

The mobile phase consisted of 45% acetonitrile in 55% potassium dihydrogen phosphate (0.6%, w/v) adjusted to pH 3 with phosphoric acid. The flow-rate was 0.9 ml/min. The mobile phase was membrane-filtered (pore size 0.45 μm) and kept in an ultrasonic bath for 15 min immediately before use.

Printing of the chromatograms was performed by an electronic integrator (No. 3080) from Hewlett-Packard, Avondale, PA, U.S.A. The peak heights were measured manually.

Procedure

To 2.00 ml of serum add 200 μl of a 0.5 *M* solution of sodium hydroxide and 10.00 ml of a mixture of *n*-hexane–dichloromethane (9:1). Rotate for 15 min (30 rpm) in a rotating device and, after centrifuging (ca. 950 *g*) for 5 min at 5°C, remove a 7.00 ml volume from the organic phase. Evaporate to dryness at 20°C with a gentle stream of nitrogen. The residue is dissolved in 100 μl of mobile phase in an ultrasonic bath and 50 μl are injected into the chromatograph.

Sampling

One female volunteer, who fasted overnight, received four tablets of noscapine 25 mg (Longatin®). Blood was collected immediately before, and 15, 35, 45, 60, 75, 90, 105, 120, 150 and 180 min after administration. Serum was centrifuged off and kept at −18°C until analysis.

RESULTS AND DISCUSSION

This study describes a liquid chromatographic method in which noscapine is determined without previous derivatization of the compound.

TABLE I

SLOPE, INTERCEPT AND CORRELATION COEFFICIENTS FOR FOUR DIFFERENT CALIBRATION GRAPHS

	Slope	Intercept	Correlation coefficient
I	0.487	15.6	0.988
II	0.496	13.5	0.996
III	0.540	9.2	0.993
IV	0.504	5.9	0.996

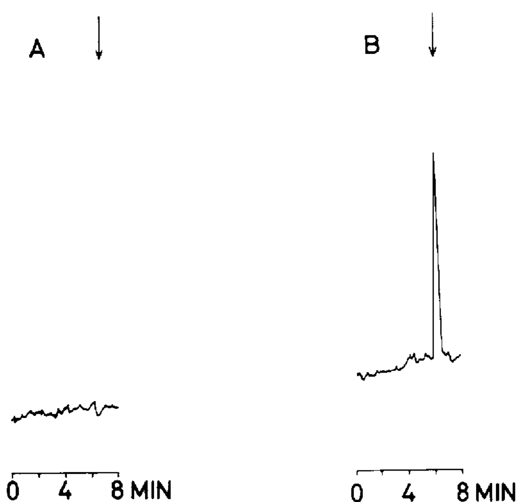

Fig. 2. Chromatograms of (A) mobile phase, and (B) mobile phase + noscapine (50 ng) (retention time: 5.9 min).

In Table I is shown slope, intercept and correlation coefficient for four calibration graphs from four different days.

Fig. 2 illustrates the chromatographic response to 50 μl of mobile phase and to 50 μl of mobile phase with 50 ng of noscapine added.

Chromatograms of control serum and of control serum with 25 ng/ml or 100 ng/ml noscapine added are shown in Fig. 3.

Fig. 4 depicts chromatograms of control serum measured at 254 nm (UV detector with fixed wavelength) and at 230 nm (detector with variable wavelength).

The recovery of noscapine from serum samples (10—250 ng/ml) by extraction with *n*-hexane or with dichloromethane added, was found to be 50% (*n*-hexane), 100% [*n*-hexane—dichloromethane (4:1)], and 90% [*n*-hexane—dichloromethane (9:1)]. The last mixture was preferred, because addition of more than 10% dichloromethane to *n*-hexane produced an unsatisfactory separation of the noscapine peak from other interferences.

The precision of the method was controlled by analysis of control serum with 75 ng/ml noscapine added. The coefficient of variation was found to be 3% (n = 10). Vedsø [12] reported the S.E.M. to be 5.4% (50—1360 ng/ml)

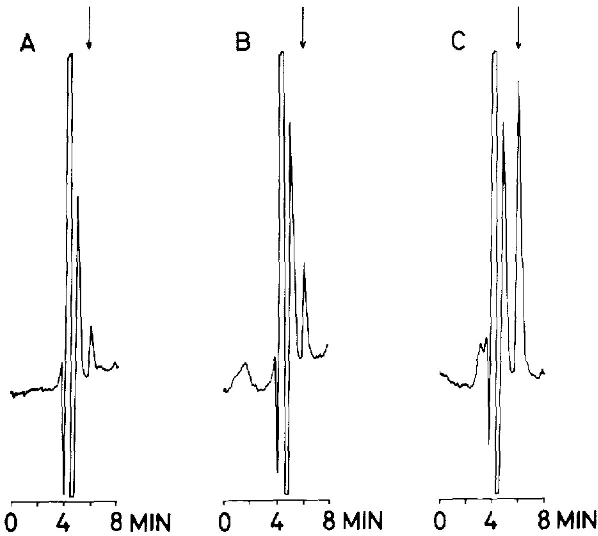

Fig. 3. Chromatograms of (A) control serum, (B) control serum with noscapine (25 ng/ml added), and (C) control serum with noscapine (100 ng/ml added) (retention time: 5.9 min).

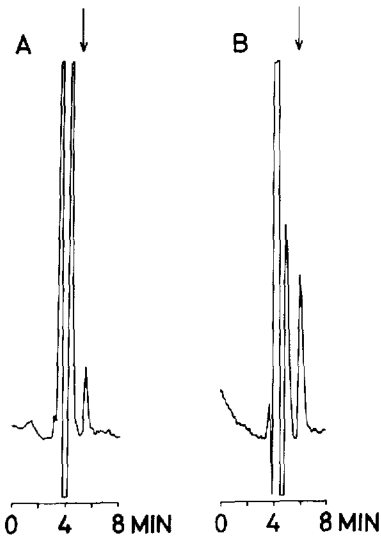

Fig. 4. Chromatograms of control serum with noscapine (50 ng/ml added) (retention time: 5.9 min), measured at (A) 254 nm and (B) 230 nm.

and according to the method by Johansson et al. [16] the coefficient of variation was 3.2% (89 ng/ml).

The minimal concentration detectable is 10 ng/ml of serum. In comparison, the detection limit reported by Vedsø [12] is 50 ng/ml and by Johansson et al. [16] 5 ng/ml.

The amount of sample volume injected into the chromatograph affects the peak heights. In this study a volume of 50 μl was chosen, because the low

serum concentrations obtainable necessitated injection of as big a fraction of the sample volume as possible.

It has been reported by Pawelczyk et al. [17—19] that an alkaline environment easily produces a lactone ring opening of the noscapine molecule, which is thereby transformed to noscapinic acid. Consequently, there is a risk that this transformation may take place during the extraction procedure, which involves alkalization of the serum (pH 10—11).

The stability of noscapine during the analysis procedure was examined. A 2-ml volume of serum with 200 μl of 0.5 N sodium hydroxide and 100 or 200 ng of noscapine added was left at room temperature for up to 24 h, and during this period no decomposition of the compound was observed.

Noscapinic acid, if present, is not determined by the prescribed method, and since no reduction in content of noscapine was observed in alkaline serum left at room temperature for 24 h, this may suggest that the noscapine molecule is protected in the serum against the opening of the lactone ring.

Various drugs which might interfere with the analysis were injected into the chromatograph. The retention times relative to noscapine are listed in Table II.

TABLE II

RELATIVE RETENTION TIMES FOR SOME COMPOUNDS

Compound	Relative retention time
Noscapine	1.00
Nitrazepam	1.38
Imipramine-N-oxide	1.51
Nortriptyline	1.52
Imipramine	1.57
Desmethylimipramine	1.62
Amitriptyline-N-oxide	1.66
Amitriptyline	1.69
Diazepam	2.25
2-Amino-5-nitrobenzophenone	2.45

Application to clinical samples

Chromatograms of serum drawn from one volunteer immediately before and 45 and 120 min after oral administration of 100 mg of noscapine are depicted in Fig. 5. Fig. 6 shows the serum concentration course in the same volunteer during the first 3 h after administration.

The peak serum concentration after 1 h was 135 ng/ml; the estimated half-life was 1.5 h (calculated on the basis of serum concentrations obtained 2, 2.5 and 3 h after administration). Vedsø [12] found serum concentrations of 300—1500 ng/ml after 1 h and 100 ng/ml after 4 h following oral administration of 250—300 mg of noscapine (Longatin®) to volunteers. Peak serum concentrations of an average 94 ng/ml after 2.5 h were reported by Dahlström et al. [21] following oral administration of 150 mg of noscapine (Longatin®). The half-life in the elimination phase was calculated to be approximately 2 h. However, great individual differences were observed, the same authors reporting an absolute bioavailability with a factor 4.8 variation among the volunteers.

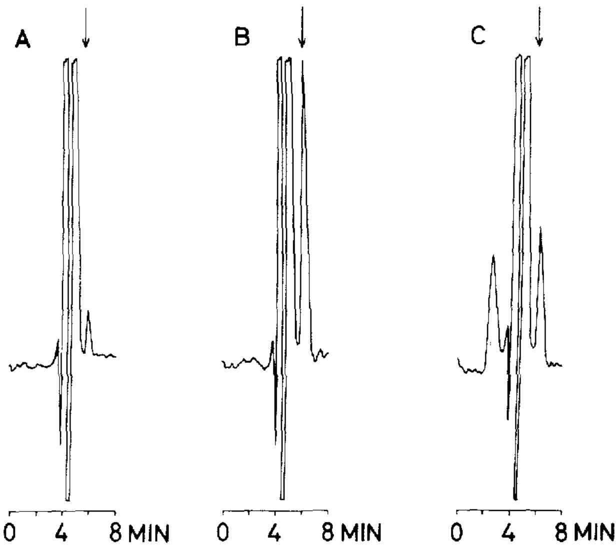

Fig. 5. Chromatograms of serum from a volunteer following an oral dose of noscapine, 100 mg: (A) before the dose, (B) 45 min after the dose, (C) 2 h after the dose (retention time of noscapine: 5.9 min).

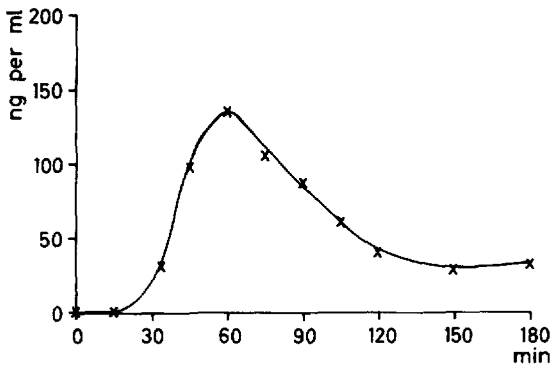

Fig. 6. Serum concentrations of noscapine in a volunteer following an oral dose of noscapine, 100 mg.

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