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Note

Rapid high-performance liquid chromatography determination of noscapine hydrogen embonate

VEIKKO HAIKALA
University Pharmacy, Quality Control Department, Kalliolanrinne 6, SF-00510 Helsinki (Finland)
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Noscapine hydrogen embonate (Fig. 1) is a new poorly soluble prodrug of noscapine. Its solubility data as well as some of its pharmacokinetic properties differ considerably from those of noscapine hydrochloride[1,2], which is the most commonly used noscapine salt in cough medicines.

Quantitation of noscapine in serum following oral doses of noscapine hydrogen embonate has previously been carried out by high-performance liquid chromatography (HPLC)[3]. Sedimentation of noscapine hydrogen embonate in suspension as also been monitored by HPLC[2]. Both methods, however, quantify only noscapine, and give no information about embonic acid. Spectrophotometry has been used to determine the solubility of noscapine hydrogen embonate as a function of pH[2]. This method quantifies both the anionic and cationic parts of the salt, but is non-specific and requires pre-treatment of the sample as do the chromatographic methods.

The present paper describes a rapid reversed-phase HPLC method for the analysis of noscapine hydrogen embonate. This method permits the simultaneous quantitation of both salt components as well as the detection of three degradation products of noscapine hydrogen embonate. It is simple, precise and accurate with minimum sample handling, and is suitable for the determination of the purity of noscapine hydrogen embonate in bulk and for stability studies of the salt. In order to demonstrate the feasibility of the method for determination of noscapine hydrogen

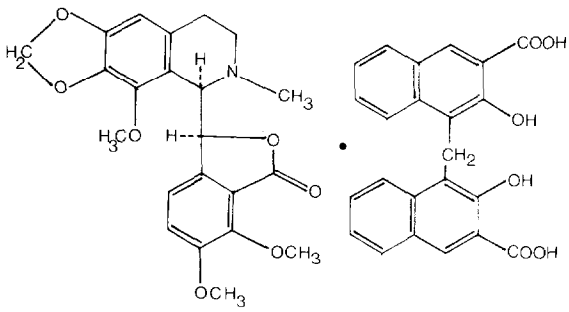

Fig. 1. Structure of noscapine hydrogen embonate.

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embonate in dosage forms, the procedure was also applied to the analysis of noscapine hydrogen embonate capsules.

EXPERIMENTAL

Chemicals

The noscapine conformed to European Pharmacopoeia requirements. The embonic acid was of purum grade (Fluka, Art. No. 45150). Both compounds were dried at 105°C before use. Noscapine hydrogen embonate monohydrate was synthesized at the analytical laboratory of the University Pharmacy and had a noscapine content of 50.4% and an embonic acid content of 47.4% as determined by UV spectrophotometry[2]. The water content was 2.2% (Karl-Fischer method). Cotarnine and opianic acid were synthesizd by the method described by Marshall *et al.*[4] and meconine by that of Rabe and McMillian[5]. Talc, lactose and magnesium stearate were used according to pharmacopoeial standards. All other chemicals were of commercial analytical grade.

Composition of the capsules examined

The capsules were prepared to contain 40.0 mg of noscapine hydrogen embonate, about 250 mg of lactose, 3 mg of talc and 1 mg of magnesium stearate. The excipients were those most commonly used in capsules prepared by the University Pharmacy.

Chromatographic conditions

The HPLC system consisted of a Pye Unicam PU 4010 pump, LC-XP gradient programmer, PU 4020 UV detector and CDP 1 computing integrator. The injections were made through an injector equipped with a 10-μl sample loop (Rheodyne Model 7125). The chromatograms were recorded on a PU 8251 single-pen recorder. A 125 mm × 4 mm Hibar LiChrosorb CN column (particle size 5 μm) was used at a flow-rate of 1.0 ml/min. Detection was monitored at 304 nm and the sensitivity was on a full scale of 0.08 absorbance units.

The mobile phase consisted of 10% (v/v) N,N-dimethylformamide and 90% (v/v) of 0.05 *M* sodium dihydrogenphosphate and 0.1 *M* sodium perchlorate adjusted to pH 5.5. Prior to injection the column was stabilized with the eluent at a flow-rate of 0.7 ml/min for 2 h.

Preparation of the standard curve

A 30.0-mg amount of noscapine and 30.0 mg of embonic acid were weighed into a 100-ml volumetric flask. Dimethylformamide (30 ml) was added and the flask was shaken for 2 min. About 60 ml of phosphate buffer containing 0.05 *M* sodium dihydrogenphosphate and 0.1 *M* sodium perchlorate (pH 5.5) were added and the flask was cooled to room temperature and made up to volume with the phosphate buffer. Dilutions of this stock solution in dimethylformamide–phosphate buffer (3:7) were made to yield 0.3, 0.24, 0.18, 0.12, 0.09, 0.06 and 0.03 mg/ml of embonic acid and noscapine. The solutions should be freshly prepared.

Sample preparation

Bulk material. About 40 mg of noscapine hydrogen embonate were weighed accurately into a 100-ml volumetric flask. Dimethylformamide (30 ml) was added and the flask shaken for 2 min. A 60-ml volume of the phosphate buffer (see above) was added and the flask was cooled to room temperature and made up to volume.

Noscapine hydrogen embonate capsules. The contents of each capsule were transferred to a 100-ml volumetric flask. The sample was then prepared as described under Bulk material with additional filtration through a quantitative filter (Rota Duren No. 640 d) after cooling to room temperature.

Method of quantitation

The peak areas for noscapine and embonic acid were measured by a computer (Pye Unicam CDP 1). A calibration curve for known concentrations was prepared by plotting the peak area for each concentration. Unknown concentrations in specimens were calculated from the areas exhibited by calibration solutions having similar concentrations to those expected in the samples. All injections were carried out on the same day in sequence: one standard, three samples, one standard, etc.

RESULTS AND DISCUSSION

Chromatographic separation

In most cases, phosphate buffers in aqueous methanol or acetonitrile are used as the mobile phase for the determination of noscapine and related compounds by reversed-phase chromatography. Octadecyl-silica is usually employed as the stationary phase[6–8], although a bonded nitrile phase has also been reported[3]. The solubility of noscapine hydrogen embonate in water–methanol or water–acetonitrile mixtures is very poor, however, which causes problems in the simultaneous separation of the salt components.

Aqueous dimethylformamide is an excellent solvent over a wide pH range for noscapine hydrogen embonate as well as for both salt components. In this study, a medium polar cyano column was successfully used with this polar eluent to separate noscapine and embonic acid by the reversed-phase mechanism. The eluent was buffered with phosphate and sodium perchlorate was used as the ion-pair reagent.

The pH of the buffer was selected as 5.5 to ensure proper separation of the salt components. A decrease in pH shortens the retention time of noscapine while the retention of embonic acid is increased (Fig. 2.3). With sodium perchlorate the retention of both components is somewhat decreased, particularly that of embonic acid, with an advantageous effect on the symmetry of the peaks (Fig. 2.4) which is indicative of ion-pair formation during elution.

Noscapine is easily oxidized yielding cotarnine, meconine and opianic acid[9]. These three degradation products were also found in solutions of noscapine hydrogen embonate which had been standing for several days. The separation of these compounds can be controlled by the dimethylformamide concentration of the eluent rather than by a change in pH (Figs. 2.2 and 3). Usually, in quality-control analysis, a dimethylformamide concentration of 10% is adequate (Fig. 2.1). When the concentration of cotarnine in the sample is more than about 5%, however, quantitation of embonic acid may become inaccurate. A decrease in the dimethylformamide con-

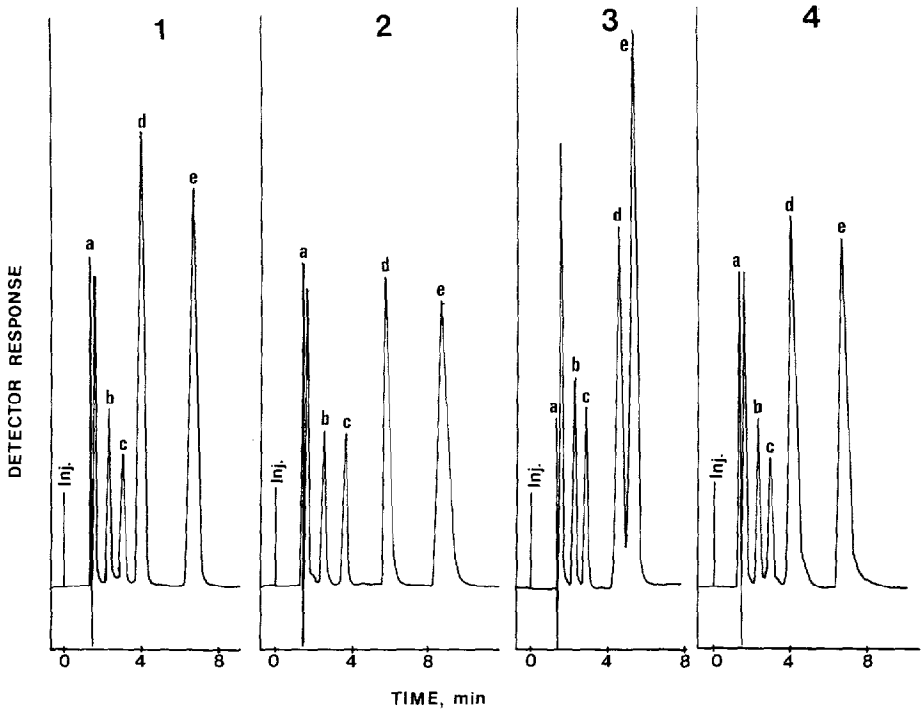

Fig. 2. Effect of eluent composition on the separation of noscapine, embonic acid and the three degradation products of noscapine hydrogen embonate. (1) Chromatographic conditions as recommended in the Experimental section. (2) Conditions as in (1) but with dimethylformamide concentration changed to 6.3% (v/v). (3) Conditions as in (1) but with buffer pH changed to 4.0. (4) Conditions as in (1) but with buffer sodium perchlorate concentration changed to 0.01 *M*. Peaks: a = opianic acid (0.01 mg/ml); b = meconine (0.01 mg/ml); c = cotarnine (0.01 mg/ml); d = embonic acid (0.18 mg/ml); e = noscapine (0.19 mg/ml).

centration ensures complete separation of cotarnine from embonic acid as well as from meconine.

It is also evident from Figs. 2 and 3 that the retention of opianic acid is not affected by the eluent composition. As a result, the specificity of the method for this highly mobile compound is always poor.

Linearity of the HPLC method for the salt components

A linear response was obtained for both salt components injected in quantities of 0.3–3 μg. The equation corresponding to a typical calibration plot for noscapine is expressed as $Y = 47558X - 62$ where $r = 0.9998$ and that for embonic acid as $Y = 37363X + 23$ where $r = 0.9999$; X is the amount (μg) of salt component injected and Y the corresponding area (integrator units). Because of the neglible intercept with the usual concentrations injected, the concentration is in practice directly proportional to the corresponding area.

Day-to-day reproducibility of peak area and retention time

The precision of the peak area and retention time for both salt components was determined by eight injections of a single freshly prepared solution of noscapine

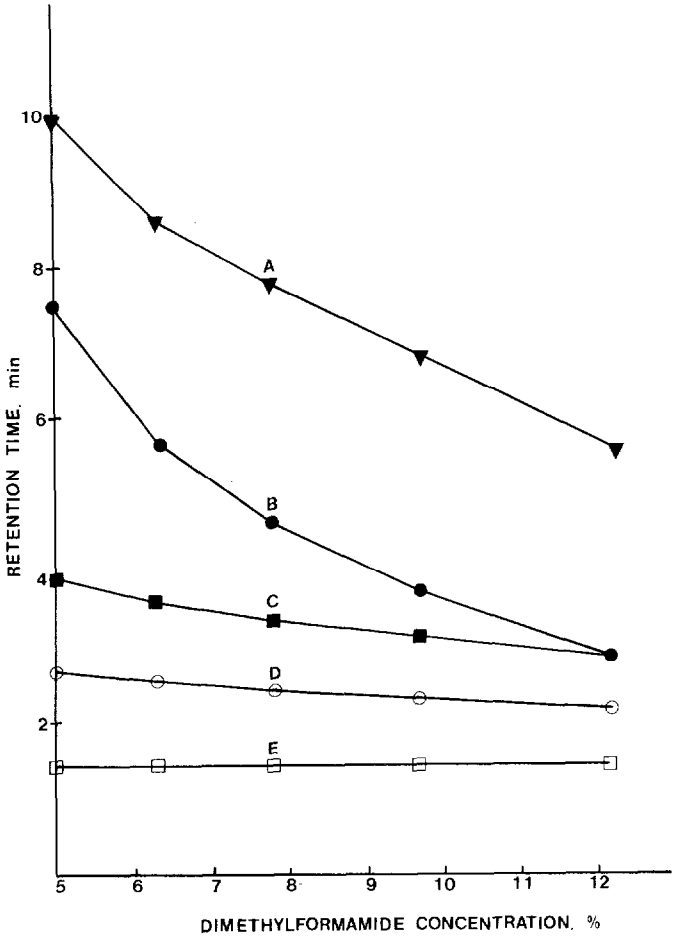

Fig. 3. Effect of dimethylformamide concentration on the retention times of noscapine, embonic acid and three degradation products of noscapine hydrogen embonate. A = Noscapine; B = embonic acid; C = cotarnine; D = meconine; E = opianic acid. Other chromatographic conditions as in the Experimental section.

hydrogen embonate monohydrate (40.0 mg per 100 ml) on each of 3 days. The precision was calculated each day as the relative standard deviation. The chromatographic precision of the peak area was taken to be the average of the three relative standard deviations, *i.e.*, 0.43% for noscapine and 0.66% for embonic acid (Table I). The mean retention time for noscapine was 6.57 min and for embonic acid, 3.84 min. The precision of the retention time on each day for both components was better than 1%.

Accuracy and assay precision

An estimate of the accuracy and procedural precision was obtained by assaying six samples (each 40.0 mg) of noscapine hydrogen embonate *versus* a standard. The recovery of the salt was 100.1%, and the relative standard deviations for noscapine,

TABLE I

DAY-TO-DAY REPRODUCIBILITY OF PEAK AREA AND RETENTION TIME BY INJECTIONS OF REPLICATE SAMPLES (n = 8) OF NOSCAPINE HYDROGEN EMBONATE MONOHYDRATE (40.0 mg/100 ml)

Day	*Salt component*	*Peak area*		*Peak retention time*	
		Mean (integrator units)	*R.S.D. (%)*	*Mean (min)*	*R.S.D. (%)*
1	Noscapine	96205	0.38	6.57	0.56
2	Noscapine	95229	0.46	6.58	0.83
3	Noscapine	96193	0.46	6.55	0.99
1	Embonic acid	71457	0.42	3.88	0.68
2	Embonic acid	70393	0.70	3.83	0.97
3	Embonic acid	70669	0.87	3.80	0.98

embonic acid and noscapine hydrogen embonate were 0.40, 0.43 and 0.50%, respectively (Table II).

Analysis of noscapine hydrogen embonate capsules

The results of the capsule analysis are shown in Table III. No extra peaks were produced in the chromatograms by the excipients themselves.

Detection limits for the degradation products of noscapine hydrogen embonate

Based on a signal-to-noise ratio of 3:1, about 1 ng of cotarnine, 1.4 ng of meconine and 2.6 ng of opianic acid were detected at the highest detector sensitivity ($\times$ 0.005 a.u.f.s.). This indicates that if the analysis is carried out with a 40-mg sample and an injection volume of 10 μl, as recommended, 0.025% of the cotarnine,

TABLE II

DETERMINATION OF NOSCAPINE HYDROGEN EMBONATE MONOHYDRATE: ACCURACY AND ASSAY PRECISION

The noscapine content of each sample corresponds to 20.2 mg and that of embonic acid to 19.0 mg.

Sample	*Found (mg)*			*Recovery of the salt (%)*
	Noscapine	*Embonic acid*	*Noscapine hydrogen embonate (anhydrous)*	
1	20.3	19.0	39.3	100.5
2	20.3	18.9	39.2	100.3
3	20.2	18.9	38.9	99.5
4	20.3	19.0	39.3	100.5
5	20.1	18.8	38.9	99.5
6	20.2	19.0	39.3	100.5
Mean ± S.D.	20.2 ± 0.082	18.9 ± 0.082	39.2 ± 0.020	100.1 ± 0.50

TABLE III

ANALYSIS OF NOSCAPINE HYDROGEN EMBONATE MONOHYDRATE CAPSULES

The noscapine content of each capsule corresponds to 20.2 mg and that of embonic acid to 19.0 mg. The other ingredients were lactose (250 mg), talc (3 mg) and magnesium stearate (1 mg).

Capsule	*Found (mg)*			*Recovery of the salt (%)*
	Noscapine	*Embonic acid*	*Noscapine hydrogen embonate (anhydrous)*	
1	20.1	18.6	38.7	98.9
2	20.4	19.1	39.5	101.0
3	20.0	19.0	39.1	99.9
4	20.4	18.8	39.2	100.2
5	20.1	18.8	38.9	99.4
6	20.1	18.8	38.9	99.4
7	20.1	18.8	38.9	99.4
8	20.2	18.9	39.1	99.9
Mean ± S.D.	20.2 ± 0.15	18.9 ± 0.15	39.0 ± 0.24	99.8 ± 0.64

0.035% of the meconine and 0.065% of the opianic acid present in the material still produce signals of reasonable strength for the detection of these compounds.

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