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Note

Rapid and sensitive determination of ambroxol in human plasma and urine by high-performance liquid chromatography

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Ambroxol hydrochloride [*trans*-4-(2-amino-3,5-dibromobenzyl)aminocyclohexanol hydrochloride] is an expectorant drug, promoting bronchial secretion and having mucolytic properties. Ambroxol is a metabolite of bromhexine [1].

For the determination of ambroxol in biological fluids, a high-performance liquid chromatographic (HPLC) method using UV detection [2] and a capillary gas chromatographic method using electron-capture detection [3] were recently published. Both methods comprise single-step extraction and derivatization with formaldehyde. The methods are time-consuming and need a relatively large amount of sample (1 ml) to reach a low detection limit. Furthermore, a radiochemical derivatization of ambroxol with [^{14}C]formaldehyde has been described [1,4]. Because of the thin-layer detection involved this method not suitable for kinetic studies in which a lot of samples must be analysed.

This paper describes a rapid HPLC method with UV detection for the determination of ambroxol. A relatively small amount of plasma or urine sample is required and ambroxol in plasma and urine can be determined in the low ng/ml range.

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EXPERIMENTAL

Apparatus

The Waters Assoc. chromatographic system consisted of a Model 510 solvent-delivery system and Model 481 UV detector connected to a 10-mV BBC SE-120 recorder. A Rheodyne Model 7125 injection valve with a 50-μl injection loop was used.

Chromatography

The analytical column (250 mm$\times$4.6 mm I.D., 5 μm particle size) was pre-packed with Hypersil 5-ODS (Chrompack, Middelburg, The Netherlands). A stainless-steel column (100 mm$\times$2.0 mm I.D.) packed with pellicular reversed phase (Chrompack) was used as guard column.

The mobile phase, acetonitrile–methanol–0.01 *M* phosphate buffer (pH 7.0)–tetrahydrofuran (35:35:27.5:2.5, v/v), was filtered through a Millipore 0.5-μm filter, type FH (Milford, MA, U.S.A.) and degassed ultrasonically before use. The flow-rate was 2.0 ml/min and the chromatography was performed at room temperature. Detection was effected at 242 nm.

Chemicals

For the quantitative analyses, pure ambroxol hydrochloride Lot MP/0253/2 (supplied by Euderma, Italy) was used. Diethyl ether, methanol, acetonitrile, tetrahydrofuran and water were all of HPLC grade (Fisons, Loughborough, U.K.).

The 0.01 *M* phosphate buffer (pH 7.0) was prepared by dissolving 0.54 g of potassium dihydrogenphosphate and 1.07 g of disodium hydrogenphosphate dihydrate in 1000 ml of water.

Buffer pH 10 (Titrisol®) was obtained from Merck (Darmstadt, F.R.G.). The 0.01 *M* hydrochloric acid was prepared by diluting 0.83 ml of concentrated hydrochloric acid (12 *M*) to 1000 ml with water.

The extraction tubes for plasma were 10-ml conical polypropylene tubes with polypropylene stoppers and 5-ml glass tubes. For urine only the 5-ml glass tubes were used.

Standard solutions

Standard stock solution was made by dissolving 15.5 mg of ambroxol hydrochloride in 20 ml of methanol. For plasma the standard stock solution was diluted 2000-fold with methanol. This diluted solution was used to make a calibration curve for human blank plasma with concentrations ranging from 10 to 200 ng/ml. For urine the standard stock solution was diluted with human blank urine to make a calibration curve with concentrations in the range 1–30 μg/ml.

Procedure

Plasma. To 0.5 ml of plasma were added 0.5 ml of buffer pH 10 and 4.0 ml of diethyl ether. The mixture was vortexed for 60 s and then centrifuged at 2600 *g* for 6 min. The diethyl ether layer was transferred to a clean glass tube, and 200 μl of 0.01 *M* hydrochloric acid were added. The mixture was vortexed for 60 s and

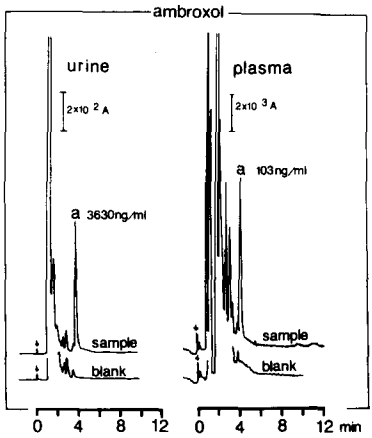

Fig. 1. HPLC of ambroxol (a) in plasma and urine samples obtained from a volunteer after the intake of 75 mg of ambroxol hydrochloride and their respective blanks, sampled prior to drug intake.

centrifuged at 2600 *g* for 6 min. The diethyl ether layer was discarded, and 50 µl of the acid layer were injected onto the column.

Urine. To 0.1 ml of urine were added 0.1 ml of buffer pH 10 and 2.0 ml of diethyl ether. Extraction was performed as for plasma.

The retention time of ambroxol was 4.3 min.

Standard solutions of ambroxol hydrochloride in plasma and urine were treated and analysed simultaneously with samples. The sample concentrations were calculated on the basis of peak height by reference to standard curves obtained by linear regression.

Testing of the analytical procedure

The accuracy, precision and linearity of the method were determined using spiked samples of human plasma and urine analysed at random. Total recovery of ambroxol was determined as the response from extracted standards relative to the response of standards in 0.01 *M* hydrochloric acid injected directly onto the column.

RESULTS

Fig. 1 shows representative chromatograms of the determination of ambroxol in urine and plasma samples. These samples were obtained from a volunteer after oral administration of 75 mg of ambroxol hydrochloride slow release capsules manufactured by Euderma. Blank samples gave no interfering peaks and no interference was observed with other (mucolytic) drugs.

The analytical procedure for ambroxol in urine and plasma was found to be accurate and precise (Table I). The accuracy was 100.1% (range 93.6–105.2%) for plasma and 97.7% (range 94.0–100.9%) for urine, calculated as percentage found on the linear standard curve. The precision, expressed as the relative stan-

TABLE I

ACCURACY AND PRECISION OF THE DETERMINATION OF AMBROXOL

Spiked human plasma and urine were used for six determinations at each concentration.

Sample	Amount added	Mean amount found	Accuracy (%)	Precision (R.S.D.) (%)
Plasma	194	190	97.9	4.2
(ng/ml)	116	120	103.5	4.2
	58	61	105.2	3.3
	31	29	93.6	2.8
Mean			100.1	3.6
Urine	46.5	46.9	100.9	3.9
(μg/ml)	23.3	22.9	98.3	5.5
	11.6	10.9	94.0	4.8
	3.9	3.8	97.4	2.9
Mean			97.7	4.3

dard deviation (R.S.D., $n=6$) was 3.6% (range 2.8–4.2%) for plasma and 4.3% (range 2.9–5.5%) for urine. The calibration curves were linear for both plasma and urine ($r=0.9990$ and 0.9994, respectively).

Total recovery of ambroxol was $87 \pm 3\%$ for plasma and $91.7 \pm 5.6\%$ for urine.

Fig. 2 shows the plasma concentration–time curve and the renal excretion rate–time profile of urine of ambroxol in one volunteer after an oral dose of 75 mg of ambroxol. Some pharmacokinetic parameters are summarized in Table II.

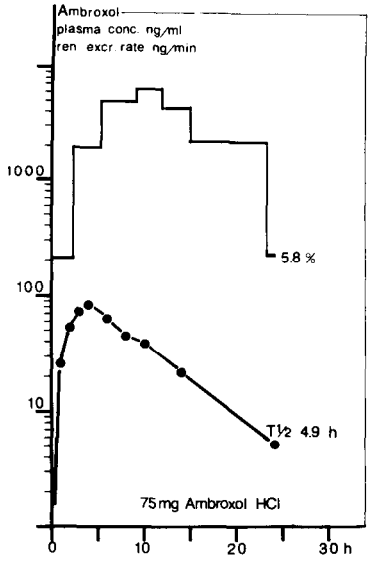

Fig. 2. Pharmacokinetics of ambroxol after an oral dose of 75 mg of ambroxol hydrochloride (slow release) to a volunteer.

TABLE II

SOME PHARMACOKINETIC ON PARAMETERS OF AMBROXOL AFTER ORAL ADMINISTRATION OF 75 mg IN A VOLUNTEER

Parameter	Value
Elimination half-life, $T_{1/2}$ (h)	4.9
Maximum plasma concentration, C_{max} (ng/ml)	84
Time to reach C_{max}, T_{max} (h)	4.0
Area under plasma concentration-time curve, AUC_{0-24} (ng/ml h)	804
Amount of drug excreted in urine (% of dose)	5.8

DISCUSSION

The HPLC method described in this paper allows the determination of ambroxol in biological fluids at concentrations as low as 5 ng/ml using a small amount of sample. This method is faster than the derivatization method, and ca. 50–60 samples a day can be handled. Also, the low detection limit (5 ng/ml) can be reached with a small amount of sample (0.5 ml of plasma and 0.1 ml of urine) whereas the other method needs 1.0 ml of plasma to reach a detection limit of 10 ng/ml.

This method can be used for kinetic studies, as shown in Fig. 2. Detailed results of the bioavailability of ambroxol hydrochloride preparations and the clinical implications will be published elsewhere.

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