Determination of Dextromethorphan in Biological Fluids by Liquid Chromatography by Using Semi-microbore Columns

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Abstract □ A sensitive, specific procedure for the determination of dextromethorphan in plasma and urine in dogs is described. The use of a semi-microbore column, 2.1 mm i.d. × 25 cm resulted in a detection limit of 1 ng/mL in plasma and 25 ng/mL in urine. Plasma (3 mL) is extracted in CCl_4 and the drug is back-extracted with 3% acetic acid. The chromatographic separation is carried out using a 5-μm particle size octadecyl-bonded column, and the drug is monitored by a fluorescence detector. The results after 10-mg oral administrations of dextromethorphan to dogs are provided.

Keyphrases □ Dextromethorphan—HPLC determination, semi-microbore column, dog plasma, urine

Dextromethorphan is a widely used synthetic antitussive agent. Due to significant first-pass hepatic metabolism, only a trace amount of unmetabolized drug is found in circulation, usually <5 ng/mL after oral administration (1, 2). Previous reports of dextromethorphan analysis involved electron-capture GC, nitrogen-phosphorus detection GC and RIA (1–3). There has been no report in the literature on the assay of dextromethorphan in biological fluids by high-performance liquid chromatography (HPLC) due to a lack of sensitivity of the instrument at this level. We report here an HPLC method for determining dextromethorphan using semi-microbore columns capable of measuring the drug in amounts as low as 1 ng/mL.

EXPERIMENTAL SECTION

Materials—All chromatographic solvents were HPLC grade, reagents were ACS, U.S.P., or N.F. quality and were used as received without further purification. Dextromethorphan HBr[1] was U.S.P. grade and was used as received. The apparatus was a modular unit consisting of a reciprocating pump[2], a fluorescence detector[3], an autoinjector[4], and a strip-chart recorder[5]. All columns were prepacked and octadecylsilane bonded[6]. The average particle size of the packing material was 5 μm. The mobile phase was acetonitrile–water (45:55) containing 0.01 M monobasic ammonium phosphate and 0.005 M sodium lauryl sulfonate. The pH was adjusted to 3.0 using phosphoric acid. The flow rate was maintained at 300 μL/min.

Sample Preparation—*Plasma Extraction Procedure*—Plasma (3 mL) was transferred into a screw-cap disposable tube (16 mm × 125 mm), 0.5 mL of 6 M HCl was added, and the mixture was vortexed; 10 M NaOH (0.4 mL) was then added and mixed until a clear mixture was obtained. Immediately, 10 mL of CCl_4 was added and the mixture was first handshaken gently a few times and then shaken for 30 min on a mechanical horizontal shaker. After a 10-min centrifugation at 2500 rpm, the top aqueous layer of the sample was completely removed by suction and discarded. The organic layer was poured into a 20 × 150 mm wide-mouth test tube. The carbon tetrachloride layer (8 mL) was transferred into another 16 × 125 mm disposable screw-cap tube. (In this step, it is essential to avoid having any of the residual aqueous layer enter the pipette.) Acetic acid (3%) was added (0.5 mL) and shaken for 30 min on a horizontal shaker. The tube was centrifuged for 5 min at 2500 rpm and a portion of the acid layer was removed for analysis.

A urine sample (5 mL) was diluted to 50 mL with 3% acetic acid. The diluted urine was injected directly into the liquid chromatograph for dextromethorphan analysis. Three healthy beagle dogs (weight, ~10 kg) were dosed orally with 10 mg of dextromethorphan hydrobromide in a physiological saline solution. Blood (8 mL) was withdrawn with a heparinized syringe at predose, 0.5, 1, 2, 4, 8, and 24 h after dosing. The blood was immediately centrifuged and the plasma was extracted for dextromethorphan analysis as described above. Urine samples were collected over a 24-h period and pooled.

Standard Curve and Calibration—Aqueous solutions of dextromethorphan hydrobromide were added to blank plasma samples such that the plasma contained 1–5 ng/mL of dextromethorphan hydrobromide. These standard plasma samples were extracted as described under sample preparations. Calibration curves were plotted using peak height *versus* the plasma drug concentration using the least-squares method. The concentrations of the drug in samples were interpolated from the standard curve.

Aqueous solutions of dextromethorphan hydrobromide were added to the blank urine samples to yield concentrations of 50–500 ng/mL dextromethorphan hydrobromide. These samples were diluted 5–50 mL with 3% acetic acid solution.

RESULTS AND DISCUSSION

Dextromethorphan has a UV maximum at 280 nm, so the initial experiments were carried out at this wavelength. The detection limit at 280 nm was not satisfactory for plasma analysis. By changing the wavelength to 200 nm, a 20-fold increase in sensitivity was obtained, but endogenous materials from plasma interfered. Since dextromethorphan has good fluorescent properties, the detection mode was changed from UV to fluorescence. Although the detection limit by fluorescence was similar to that by UV at 200 nm, the specificity improved significantly. The plasma blank showed no interference due to endogenous substances in the dextromethorphan elution region of the chromatogram. The use of semi-microbore columns (2.1 mm i.d.) increased the sensitivity of dextromethorphan fivefold with conventional 4.6 mm i.d. columns.

The chromatograms of the extracts of both human plasma and canine plasma were identical. For this reason, pooled human plasma was used to obtain a standard curve for dog plasma analysis since blank dog plasma was in short supply. Figure 1 shows the chromatograms of unspiked and dextromethorphan hydrobromide-spiked (1 ng/mL) human plasma. Figure 2 shows the chromatograms of dog urine blank and that spiked with 50 ng/mL. The detection limit in the urine samples is ~20 ng/mL.

Linearity—A linearity study was conducted on plasma samples by spiking with known amounts of dextromethorphan hydrobromide; levels of 0, 1, 2, 3, and 5 ng/mL were investigated. Between-day variation was examined by an analysis of variance of slopes and intercepts from 11 standard curves run over a period of 3 weeks. The slopes of these curves had an *RSD* of ±8.7%. This variation is not unusual and is related to a slight change in the chromatographic column performance over a period of time. A small interference is indicated by a positive intercept which amounted to an equivalent of 0.30 ng/mL of dextromethorphan hydrobromide. The data are summarized in Table I.

A linearity study on the urine sample was done by spiking blank urine with dextromethorphan hydrobromide at levels of 50–500 ng/mL. A correlation coefficient of 0.9996 was found. This much improved correlation for the urine standards as compared to the plasma standards is due to the fact that no extraction was involved in urine standards.

Absolute Recovery of Dextromethorphan from Biological Fluids—Recovery studies of dextromethorphan from spiked plasma were carried out. Recovery data were calculated against an unextracted standard (Table II). A small interference was found in the blank plasma and this peak height was subtracted from the sample peak height for the calculation of the recovery data. The *RSD* values were ~7–9% at the 2–5 ng/mL level, and 15% at 1 ng/mL, which is about the lower limit of detection by this method.

[1] Hoffman-La Roche, Nutley, N.J.
[2] Model 6000; Waters Associates, Milford, Mass.
[3] Model GM 970; Kratos, Ramsey, N.J.
[4] Model WISP 710; Waters Associates.
[5] Model 56; Perkin-Elmer Corp., Norwalk, Conn.
[6] 2.1 mm × 25 cm, packed with Partisil ODS-3; Alltech Assoc., Deerfield, Ill.

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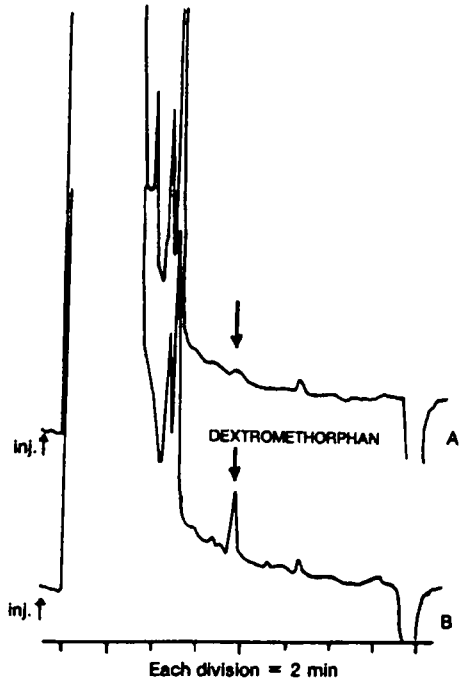

Figure 1—*Chromatograms of plasma blank (A) and plasma spiked with 1 ng/mL of dextromethorphan hydrobromide (B). Arrows indicate dextromethorphan elution areas.*

Recoveries of dextromethorphan hydrobromide from urine samples were essentially 100% since the fluorescence responses in the spiked urine samples were similar to those of the aqueous standards at the same concentration. The results were expected since there was no simple extraction involved. The urine samples were simply diluted with 3% acetic acid solution and then injected into the HPLC system.

Dextromethorphan Level After Oral Administration To Dogs—A typical plasma dextromethorphan concentration *versus* time curve in dogs after 1 mg/kg oral administration is shown in Fig. 3. These results compare well with those found by Barnhart and Massad (1, 4) who found 2.1 ng/mL peak maximum in dog plasma after 1 mg/kg oral administration. No detectable

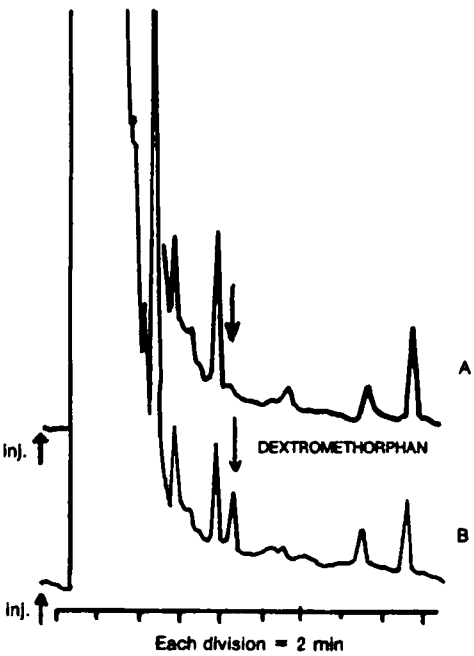

Figure 2—*Chromatograms of dog urine blank (A) and urine spiked with dextromethorphan hydrobromide at 50 ng/mL (B). Arrows indicate dextromethorphan elution areas.*

Table I—Between-Day Variation of Dextromethorphan Linearity [a]

n	ng/mL	Peak Height, mm ± *SD*
9	0	4.3 ± 1.4
8	1	15.6 ± 1.4
9	2	29.6 ± 1.3
9	3	44.1 ± 3.7
11	5	72.7 ± 6.3

[a] The regression yielded $r = 0.9965$ and $y = 13.8x + 3.2$.

Table II—Recovery of Dextromethorphan From Plasma

n	Spiked Plasma, ng Dextromethorphan Hydrobromide/mL	Recovery, % ± *SD*
8	1	41.1 ± 5.8
9	2	48.7 ± 3.8
9	3	51.6 ± 3.3
11	5	53.3 ± 4.6

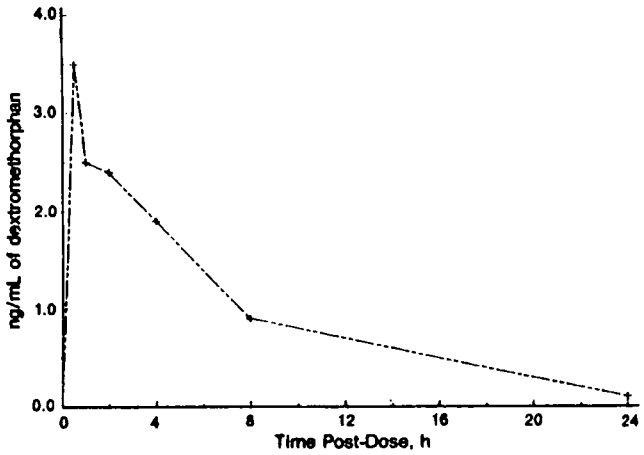

Figure 3—*Plasma dextromethorphan concentration* versus *time curve in the dog after 1-mg/kg oral administration of dextromethorphan hydrobromide.*

amount of dextromethorphan was found in pooled urine. This finding is also consistent with that found by Barnhart and Massad who recovered only a trace of the drug (0.12% of the total dose) after a 1.073 mg/kg oral administration.

The method described above is simple, accurate, and specific for determining dextromethorphan in plasma and urine. The use of semi-microbore columns allows detection limits of 1 ng of dextromethorphan/mL of plasma and 20 ng/mL of urine.

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