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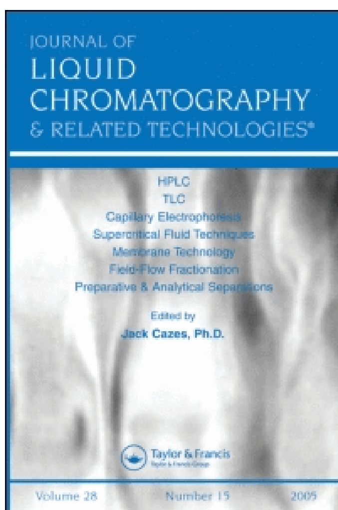

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Simultaneous Determination of Dextromethorphan and Dextrorphan in Human Plasma, Urine and Cerebrospinal Fluid by HPLC with Fluorescence Detection

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SIMULTANEOUS DETERMINATION OF DEXTROMETHORPHAN AND DEXTRORPHAN IN HUMAN PLASMA, URINE AND CEREBROSPINAL FLUID BY HPLC WITH FLUORESCENCE DETECTION

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ABSTRACT

Dextromethorphan is a non-narcotic antitussive drug that recently attracted interest because of its anticonvulsant and neuroprotective properties. Dextromethorphan undergoes polymorphic oxidation and 5-10% of human subjects are characterised as poor metabolizers on the basis of the formation rate of its O-demethylated metabolite, dextrorphan catalysed by isozyme CYP2D6.

A sensitive, selective and reliable high performance liquid chromatographic (HPLC) method for the simultaneous determination of dextromethorphan and dextrorphan in human

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plasma, urine, and cerebrospinal fluid, is described, using levallorphan as the internal standard. The analytes were extracted from biological fluids by a liquid-liquid extraction procedure. The chromatographic separation was performed on a 5-μm cyano analytical column (220 X 4.6 mm) using a mixture of acetonitrile/distilled water/n-octylamine (19/80.95/0.05, v/v) pH 2.8 as the mobile phase with a flow rate of 1.0 mL/min at 40^{0}C and the chromatographic peaks were measured by fluorescence detection.

The calibration curves were linear with a correlation coefficient of 0.999 or better from 1-100, 5-500, and 1-100 ng/mL for both drugs in plasma, urine, and cerebrospinal fluid, respectively. The method was not interfered with by other endogenous components or concurrent antiepileptic drugs. The within-day precision of the method was evaluated for three concentrations and was found to be less than 6%, and the accuracy was nearly 100%.

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INTRODUCTION

Dextromethorphan is a widely used and effective non-narcotic antitussive drug. It is a centrally acting opioid congener and its potency is nearly equal to that of codeine and unlike the latter, it does not cause any addiction or respiratory depression.[1,2] Recently dextromethorphan has attracted interest because of its anticonvulsant and neuroprotective properties.[3,4]

Dextromethorphan is O-demethylated to dextrorphan, its main active metabolite, which is mainly in conjugated form as the glucuronide. The O-demethylation of dextromethorphan in man is catalysed by a specific isozyme (CYP2D6) and it is under genetic control, cosegregating with the well-known polymorphic debrisoquine 4-hydroxylation.[5,6] This particular isozyme is also responsible for the metabolism of other commonly used drugs.[7,8] CYP2D6 may be absent or deficient in a certain percentage of people. Most subjects are characterised as "extensive metabolizers", whereas the rest (5-10%) as "poor metabolizers". Dextromethorphan is used as a marker for the CYP2D6 isozyme and the ratio of parent drug to its metabolite dextrorphan defines a subject as an "extensive" or "poor" metabolizer.[9,10]

This paper describes a selective, sensitive, precise, and accurate high performance liquid chromatographic assay for the simultaneous determination of

dextromethorphan and its major active metabolite dextrorphan, in human plasma, urine, and cerebrospinal fluid, with advantages over previously published HPLC methods.[5,9,11-17] This method has been applied to the pharmacokinetic study in epileptic patients receiving dextromethorphan in combination with other antiepileptic drugs and for determining phenotype status for the CYP2D6 isozyme.[18]

MATERIALS AND METHODS

Chemicals

HPLC-grade acetonitrile and hexane and analytical-reagent grade 85% phosphoric acid, sodium carbonate and n-octylamine were obtained from Merck (Darmstadt, Germany). Water was deionized and distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a 0.45 µm pore size nylon filter (Alltech, Deerfield, IL, USA) and vacuum degassed by sonication before use. Dextromethorphan hydrobromide, dextrorphan tartrate and levallorphan tartrate (internal standard) were kindly supplied by Hoffman-La Roche (Basle, Switzerland).

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Apparatus

A model 2510 liquid chromatographic system (Varian, Palo Alto, CA, USA), equipped with a 7125 Rheodyne manual injection valve with a fixed 100 µL loop (Cotati, CA, USA), a model 502 variable fluorescence detector (Scientific Systems Inc., State College, PE, USA) coupled with a 4290 Varian integrator was used. Separation was performed using a Brownlee Spheri-5 cyano analytical column (5 µm, 220 X 4.6 mm, ID) (Applied Biosystems, Foster City, CA, USA) proceeded by a cyano guard column (5 µm, 10 X 4.6 mm, ID).

Chromatographic Conditions

The mobile phase consisted of acetonitrile/distilled water/n-octylamine (19/80.95/0.05, v/v), adjusted to pH 2.8 with phosphoric acid. The chromatographic system was operated isocratically at a flow rate of 1.0 mL/min at 40^{0}C, resulting in an inlet pressure of approximately 1600 psi, and the chromatographic peaks were measured by fluorescence detection at 230 and 330 nm for λ_{ex} and λ_{em}, respectively.

Sample Preparation

Venous blood samples were collected in heparinized glass tubes and centrifuged at 2000 g for 10 min, and the plasma fractions were removed. Urine samples were collected via spontaneous voiding, the total volume of urine was recorded and an aliquot was kept. Cerebrospinal fluid samples were obtained using a standard lumbar puncture technique. All plasma, urine, and cerebrospinal fluid samples were stored at -70^{0}C and thawed and vortex mixed before analysis.

To a 15-mL stoppered silanized glass centrifuge tube containing 1 mL of plasma, urine (diluted 3:1 or more with distilled water), or cerebrospinal fluid, were added 100 μL of internal standard solution (0.1 μg/mL). After vortexing for a few seconds, 0.5 mL of saturated sodium carbonate solution was added, the sample briefly mixed and 5 mL of hexane containing 0.1% n-octylamine was added. After mixing on a vortex mixer for 60 sec, the sample was centrifuged at 2000 g for 10 min and the organic phase was transferred into a clean centrifuge tube. The aqueous phase was re-extracted with 5 mL of hexane containing 0.1% n-octylamine and the combined hexane extracts were evaporated to dryness under nitrogen in a 50^{0}C water bath. The residue was reconstituted with 150 μL of 0.1 M HCl and a 100-μL aliquot was injected into the HPLC system.

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Working standard solutions of dextromethorphan, dextrorphan and internal standard, levallorphan were prepared in distilled water (1 μg/mL, as base). The procedure was standardized by analysing drug-free plasma, urine, or cerebrospinal fluid samples, spiked with internal standard solution and appropriate volumes of working standard solutions to formulate dextromethorphan and dextrorphan concentrations of 1-100 ng/mL, 5-500 ng/mL, and 1-100 ng/mL for plasma, urine, and cerebrospinal fluid, respectively, as in the sample preparation procedure.

The concentrations of dextromethorphan and dextrorphan in plasma, urine, and cerebrospinal fluid samples, were calculated by interpolation from the linear least-squares regression line of the standard curve plot of peak height ratios of dextromethorphan/internal standard and dextrorphan/internal standard, versus dextromethorphan and dextrorphan concentrations in the calibration standards.

RESULTS AND DISCUSSION

The peaks of the studied analytes were well separated from each other giving retention times of 5.3, 7.4 and 10.7 min for dextrorphan, levallorphan

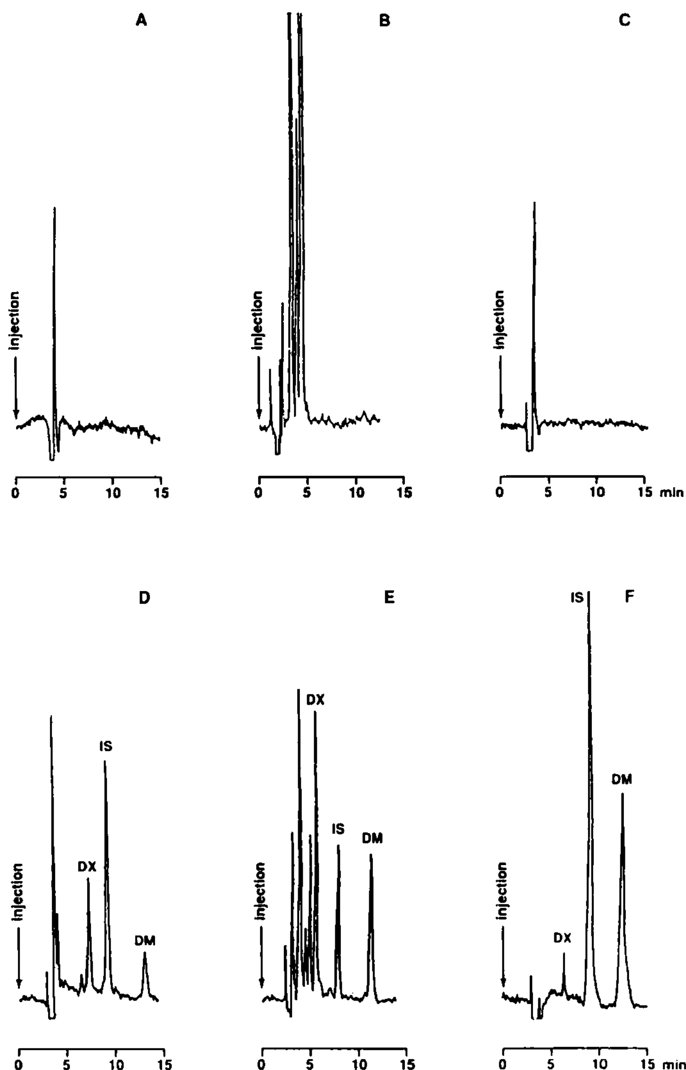

Figure 1. Chromatograms of (A) drug-free plasma; (B) drug-free urine; (C) drug-free cerebrospinal fluid; (D) plasma sample from an epileptic patient after the last steady-state oral dose of 50mg/6h of dextromethorphan hydrobromide; (E) urine sample from the same patient; (F) cerebrospinal fluid sample from the same patient. Peaks: DX=dextrorphan (retention time=5.3 min); IS=internal standard levallorphan (retention time=7.4 min); DM=dextromethorphan (retention time 10.7 min).

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Table 1

Within-day Precision and Accuracy Data of the Determination of Dextromethorphan and Dextrorphan in Human Plasma, Urine and Cerebrospinal Fluid

Concentration Added (ng/mL)	Concentration Found (ng/mL)	Precision CV (%) (n=6)	Accuracy Concentration Found/Added (%)
Plasma			
Dextromethorphan			
5.0	5.2	5.4	104
50.0	48.3	3.3	97
100.0	98.8	2.6	99
Dextrorphan			
5.0	4.7	5.8	94
50.0	51.4	3.5	103
100.0	101.7	2.8	102
Urine			
Dextromethorphan			
10.0	9.8	5.6	98
200.0	203.1	2.9	102
400.0	395.6	2.6	99
Dextrorphan			
10.0	10.3	5.9	103
200.0	197.2	3.1	99
400.0	403.8	2.9	101
Cerebrospinal Fluid			
Dextromethorphan			
5.0	4.8	5.3	96
50.0	51.5	3.6	103
100.0	102.1	2.9	102
Dextrorphan			
5.0	5.3	5.9	106
50.0	48.6	3.8	97
100.0	99.3	3.1	99

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(internal standard), and dextromethorphan, respectively. The retention times were found to be reproducible and the coefficients of variation were less than 0.5%. Fig. 1 shows representative chromatograms for dextrorphan, levallorphan, and dextromethorphan in human plasma, urine, and cerebrospinal fluid samples. Fig. 1A, 1B, and 1C show the chromatograms from the analysis of drug-free human plasma, urine, and cerebrospinal fluid, respectively.

Fig. 1D, 1E, and 1F show typical chromatograms of the plasma, urine, and cerebrospinal fluid samples (at designated intervals) from an epileptic patient, receiving concurrent antiepileptic drugs (phenytoin and carbamazepine), after the last steady-state oral dose of 50mg/6h of dextromethorphan hydrobromide. In these chromatograms there were no interfering peaks due to endogenous substances or antiepileptic drugs at the retention times of dextrorphan, levallorphan, and dextromethorphan.

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A least-squares linear regression analysis was used to calculate the equation relating the peak height ratio of dextromethorphan and dextrorphan to internal standard (Y) versus drug concentration (ng/mL) in spiked plasma, urine or cerebrospinal fluid samples (X). The calibration curves were linear with a correlation coefficient of 0.999 or better for concentrations ranging from 1 to 100 ng/mL, 5 to 500 ng/mL, and from 1-100 ng/mL for both analytes in plasma, urine, and cerebrospinal fluid, respectively. The detection limit calculated for a signal-to-noise ratio of 4 was *ca.* 1 ng/mL and 0.5 ng/mL for dextrorphan and dextromethorphan, respectively.

The within-day precision and accuracy data of the method were evaluated for three concentrations of each drug in spiked plasma, urine, and cerebrospinal fluid samples. The results, expressed as the mean of six determinations are presented in Table 1. The within-day precision of the assay, expressed as the coefficients of variation for the determined concentrations of both analytes in human plasma, urine, and cerebrospinal fluid was less than 6% and the accuracy, assessed as the percent of the estimated concentration divided by the nominal concentration was nearly 100%.

In conclusion, the HPLC method we developed for the simultaneous quantitation of dextromethorphan and its main active metabolite, dextrorphan, in human plasma, urine, and cerebrospinal fluid, is precise, accurate, selective, and sufficiently sensitive and seems well suited to characterize dextromethorphan and dextrorphan pharmacokinetics, as well as for determining "extensive" and "poor" metabolizers of the debrisoquine/sparteine oxidative polymorphism involving CYP2D6 isozyme.

REFERENCES

1. J. R. Woodworth, S. R. K. Dennis, O. N. Hinsvark, L. P. Amsel, K. S. Rotenberg, J. Clin. Pharmacol., **27**, 133-138 (1987).

2. H. Char, S. Kumar, S. Patel, D. Piemontese, K. Iqbal, A. Waseem, R. A. Salvador, C. R. Behl, J. Pharm. Sci., **81**, 750-753 (1992).

3. D. W. Choi, Brain Res., **403**, 333-336 (1988).

4. F. C. Tortella, M. Pellicano, N. G. Bowery, Trends Pharmacol. Sci., **10**, 501-507 (1989).

5. B. Schmid, R. Bircher, A. Kupfer, Clin. Pharmacol. Ther., **38**, 618-624 (1985).

6. A. Kupfer, B. Schmid, R. Preisig, G. Pfaff, Lancet, **2**, 517-518 (1984).

7. K. Brosen, Clin. Pharmacokinet., **18**, 220-239 (1990).

8. U. A. Meyer, Clin. Pharmacol., **4**, 595-615 (1990).

9. E. Jacqz, H. Dulac, H. Mathieu, Eur. J. Clin. Pharmacol., **35**, 167-171 (1986).

10. R. J. Guttendorf, M. Britto, R. A. Blouin, T. S. Foster, W. John, K. A. Pittman, P. J. Wedlund, Br. J. Clin. Pharmacol., **29**, 373-380 (1990).

11. Y. H. Par, M. P. Kullberg, O.N. Hinsvark, J. Pharm. Sci., **73**, 24-29 (1984).

12. T. East, D. Dye, J. Chromatogr., **338**, 99-112 (1985).

13. O. Mortimer, B. Lindstrom, H. Laurell, A. Rane, U. Bergman, Br. J. Clin. Pharmacol., **27**, 223-227 (1989).

14. Z. R. Chen, A. A. Somogyi, F. Bochner, Ther. Drug Monit., **12**, 97-104 (1990).

15. S. Jaruratanasirikul, A. D. Cooper and T. F. Blaschke, Drug Metab. Disp., **20**, 379-382 (1992).

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16. P. S. Marshall, R. J. Straka, K. Johnson K, Ther. Drug Monit., **14**, 402-407 (1992).

17. Y. W. F. Lam, S. Y. Rodriguez, Ther. Drug Monit., **15**, 300-304 (1993).

18. A. D. Kazis, V. K. Kimiskidis, I. Niopas, Acta Neurol. Scand., in press (1996).

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