Journal of Chromatography, 420 (1987) 217–222
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3743

Note

High-performance liquid chromatographic determination of dextrorphan and 3-hydroxymorphinan in human plasma based on a selective pre-column sample clean-up

HERMANN MASCHER

Pharm-Analyt, P.O. Box 4, A-2514 Traiskirchen, Vienna (Austria)

(First received February 9th, 1987; revised manuscript received April 6th, 1987)

Dextromethorphan is a widely used non-narcotic antitussive agent. Its major metabolites are dextrorphan (I) and 3-hydroxymorphinan (II) [1,2]. Since both substances are found in human plasma almost exclusively as glucuronides and sulphates, enzymic hydrolysis is a necessary part of the determination process. Several methods have been described for the determination of I in human plasma [1–5], and one of these has also been used for the determination of II [2]. The assays were based on gas chromatography with flame-ionization detection [1], high-performance liquid chromatography (HPLC) with fluorescence detection [2,5], HPLC with electrochemical detection [3] and fluorimetry [4]. Some of these procedures are very time-consuming, involving extraction, evaporation and lengthy chromatography; moreover, some of them lack selectivity and sensitivity.

This paper describes a procedure for the determination of I and II in human plasma, based on pre-column clean-up following enzymic hydrolysis. The first step is a selective sample clean-up on-line on a pre-column, the second step is the separation on a reversed-phase column with fluorescence detection. This procedure is highly sensitive: the limit of detection for I and II is as low as 0.2 ng/ml plasma. Moreover, this procedure is rapid, because the time-consuming processes of extraction and evaporation are eliminated. The total analysis time after enzymic hydrolysis is ca. 5 min, which permits analysis of as many as 80 samples per day.

EXPERIMENTAL

Reagents and materials

All reagents were of analytical-reagent grade. Solvents were supplied by Zinsser (Frankfurt/M., F.R.G.) and by E. Merck (Darmstadt, F.R.G.). Dextrorphan

0378-4347/87/$03.50

was kindly supplied by Hoffmann-La Roche (Basle, Switzerland) and 3-hydroxymorphinan by H. Mack (Illertissen, F.R.G.). Glucuronidase/arylsulphatase (127698) was supplied by Boehringer Mannheim (Mannheim, F.R.G.).

High-performance liquid chromatography

The HPLC system consisted of an LC 420 pump (Kontron, Zürich, Switzerland), a Rheodyne valve 7126 (Cotati, CA, U.S.A.), a fluorimeter F1000 (Merck-Hitachi, Darmstadt, F.R.G.) and an integrator CI-10B (LDC, Shannon, Ireland).

The pre-column (10×4 mm I.D.; Bischoff, Leonberg, F.R.G.) was packed with Nucleosil C_{18} (30 μm). The analytical column (125×4 mm I.D., SRD-Pannosch, Vienna, Austria) was packed with Polygosil C_8 (5 μm, Macherey-Nagel, Düren, F.R.G.).

The mobile phase consisted of acetonitrile–0.05 *M* perchloric acid/0.01 *M* triethylamine (20:80, v/v). The flow-rate was 2.0 ml/min. The column was kept at room temperature.

The fluorescence detector was set at an excitation wavelength of 275 nm and an emission wavelength of 305 nm (both bandwidths 15 nm). The 1-ml gas-tight syringes were from Hamilton (Bonaduz, Switzerland).

Plasma preparation

A 0.1-ml volume of 1 *M* acetate buffer (pH 5.2) and 50 μl of enzymic solution (5000 Fishman U of β-glucuronidase and 40 000 Roy U of arylsulphatase) were added to 1 ml of plasma. The mixture was kept overnight at a constant temperature of 37°C. Prior to the HPLC analysis, 1 ml of 0.2 *M* ammonium carbamate solution was added, and the mixture was centrifuged (more than 2000 *g*) for 2 min.

Pre-column clean-up

The pre-column was used instead of a loop connected to the injector. The sample and clean-up solutions were applied manually onto the pre-column, but this could also be done with some auto-sampling injectors (e.g. Gilson, Model 231, Villiers le Bel, France).

The pre-column was first conditioned with 0.5 ml of A (0.02 *M* ammonium carbamate solution). Then 1 ml of the supernatant of the sample was applied, followed by 0.5 ml of A, 1.0 ml of B (mixture of A and acetonitrile, 1:9, v/v) and a third aliquot of 0.5 ml of A. The injection valve was then switched to the inject position for 1 min, and the substances (I and II) were eluted by the mobile phase onto the analytical column. After switching to the load position, the pre-column is ready for the next sample. This clean-up procedure takes ca. 2 min.

Calculations

An external standardization was performed: 6–860 ng/ml I and 5–500 ng/ml II were added as diluted stock solutions in different volumes for each 1 ml of blank plasma and then treated in the manner described above.

TABLE I

REPRODUCIBILITY OF I IN SPIKED PLASMA SAMPLES

Samples analysed in triplicate. Linear regression $r=0.9999$; $n=15$; $y=0.2108x+0.08$. The accuracy of the assay ranged from 98.6 to 102.5% of the theoretical value (concentration range 10–600 ng/ml of plasma).

Concentration (ng/ml)	S.D.	C.V. (%)
6.1	0.17	2.8
24.6	1.04	4.2
92.1	3.37	3.7
245.6	2.10	0.9
859.6	9.44	1.1

RESULTS

Pre-column clean-up

Under the conditions described at different concentrations (1–300 ng) more than 97% of I and II were retained on the pre-column and then eluted by the mobile phase onto the analytical column.

Calibration

Peak areas were plotted against concentration. Spiked plasma samples of I at different concentrations yielded a good reproducibility and linear regression (Table I). The calibration and reproducibility for II were almost as good as those for I.

Chromatography

Under the conditions described, the substances have the following retention times: II, 3.4 min; I, 3.9 min (Fig. 1). In a test series for a bioavailability study, dextromethorphan was administered to volunteers in the form of tablets or solu-

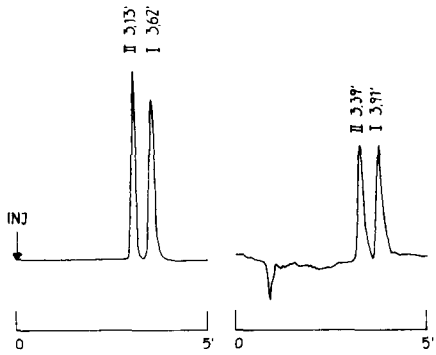

Fig. 1. Chromatograms obtained using diluted stock solutions of I and II, introduced by (left) a 20-μl loop and (right) the described clean-up procedure. In both cases the resolution is satisfactory. The sensitivity settings were 0.2 and 2, respectively, and the amounts of substance present were ca. 20 ng each of I and II (left) and 1.5 ng I and 1.2 ng II (right).

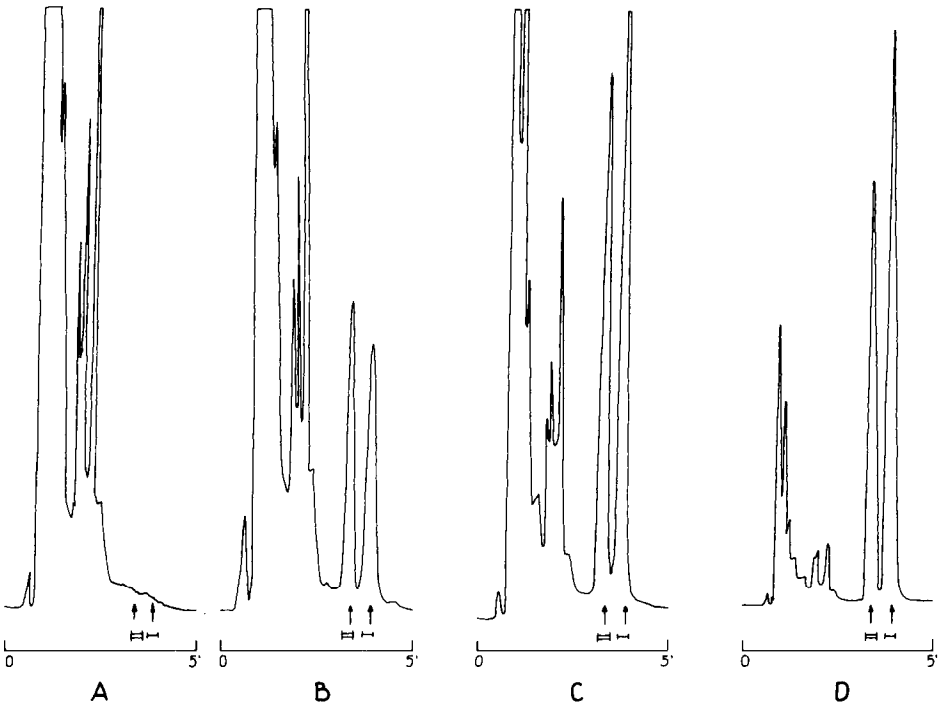

Fig. 2. Chromatograms of I and II in plasma samples from volunteers. (A) Before administration of dextromethorphan (sensitivity 1); (B) 12 h after administration: 13.8 ng/ml I and 9.6 ng/ml II (sensitivity 1); (C) 8 h after administration: 61.6 ng/ml I, 38.2 ng/ml II (sensitivity 0.5); (D) 3 h after administration: 339.2 ng/ml I, 196.8 ng/ml II (sensitivity 0.1).

TABLE II

PRECISION AFTER MULTIPLE DETERMINATIONS OF I AND II IN PLASMA SAMPLES FROM VOLUNTEERS

Concentration (ng/ml)	*n*	C.V. (%)	Median C.V. (%)
Dextrorphan			
7.9- 34.6	7	0.5-3.6	2.0
52.4-190.6	7	0.6-2.8	1.3
263.2-476.1	7	0.3-2.1	1.5
3-Hydroxymorphinan			
1.1- 19.0	6	0.8-6.0	2.2
23.2- 84.0	10	0.1-9.2	1.7
101.0-189.0	4	0.3-1.2	0.7

tion (corresponding to 25 mg of dextromethorphan base). The resulting chromatograms are shown in Fig. 2.

Precision after multiple determinations

Multiple determinations of I and II in plasma samples from volunteers pro-

TABLE III

ELIMINATION HALF-LIVES FOR I AND II

Values from eight volunteers.

	Half-life I (h)	Half-life II (h)
Solution		
α-phase	1.49 ± 0.27	2.2 ± 0.7
β-phase	7.0	
Tablet		
α-phase	1.34 ± 0.26	2.1 ± 0.4
β-phase	6.5	

duced a very low coefficient of variation (Table II). The precision is very good, although no internal standard was used.

Selectivity of assay

Dextromethorphan and a further metabolite, 3-methoxymorphinan, would have retention times of more than 15 min but they were eliminated during the pre-

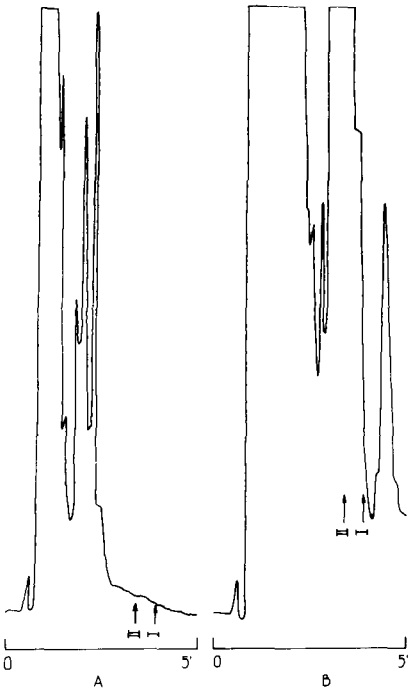

Fig. 3. (A) Chromatogram of blank plasma following the clean-up procedure with 90% acetonitrile: a clear identification of I and II is possible, demonstrating the effectiveness of the clean-up procedure. (B) Chromatogram of blank plasma following the clean-up procedure without 90% acetonitrile; many endogenous substances interfere with the identification of I and II.

column clean-up. Codeine, a common antitussive, and morphine have retention times of less than 1.5 min.

Pharmacokinetic data

Following administration of a tablet and solution of dextromethorphan hydrobromide (corresponding to 25 mg of base) similar elimination half-lives were obtained (Table III).

DISCUSSION

The most important aspect of this procedure is the selective sample clean-up on a pre-column in combination with an enrichment step on this pre-column. I and II (as free bases) are strongly retained on the pre-column, thus making it possible to rinse the pre-column with 90% acetonitrile without eluting I and II. This rinsing step permitted the elimination of all interfering neutral, acidic and basic substances (Fig. 3). Afterwards I and II were eluted by the mobile phase under acidic conditions onto the analytical column, where they were separated. Thus this method constitutes an improvement with regard to analysis time and sensitivity. So far it has been successfully performed with more than 400 plasma samples.

REFERENCES

1 G. Pfaff, P. Briegel and I. Lamprecht, Int. J. Pharm., 14 (1983) 173.
2 T. East and D. Dye, J. Chromatogr., 338 (1985) 99.
3 R. Lucek and R. Dixon, J. Chromatogr., 341 (1985) 239.
4 G. Ramachander, F.D. Williams and J.F. Emele, J. Pharm. Sci., 66 (1977) 1047.
5 R. Gillilan, R.C. Lanman and W.D. Mason, Anal. Lett., 13 (B5) (1980) 381.