ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 827–838

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Simultaneous determination of dextromethorphan and its metabolites in human plasma by capillary electrophoresis

Helle T. Kristensen *

Pharmacia and Upjohn Hillerød AS, Herredsvejen 2, DK-3400 Hillerød, Denmark

Received 15 May 1998; received in revised form 28 August 1998; accepted 13 September 1998

Abstract

A sensitive capillary electrophoretic method was developed and validated for the simultaneous determination of dextromethorphan and its metabolites, dextrorphan, 3-hydroxymorphinan, and 3-methoxymorphinan, in human plasma. After cleavage of conjugates by enzymatic hydrolysis with β-glucuronidase, dextromethorphan and its metabolites were extracted from 1.5 ml of plasma by a liquid–liquid extraction procedure using heptane–ethylacetate (50:50, v/v) and re-extracted to aqueous phase. The compounds were separated within 8 min on a fused silica capillary, 75 μm internal diameter using sodium borate (pH 9.4; 50 mM) as running buffer, and measured by UV-detection at 195 nm using a detection cell with a path length of 1.2 mm. The method was accurate and precise. Linear relationships were observed between the peak response and the concentration in the range of 1–400 ng ml^{-1} plasma with correlation coefficients above 0.998. The limit of detection was 0.5–1 ng ml^{-1} plasma for all compounds. The method was used for determination of plasma levels of dextromethorphan and its metabolites after transdermal and oral administration of dextromethorphan.

Keywords: Dextromethorphan; Metabolites; Plasma; Capillary electrophoresis; Transdermal and oral administration

1. Introduction

Dextromethorphan is widely used as an effective non-narcotic antitussive drug. In most individuals, dextromethorphan is rapidly and extensively metabolised by a first-pass metabolic process [1,2]. As shown in Fig. 1, the metabolism occurs via two pathways, namely, *O*-demethylation to the major active metabolite, dextrorphan and *N*-demethylation to 3-methoxymorphinan. These metabolites are further demethylated to 3-hydroxymorphinan. The 3-hydroxyl group position of dextrorphan and 3-hydroxymorphinan is rapidly conjugated via glucuronidation [3,4]. Several analytical methods have been developed for the determination of dextromethorphan and its metabolites in biological matrices. Analytical methods that are sufficiently sensitive for determination of dextromethorphan in plasma samples

* Fax.: +45-48-207102; e-mail: helle.kristensen@eu.pnu.com.

0731-7085/98/$ - see front matter
PII: S0731-7085(98)00273-8

include gas chromatography [1,5,6], high performance liquid chromatography [7–12] and thin layer chromatography [13]. Among the methods described so far only a few allow for the determination of plasma concentrations of dextromethorphan and its metabolites in a single run [9–11]. Chen et al. [10] reported a HPLC method suitable to determine the four compounds simultaneously. However, the extraction procedure included use of chloroform which is injurious to health. The other methods [9,11] required complex instrumentation.

Capillary electrophoresis (CE) has shown to be an inexpensive and highly efficient analytical technique for determination of urinary dextromethorphan and metabolites [14,15]. These CE methods, however, suffer from relatively long migration times and are not applicable to analysis of plasma samples owing to lack of sensitivity.

This paper describes a selective and sensitive CE method for simultaneous determination of dextromethorphan, dextrorphan, 3-hydroxymorphinan and 3-methoxymorphinan in plasma using UV-absorbance detection. Application of the method to determine plasma levels in human volunteers following patch and tablet administration of dextromethorphan is presented.

2. Experimental

2.1. Chemicals

Dextromethorphan hydrobromide was obtained from Hoffmann-La Roche (Basel, Switzerland). Dextrorphan d-tartrate, 3-hydroxymorphinan hydrobromide and 3-methoxymorphinan hydrochloride were purchased from Research Biochemicals International (Natick, MA) and used as received. Levallorphan tartrate was a USP Reference Standard. The β-glucuronidase (Helix pomatia, type H-1) was purchased from Sigma (St. Louis, MO) and sodium dihydrogenphosphate (analytical grade) from Fluka (Buchs, Switzerland). The other chemicals were all of analytical grade and obtained from Merck (Darmstadt, Germany).

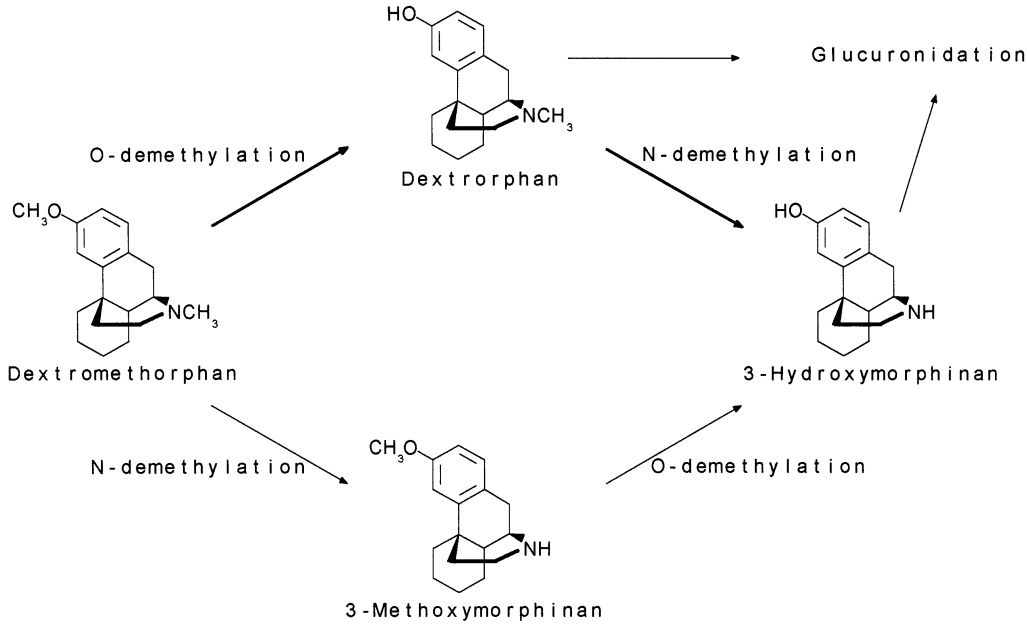

Fig. 1. Chemical structures and metabolic pathways of dextromethorphan.

2.2. Apparatus

The CE system consisted of a HP ^{3D}Capillary Electrophoresis System (Hewlett Packard, Avondale, PA) coupled to a HP Vectra XM4 5/150 computer with ChemStation version A.04.02 installed. The computer did both control the CE apparatus and record the electropherograms.

Detection was performed by UV absorption at 195 nm using a HP ^{3D}CE High Sensitivity Detection Cell (Hewlett Packard) with a path length of 1.2 mm.

Separation was performed on a fused silica capillary obtained from Hewlett Packard. The capillary was 75 μm i.d. and flared to 100 μm i.d. at the point of connection to the high sensitivity detection cell. The length of the capillary was 40.0 cm to the inlet of the cell and 8.5 cm from the outlet. The capillary temperature was maintained at 15°C.

2.3. Electrophoretic procedure

The fused silica capillary coupled to the high sensitivity detection cell was treated with sodium hydroxide (0.5 M) for 15 min and subsequently with Milli-Q water for 5 min before introducing the running buffer, sodium borate (pH 9.4; 50 mM), for 15 min. Prior to every run the capillary was rinsed with sodium hydroxide (0.5 M) for 2 min and subsequently with sodium hydroxide (0.05 M) for 1 min before introducing the running buffer for 5 min.

The sample was injected by pressure at 50.0 mbar for 5.0 s, and the run potential was 11 kV.

2.4. Standard preparation

Dextromethorphan, dextrorphan, 3-hydroxymorphinan and 3-methoxymorphinan were made up as 100 μg ml^{-1} (as base) stock standard solutions in sodium phosphate (pH 2.6; 25 mM), and were diluted to concentrations ranging from 0.025 to 1.5 μg ml^{-1}.

Levallorphan, the internal standard, was dissolved and diluted with sodium phosphate (pH 2.6; 25 mM) to concentrations of 0.25 and 0.7 μg base ml^{-1}.

Plasma calibration standards, covering a concentration range of 5–100 ng ml^{-1} for determination of total metabolites and 1–20 ng ml^{-1} for determination of unconjugated metabolites, were prepared on the day of analysis by adding 100 μl of combined standard solutions containing internal standard to 1.5 and 2.5 ml, respectively, of drug-free plasma. The standards were then prepared for analysis as described in 2.5.1 and 2.5.2.

2.5. Sample preparation

2.5.1. Determination of dextromethorphan and total (free and conjugated) metabolites

A total of 1.5 ml of human plasma in a 15 ml screwcapped polypropylene tube was added 100 μl of the 0.7 μg ml^{-1} internal standard solution and 750 μl of β-glucuronidase solution (8000 U ml^{-1} in sodium acetate (pH 5; 0.2 M)). The solution was mixed and then incubated at 37°C for 2 h in a water bath.

A total of 1.0 ml of saturated sodium carbonate solution was added. After mixing for 15 s, 6.0 ml of heptane–ethylacetate (50:50, v/v) was added, and the tube was shaken on a horizontal shaker for 10 min.

The organic and aqueous phases were separated by centrifugation at 3000 rpm for 10 min. The upper organic phase was transferred to another 15 ml screwcapped tappered polypropylene tube containing 100 μl of sodium phosphate (pH 2.6; 25 mM). The tube was shaken on a horizontal shaker for 10 min followed by centrifugation at 3000 rpm for 10 min. The upper organic phase was removed by aspiration and the aqueous phase was injected onto the CE system.

2.5.2. Determination of dextromethorphan and unconjugated metabolites

A total of 2.5 ml of human plasma in a 15 ml screwcapped polypropylene tube was added 100 μl of the 0.25 μg ml^{-1} internal standard solution and 1.0 ml of saturated sodium carbonate solution. After mixing for 15 s the procedure was starting from the addition of 6.0 ml of heptane–ethylacetate (50:50, v/v) as described above.

2.6. Validation

2.6.1. Selectivity

Plasma samples from 16 patients without restrictions regarding intake of food and drugs were studied for interference by preparing and analysing the samples as described in Section 2.5.1. The compounds, caffeine, theophylline, theobromine, ibuprofen, paracetamol and acetylsalicylic acid were also evaluated for interference to allow the volunteers to drink coffee and tea and take pain-killers during the study period.

2.6.2. Linearity, precision and accuracy

Plasma calibration standards at 4–5 different concentrations ($n = 2$) over the range of 1.2–400 ng ml^{-1} plasma for determination of total metabolites were prepared and analysed. Calibration curves were constructed for all analytes. Similarly, calibration curves were constructed over the range of 1–80 ng ml^{-1} plasma for determination of unconjugated metabolites.

Accuracy and repeatability were determined by means of spiked drug-free plasma samples at 2–3 different concentrations ($n = 4$) injected in duplicate. The concentrations of the samples were calculated using the calibration curves.

Intermediate precision was evaluated by preparing and analysing six plasma calibration standards at four different days by two different operators using different standard weighings and running buffers.

2.6.3. Recovery

Extraction recovery at different concentrations was determined by comparing the peak areas of the analytes from extracted plasma calibration standards with the peak areas of analytes from the aqueous standard solutions of the same concentrations.

2.6.4. Stability

The stability of dextromethorphan and metabolites were examined in standard solutions, plasma calibration standards and extracts of plasma calibration standard. The standard solutions and standard extracts were analysed immediately and then stored at $\div 20$°C, 5°C or room temperature for variable periods. After storage they were analysed using freshly prepared standards.

2.7. Calibration and calculation

Calibration curves were constructed by plotting peak area ratios of the drug standard to the internal standard versus the concentration of the drug. Linear regression analysis was performed and drug concentrations in plasma samples were determined by entering the peak area ratios into the regression equation. All experiments were performed twice and individual data points were the mean of two determinations.

2.8. Assay application

Healthy volunteers were treated with transdermal and oral dextromethorphan. The transdermal administration was given as patches, 30 mg dextromethorphan during 24 h, and the oral administration as tablets, 15 mg dextromethorphan hydrobromide four times daily.

Blood samples were collected immediately before administration and over a 36-h period for patches and a 24-h period for tablets. The blood was immediately placed on ice and then processed by centrifugation to yield the plasma, which was stored at $\div 20$°C pending analysis.

3. Results and discussion

3.1. Analytical procedure

As previously described by Caslavska et al. [15] separation of dextromethorphan and its metabolites could easily be performed by CE in borate buffer. In order to optimise the migration characteristics of dextromethorphan and metabolites in this study, the capillary length was shortened, the capillary temperature was reduced to 15°C and the molarity of the borate buffer was changed from 140 to 50 mM while the pH of the buffer was maintained at 9.4. A run potential of 11 kV resulted in a current of 68 μA through the capillary. An electropherogram from the extract of a plasma calibration standard was obtained with

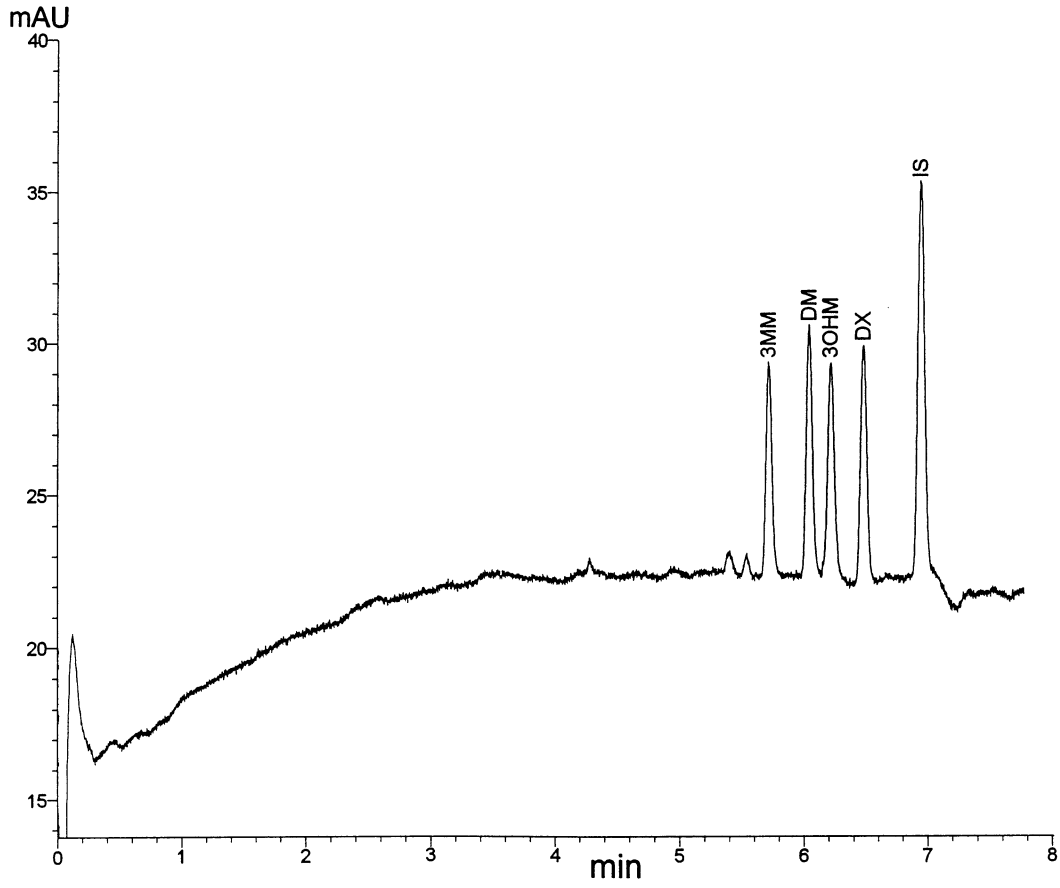

Fig. 2. Electropherogram from an extract of a hydrolysed plasma calibration standard consisting of drug-free plasma spiked with 25 ng ml^{-1} of dextromethorphan (DM), dextrorphan (DX), 3-hydroxymorphinan (3OHM) and 3-methoxymorphinan (3MM), respectively. Internal standard (IS).

good resolution of all four compounds and the internal standard within 8 min (Fig. 2).

The sensitivity of CE is often insufficient when analysing plasma samples. However, the detection sensitivity was optimised by measuring UV-absorption at 195 nm and by use of the high sensitivity detection cell. The cell was a decoupled detection cell with a path length of 1.2 mm used for direct UV-absorbance detection. The increased path length compared to the internal diameter of the capillary increased the signal-to-noise ratio by about ten times when measuring dextromethorphan.

The liquid–liquid extraction method was assessed with respect to absolute recovery of dextromethorphan and metabolites, and with respect to environmental and health aspects. Many of the methods described so far used liquids such as chloroform, diethyl ether or hexane [5–8,10,12]. Heptane–ethylacetate (50:50, v/v) was chosen as the extraction solvent because it afforded a better extraction of dextrorphan and 3-hydroxymorphinan without loss of dextromethorphan and 3-methoxymorphinan compared to heptane as extraction solvent alone. Heptane and ethylacetate are not classified as injurious to health.

3.2. Validation

Under the applied electrophoretic conditions, almost all plasma components migrated against the electroosmotic flow, and reached the detector after 8 min or more. Minor interference from endogenous plasma constituents was detected in samples from two out of 16 patients, where dextrorphan and 3-hydroxymorphinan comigrated with an unknown substance. None of the compounds, caffeine, theophylline, theobromine, ibuprofen, paracetamol and acetylsalicylic acid interferred with the electropherograms of dextromethorphan and metabolites.

The relationships between peak area ratios and plasma concentrations were linear for all analytes over the concentration range 1.2–400 ng ml^{-1} plasma for determination of total metabolites and 1–80 ng ml^{-1} plasma for determination of unconjugated metabolites. Calibration curves obtained following enzymatic hydrolysis were $y = 0.030x \div 0.003$, ($r = 0.9999$) for dextromethorphan, $y = 0.029x \div 0.08$, ($r = 0.9999$) for dextrorphan, $y = 0.024x \div 0.09$, ($r = 0.9999$) for 3-hydroxymorphinan, and $y = 0.027x \div 0.03$, ($r = 0.9999$) for 3-methoxymorphinan. Calibration curves obtained without enzymatic hydrolysis were similar and with a correlation coefficient above 0.998 for all unconjugated metabolites.

The limit of quantification was defined as the lowest concentration that could be assayed with relative standard deviation of no greater than 13%. The limits of quantification of the assay including enzymatic hydrolysis were about 1.2 ng ml^{-1} plasma and the limits of detection (defined as three times baseline noise) were 0.5–1 ng ml^{-1} plasma for all four compounds. The limits of quantification of the determination of unconjugated metabolites were estimated to be about 0.7 ng ml^{-1} plasma for all compounds.

Tables 1 and 2 show repeatability and accuracy data. The accuracy across the entire range of spiked concentrations was, in general, within 83–103% of the target value. The repeatability was less than 13% at all levels, and was typically less than 10%.

Intermediate precision of the assay was assessed by means of single-factor analysis of variance and calculation of relative standard deviation. Table 3 shows RSD for within-day, between-day and all measurements. The data are typically below 10%.

The extraction recoveries of dextromethorphan and total metabolites are presented in Table 4 and were in average about 99% for dextromethorphan, 95% for dextrorphan, 68% for 3-hydroxymorphinan and 83% for 3-methoxymorphinan.

Table 1
Accuracy and repeatability of the measurement of dextromethorphan and total metabolites in plasma

Concentration (ng ml^{-1})	n	Accuracy (%)	RSD (%)
Dextromethorphan			
5	7	88	5.9
25	8	95	1.8
100	8	103	2.2
Dextrorphan			
5	7	102	3.8
25	8	89	3.2
100	8	97	1.0
3-Hydroxymorphinan			
5	7	92	9.4
25	8	83	5.4
100	8	102	1.8
3-Methoxymorphinan			
5	7	98	4.8
25	8	90	3.4
100	8	101	0.8

Table 2
Accuracy and repeatability of the measurement of dextromethorphan and unconjugated metabolites in plasma

Concentration (ng ml^{-1})	n	Accuracy (%)	RSD (%)
Dextromethorphan			
5	7	103	2.2
20	8	100	7.9
Dextrorphan			
5	7	92	3.9
20	8	97	3.7
3-Hydroxymorphinan			
5	7	100	2.6
20	8	87	9.3
3-Methoxymorphinan			
5	7	101	2.8
20	8	88	12.8

Table 3
Intermediate precision of the measurement of dextromethorphan and total metabolites in plasma at a concentration of 25 ng ml^{-1}

Compound	RSD (%)		
	Within-day ($n = 6$)	Between-day ($n = 4$)	All ($n = 24$)
Dextromethorphan	2.8	7.0	6.8
Dextrorphan	4.5	9.6	9.6
3-Hydroxymorphinan	6.8	17.0	16.5
3-Methoxymorphinan	5.2	7.8	8.6

Regarding the internal standard, a recovery of 97% ($n = 18$) and a RSD of 4.0% were obtained. The absence of enzymatic hydrolysis resulted in recoveries of unconjugated compounds ranging between 60 and 90% determined at a concentration of 20 ng ml^{-1} plasma.

The stability of dextromethorphan and metabolites in solutions were evaluated by means of percentage recovery. The compounds were considered as stable if the recovery was about 90% or above. A stock standard solution of dextromethorphan and metabolites (100 μg base ml^{-1}) was stable for 4 months at 5°C and for 8 days at room temperature. A diluted standard solution (400 ng ml^{-1}) showed good recovery for at least 8 days at ÷20 and 5°C. A plasma calibration standard (25 ng ml^{-1}) was stable for 3 months at ÷20°C, and an extract of the plasma calibration standard for 3 days at room temperature. This allowed plasma samples to be stored at ÷20°C for 3 months pending analysis, and extracts of plasma samples to be injected and re-injected, if necessary, onto the CE system over a 3-day period.

3.3. Assay application

The assay was successfully applied to plasma samples collected prior to and after administration of dextromethorphan. Fig. 3 shows typical electropherograms from the extract of hydrolysed plasma samples collected prior to administration, 10 h after patch application and 10 h after administration of first tablet from an extensive and poor metaboliser, respectively.

Fig. 4 demonstrates the plasma levels of dextromethorphan and its metabolites after patch application for 24 h and tablet administration four times daily in an extensive and poor metaboliser, respectively. In the extensive metabolisers, the *O*-demethylated metabolite dextrorphan was the dominating drug component in plasma followed by 3-hydroxymorphinan both after transdermal and oral administration. The 3-methoxymorphinan and dextromethorphan were only present in low concentrations. In poor metabolisers dextromethorphan was the dominating drug component in plasma after transdermal as well as oral administration. This is in agreement with what has been reported by Schadel et al. [4].

Table 4
Recoveries of dextromethorphan and total metabolites in plasma

Concentration (ng ml^{-1})	n	Recovery (%)	RSD (%)
Dextromethorphan			
5	6	97	5.4
25	6	101	3.8
100	6	98	1.6
Dextrorphan			
5	6	93	7.7
25	6	97	3.0
100	6	96	1.6
3-Hydroxymorphinan			
5	6	65	10.5
25	6	69	7.5
100	6	69	3.6
3-Methoxymorphinan			
5	6	76	11.6
25	6	86	2.0
100	6	86	2.4

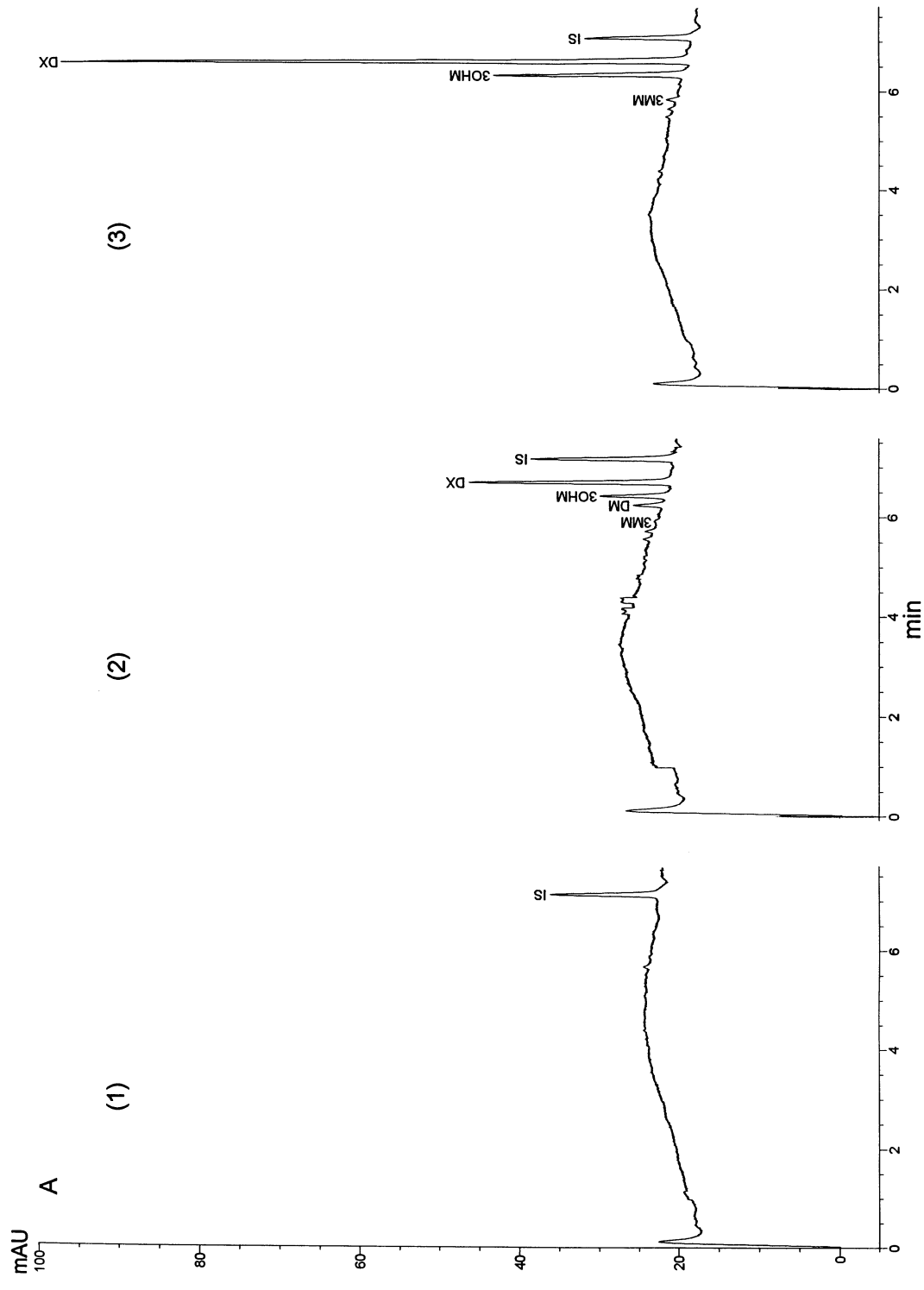

Fig. 3. Electropherograms from the extract of hydrolysed plasma samples collected (1) prior to administration, (2) 10 h after patch application and (3) 10 h after administration of first tablet from an extensive (A) and poor (B) metaboliser. Peak labels are the same as indicated in Fig. 2, and the concentrations of the various compounds appear from Fig. 4.

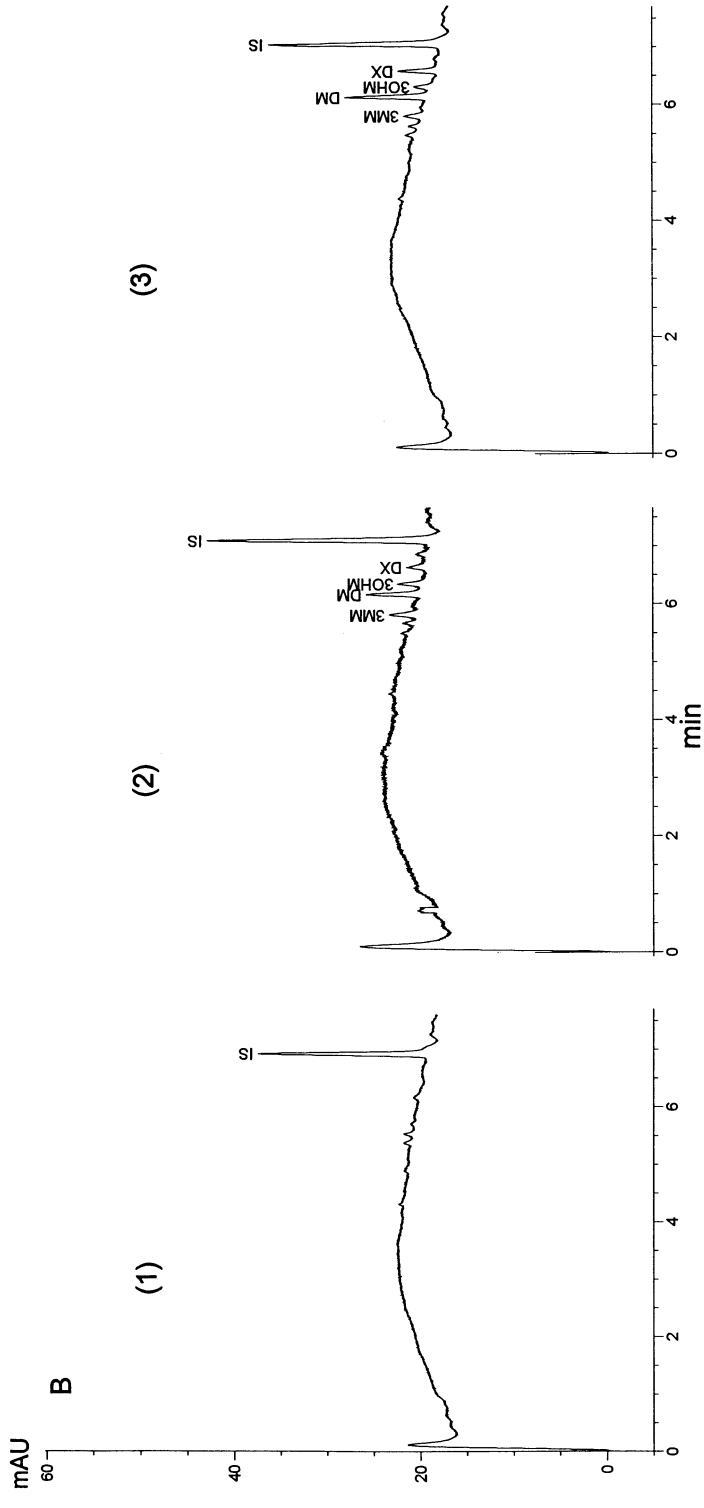

Fig. 3. (*Continued*)

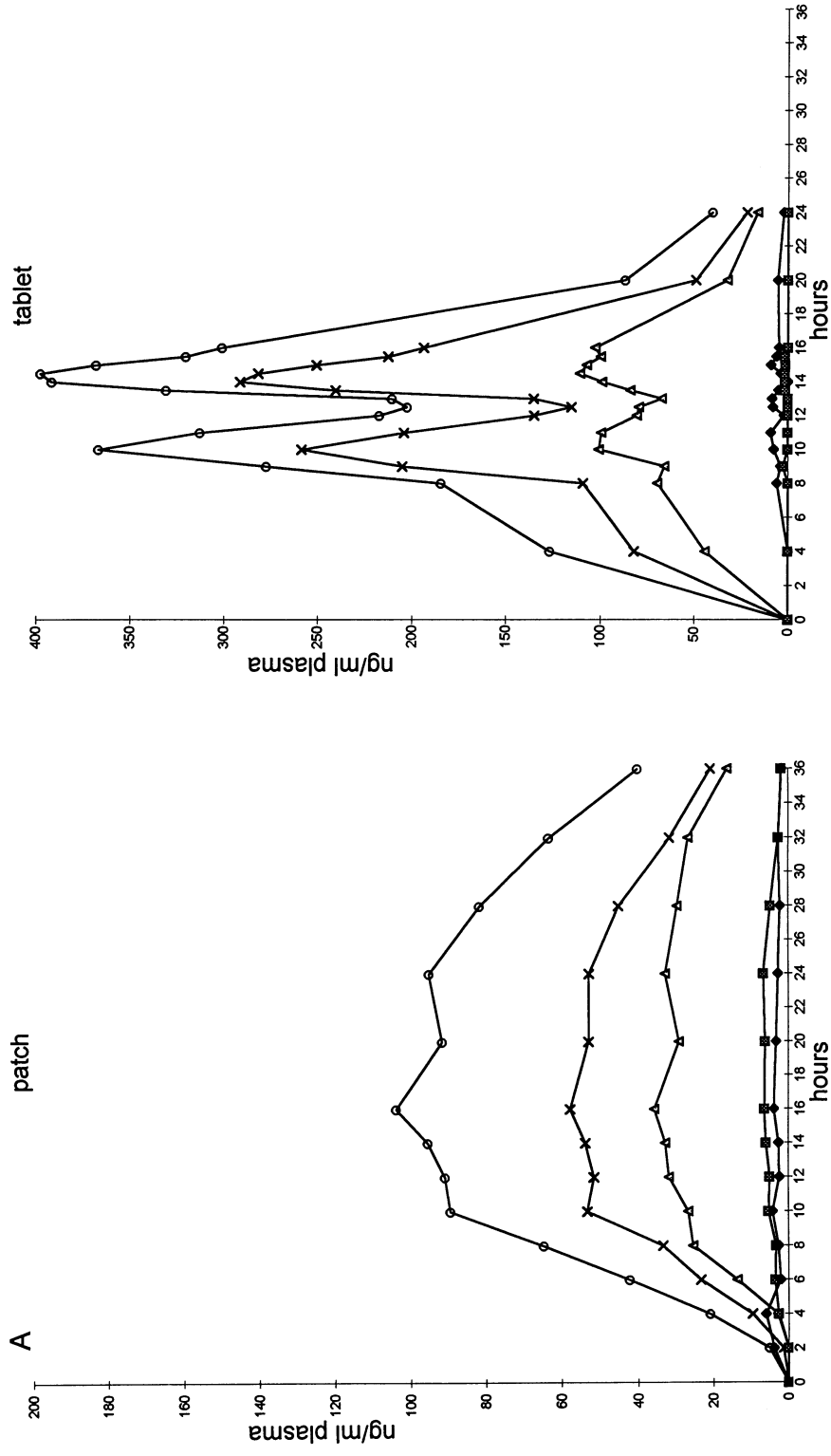

Fig. 4. Total plasma concentrations of dextromethorphan (■); dextrorphan (X); 3-hydroxymorphinan (△); 3-methoxymorphinan (◆); and the sum of all four compounds (○) in an extensive (A) and poor (B) metaboliser after patch and tablet administration.

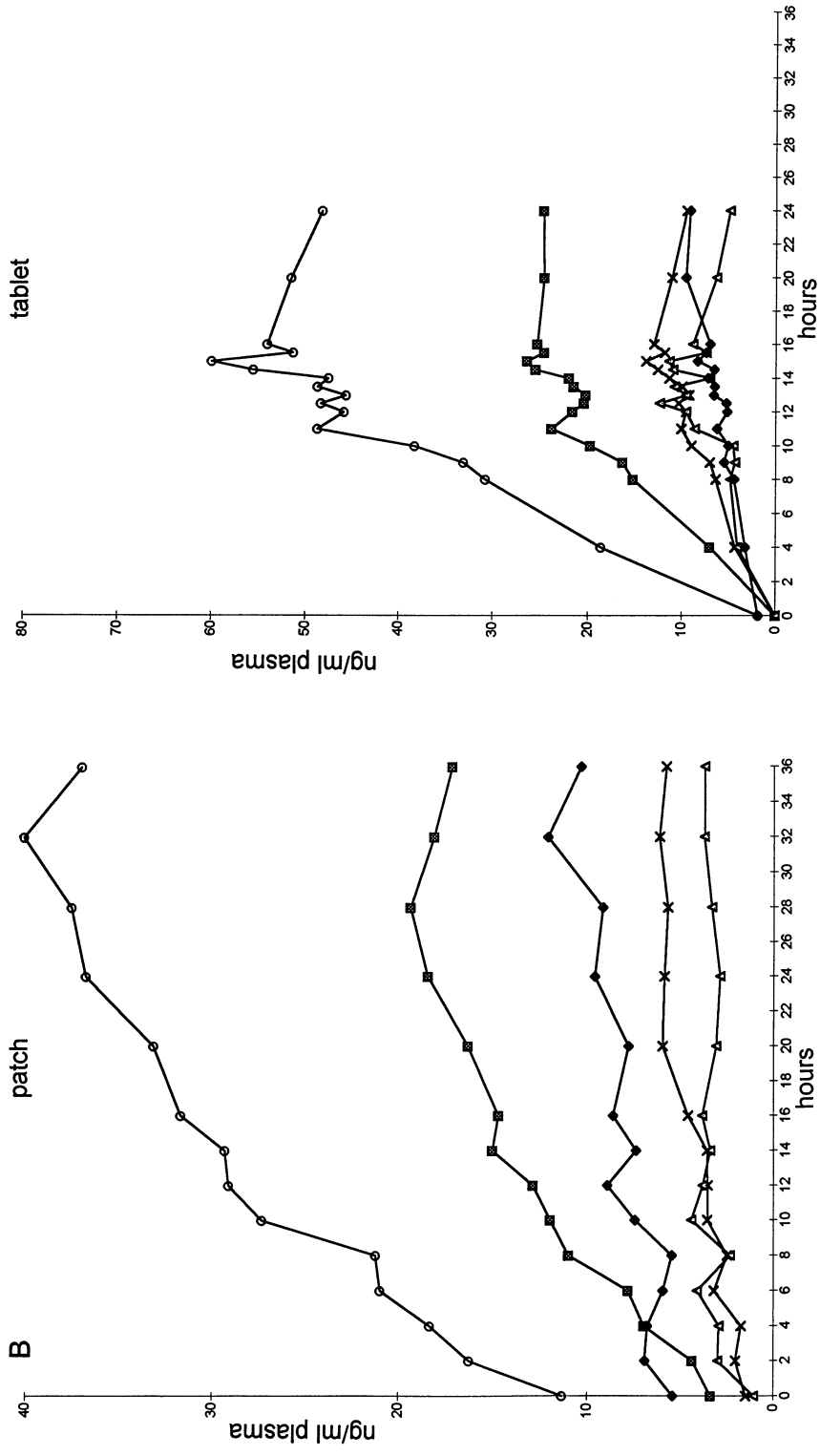

Fig. 4. (*Continued*)

Dextrorphan and 3-hydroxymorphinan were mainly present as conjugated metabolites. The concentrations of free dextrorphan and 3-hydroxymorphinan were about 100-fold less than the conjugated forms.

To conclude, the sensitive, accurate and precise CE method that has been developed is useful for determination of plasma concentration profiles of dextromethorphan and its metabolites following administration of dextromethorphan as patch and tablets, respectively.

Acknowledgements

I am grateful to Karin Møller-Holst and Githa Mastek-Petersen for valuable discussions and for performing the laboratory work.

References

[1] J.W. Barnhart, E.N. Massad, J. Chromatogr. 163 (1979) 390–395.
[2] B. Schmid, J. Bircher, R. Preisig, A. Kupfer, Clin. Pharmacol. Ther. 38 (1985) 618–624.
[3] J.C. Duche, V.Q. Ferrer, J. Barre, M. Mesangeau, J.P. Tillement, Int. J. Clin. Pharmacol. Ther. Toxicol. 31 (1993) 392–398.
[4] M. Schadel, D. Wu, S.V. Otton, W. Kalow, E.M. Sellers, J. Clin. Psychopharmacol. 15 (1995) 263–269.
[5] G. Pfaff, P. Briegel, I. Lamprecht, Int. J. Pharm. 14 (1983) 173–189.
[6] P. Baumann, M. Jonzier-Perey, Clin. Chim. Acta 171 (1988) 211–222.
[7] V.K. Kimiskidis, A.D. Kazis, I. Niopas, J. Liq. Chrom. Rel. Technol. 19 (1996) 1267–1275.
[8] T. East, D. Dye, J. Chromatogr. Biomed. Appl. 338 (1985) 99–112.
[9] M. Johansson, C. Svensson, J. Pharm. Biomed. Anal. 6 (1988) 211–220.
[10] Z. R. Chen, A.A. Somogyi, F. Bochner, Ther. Drug Monit. 12 (1990) 97–104.
[11] S. Härtter, D. Baier, J. Dingemanse, G. Ziegler, C. Hiemke, Ther. Drug Monit. 18 (1996) 297–303.
[12] T.H. Eichhold, L.J. Greenfield, S.H. Hoke II, K.R. Wermeyer, J. Mass Spectrom. 32 (1997) 1205–1211.
[13] R.A. De Zeeuw, D. Eikema, J.P. Franke, J.H. Jonkman, Pharmazie 47 (1992) 346–348.
[14] S. Li, K. Fried, I.W. Wainer, D.K. Lloyd, Chromatographia 35 (1993) 216–222.
[15] J. Caslavska, E. Hufschmid, R. Theurillat, C. Desiderio, H. Wolfisberg, W. Thormann, J. Chromatogr. Biomed. Appl. 656 (1994) 219–231.