ELSEVIER

Journal of Chromatography B, 674 (1995) 49–55

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Determination of codeine and metabolites in plasma and urine using ion-pair high-performance liquid chromatography

J.O. Svensson*, Q.Y. Yue, J. Säwe

Department of Clinical Pharmacology, Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

First received 30 September 1994; revised manuscript received 20 June 1995; accepted 3 July 1995

Abstract

A reversed-phase ion-pair high-performance liquid chromatographic method for the simultaneous determination of codeine and seven metabolites is described. The samples are purified by reversed-phase solid-phase extraction. Codeine, norcodeine, codeine-6-glucuronide, norcodeine-6-glucuronide and morphine-3-glucuronide are measured with UV detection. Detection limits are 3 nmol/l (morphine-3-glucuronide) to 20 nmol/l (codeine). Morphine, normorphine and morphine-6-glucuronide are measured with electrochemical detection. Detection limits are 0.4 nmol/l (morphine-6-glucuronide) to 1.0 nmol/l (normorphine). Correlation coefficients better than 0.998 are normally obtained for all compounds. The method was applied to the determination of the kinetics of codeine and its metabolites in plasma and urine samples from healthy volunteers.

1. Introduction

The main metabolic pathway of codeine in man is via conjugation with glucuronic acid to form codeine-6-glucuronide. Other minor pathways include O-demethylation to morphine and N-demethylation to norcodeine (Fig. 1). To what extent morphine contributes to the analgesic effects of codeine is not known. Other active metabolites are normorphine [1] and morphine-6-glucuronide [2] (Fig. 1). The formation of the O-demethylated active metabolites has been shown to be polymorphic [3] and recent studies have highlighted the importance of taking the metabolites into account when evaluating the kinetics of and response to codeine [4–7]. We have previously described a method for determination of morphine and its glucuronidated metabolites [8,9]. The method has been used to detect morphine and metabolites in plasma and urine in several studies including different patient groups [10], healthy volunteers [11], experimental animals and, with modifications, for codeine and metabolites in plasma and urine [3,6,7,12] in healthy volunteers. It has also been used to determine the formation of glucuronides of codeine [13] and morphine [14] in vitro in microsomes prepared from human and kidney specimens. The aim of this paper is to describe the method for codeine (C) and its seven metabolites codeine-6-glucuronide (C6G), morphine-3-glucuronide (M3G), morphine-6-glucuronide (M6G), morphine (M), normorphine (NM), nor-

* Corresponding author.

0378-4347/95/$09.50
SSDI 0378-4347(95)00292-8

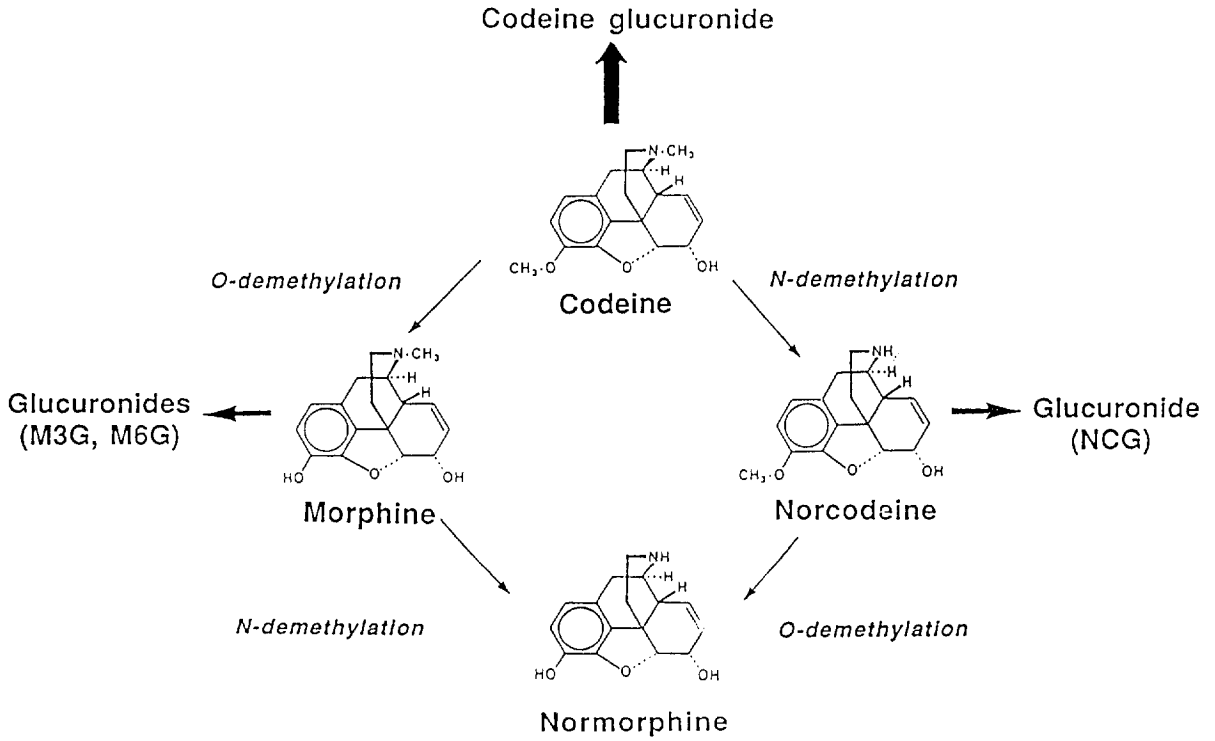

Fig. 1. Major metabolic pathways for codeine.

codeine (NC) and norcodeine glucuronide (NC6G) in detail with the latest modifications.

2. Experimental

2.1. Materials

Codeine phosphate and morphine hydrochloride were obtained from Pharmacia (Stockholm, Sweden). Norcodeine hydrochloride, codeine-6-glucuronide, normorphine hydrochloride and morphine-3-glucuronide were generously supplied by the National Institute of Drug Abuse (Bethesda, MD, USA). Morphine-6-glucuronide was obtained from Ultrafine Chemicals (Manchester, UK). 1-Dodecyl sulphate (sodium salt) was an electrophoresis purity reagent (Bio-Rad, Hercules, CA, USA). Acetonitrile was of HPLC grade. All other chemicals were of analytical-reagent grade. The water used was deionized. Sep-Pak Light C_{18} cartridges were obtained from Waters (Milford, MA, USA).

2.2. Apparatus

The equipment consisted of a Model 2150 HPLC pump (Pharmacia LKB, Bromma, Sweden), a Gilson Aspec sample preparation system (Gilson, Villiers-le-Bel, France) modified for Sep-Pak light cartridges and equipped with a 5-ml dispenser syringe and a 2-ml loop, a Spherisorb S3 ODS2 100 × 4 mm I.D. reversed phase column (3-μm particles) (Knauer, Bad Homburg, Germany), a Model 441 absorbance detector with a fixed wavelength of 214 nm (Waters), a Model 5100 A Coulochem detector with a Model 5010 detector cell (ESA, Bedford, MA, USA) with a potential of 0.25 V at the first electrode and 0.35 V at the second electrode and a C-R5A integrator (Shimadzu, Duisburg, Germany).

2.3. Chromatographic conditions

The eluent was 30 m*M* potassium dihydrogenphosphate buffer (pH 2.1) (adjusted with phos-

phoric acid) containing 3 m*M* dodecyl sulphate and 22% acetonitrile. The temperature was ambient and the flow-rate was 1.5 ml/min.

2.4. Sample purification

The samples were processed on the Gilson Aspec as follows:

1. The Sep-Pak Light C_{18} cartridges were pretreated with 1.0 ml of methanol and thereafter with 1.0 ml of water.
2. 1.0 ml of plasma or urine (if necessary diluted up to 20-fold with water) was mixed with 1.0 ml of 0.5 *M* ammonium sulphate adjusted to pH 9.3 with ammonia.
3. 1.9 ml of the mixture was passed through a first Sep-Pak cartridge at a flow-rate of 12.5 μl/s.
4. The Sep-Pak cartridge was washed with 4.0 ml of 5 m*M* ammonium sulphate adjusted to pH 9.1 with ammonia (flow-rate 25 μl/s).
5. The washing solution was displaced with 200 μl of 15% acetonitrile in 30 m*M* potassium hydrogenphosphate (pH 2.1) (flow-rate 12.5 μl/s).
6. Codeine and metabolites were eluted with 600 μl of 15% acetonitrile in 30 m*M* potassium dihydrogenphosphate buffer (pH 2.1) (flow-rate 12.5 μl/s).
7. The eluate was mixed with 1.0 ml of the 0.5 *M* ammonium buffer (pH 9.3) and the mixture was passed through a second Sep-Pak cartridge (flow-rate 12.5 μl/s).
8. As Step 4.
9. As step 5.
10. As step 6.
11. 400 μl of the eluate were injected on to the HPLC column.

3. Results and discussion

The method published in 1982 [8] used conventional Sep-Pak C_{18} cartridges. A new development is the Sep-Pak Light cartridge, with half the inner diameter (5 mm), with 130 mg of material instead of 360 mg, and with drastically reduced volumes at the inlet and outlet. The packing material is exactly the same in chemistry and particle size. The improvement effectively means that components can be eluted in 600 μl, compared with 3.0 ml, with the same reproducibility. In order to obtain good recoveries for codeine, norcodeine and codeine-glucuronide, 15% acetonitrile, instead of 10% for morphine and metabolites, was used for elution. The recovery was approximately 90% for all components, except normorphine (50%). The lower recovery of normorphine is due to loss during the washing step. The reason is probably that normorphine is more dissociated than morphine at pH 9.1 (it has a higher pK_a for the amine group). Higher sensitivity and shorter retention times were obtained by using a 100×4.0 mm I.D. HPLC column packed with Spherisorb ODS2 3-μm particles. However, the injection of large volumes sometimes led to split peaks. Changing from 10 m*M* sodium dihydrogenphosphate to 30 m*M* of the corresponding potassium salt and from 1 m*M* dodecyl sulphate to 3 m*M* in the eluent solved this problem.

On the electrochemical detector, virtually 100% of all oxidizable components (M, NM and M6G) were oxidized between 0.20 and 0.45 V. In order to suppress disturbances from the matrix, the potential of the first electrode was raised to 0.25 V and the potential of the second electrode was lowered to 0.35 V. With the change from 0.20 to 0.25 V, about 35% of the peak height was lost for M and NM and 15% for M6G. On changing from 0.45 to 0.35 V about 10% of peak height was lost for all three components.

The Gilson Aspec was originally constructed for syringe-type SPE cartridges. With the modification, the flow-rate and volume can be much better controlled, because flow through the cartridge is driven directly by the diluter syringe and not by compressed air. Differences in back-pressure due to, for example, different sample viscosities are then unimportant.

The minimum detectable concentrations, with 1 ml of plasma sample, were about 3 nmol/l for M3G, 5 nmol/l for C6G, 20 nmol/l for NC and C, 0.4 nmol/l for M6G, 1.0 nmol/l for NM and 0.5 nmol/l for M. Minimum detectable concentrations in urine are up to ten times higher.

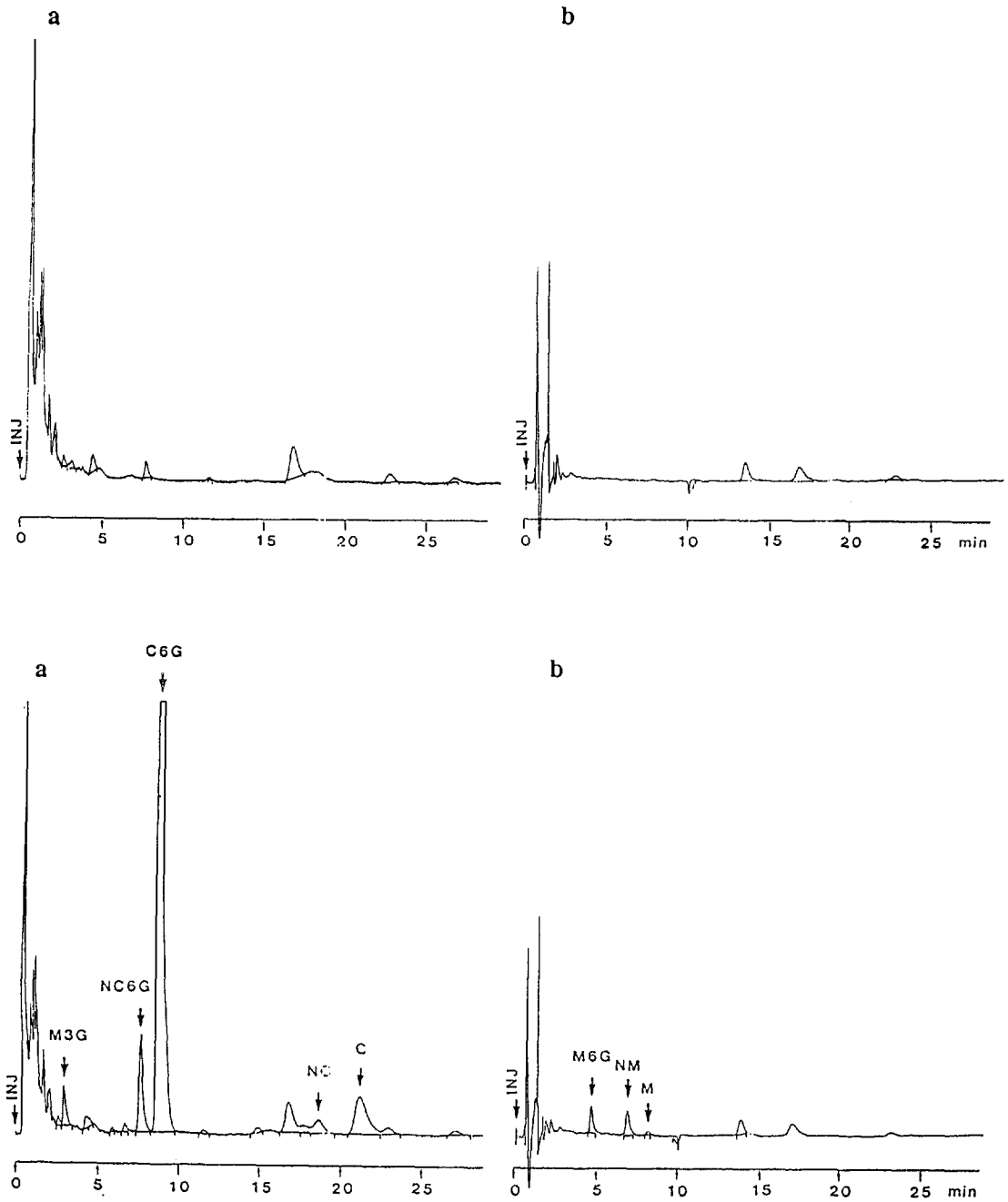

Fig. 2. Chromatograms from plasma samples taken before and 3 h after dose. (a) UV detector (0.016 AUFS); (b) electrochemical detector (400 nA full-scale) (same scale as UV). Concentrations: M3G, 31 nmol/l; NC6G, ca. 130 nmol/l; C6G, 1990 nmol/l; NC, 44 nmol/l; C, 185 nmol/l; M6G, 4.7 nmol/l; NM, 7.7 nmol/l; M, 0.9 nmol/l.

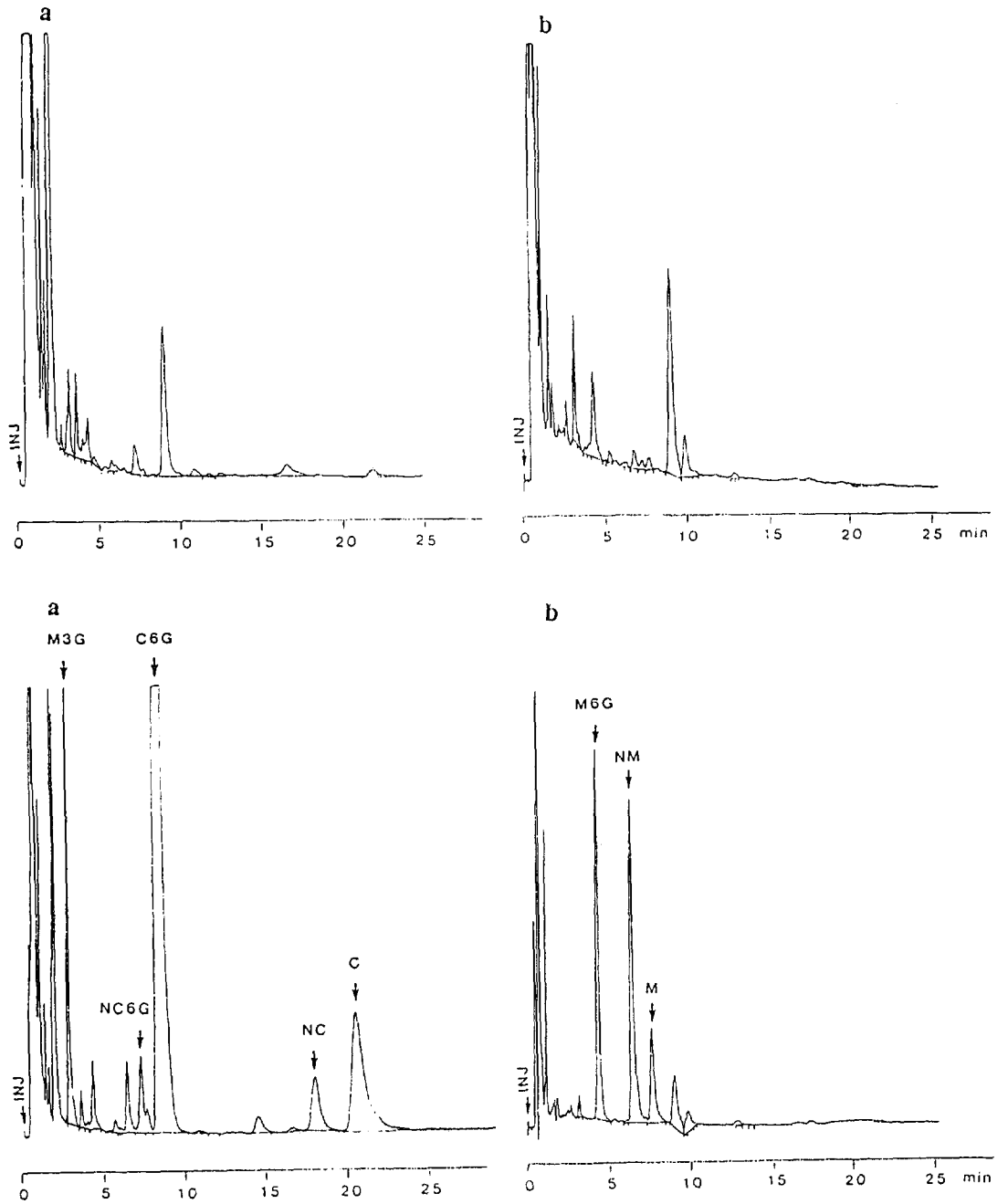

Fig. 3. Chromatograms from urine samples taken before dose and sampled 0–2 h after dose. (a) UV detector (0.016 AUFS); (b) electrochemical detector (400 nA full-scale). Concentrations: M3G, 3.73 μmol/l; NC6G, ca. 2.15 μmol/l; C6G, 98.6 μmol/l; NC, 4.24 μmol/l; C, 11.10 μmol/l; M6G, 1.21 μmol/l; NM, 1.71 μmol/l; M, 0.43 μmol/l. Urine before dose diluted fivefold. Urine after dose diluted twentyfold.

Table 1
Linear calibration graph ranges

Compound	Plasma (nmol/l)	Urine (μmol/l)
M3G	10–1600	0.1–16
C6G	60–9600	6–96
NC	25–4000	0.25–40
C	25–4000	0.25–40
M6G	2–320	0.02–3.2
NM	2–320	0.02–3.2
M	1–160	0.01–1.6

Chromatograms from plasma before and 3 h after administration of 50 mg of codeine phosphate to a healthy volunteer (extensive debrisoquine hydroxylator) are shown in Fig. 2. Chromatograms from urine before administration and sampled 0–2 h after administration of 50 mg of codeine phosphate to a healthy volunteer (extensive debrisoquine hydroxylator) are shown in Fig. 3. The identity of norcodeine-6-glucuronide (NC6G) is not established, since the substance is not available. The approximate concentrations are calculated from the calibration graph for C6G. This is possible as we know that the UV absorption curves of NC and C are similar. Calibration graphs (seven standard points, external standard) with correlation coefficients better than 0.998 were obtained for all components. The ranges are listed in Table 1. The day-to-day coefficients of variation are listed in Table 2. The urine control samples were diluted tenfold before analysis.

This method was used to determine codeine and seven metabolites in plasma and urine samples collected from healthy volunteers and in in vitro experiments with microsomes prepared from different human tissue specimens. In kinetic studies in healthy Caucasian subjects, 84–90% of the given dose was recovered in 48-h urine collections following 50 mg of codeine orally. Most of the drug (about 70%) was excreted as C6G and about 10% was metabolized by N-demethylation. The metabolites formed through O-demethylation accounted for 6% of the given dose in extensive hydroxylators of debrisoquine and only 0.3% in poor hydroxylators. Only 3% of the given dose was recovered as unchanged codeine. In Chinese subjects the total urinary recovery of the given drug was close to that found in Caucasians (82%), suggesting a similar extent of codeine absorption. However, kinetic data from Chinese subjects have shown a 50% higher codeine plasma AUC and a significantly lower total plasma clearance as well as partial metabolic clearance through glucuronidation compared with Caucasians [7,12]. Thus it has been possible to identify important inter-individual and inter-ethnic differences in the glucuronidation and O- and N-demethylation of codeine [3,6,7,12].

Table 2
Day-to-day coefficients of variation (C.V.)

Compound	Plasma			Urine		
	Concentration (nmol/l)	C.V. (%)	n	Concentration (μmol/l)	C.V. (%)	n
M3G	79.6	3.6	10	11.6	5.0	6
C6G	600	2.0	10	30.0	2.0	6
NC	100	11.7	10	14.1	2.1	6
C	100	10.2	10	12.4	4.7	6
M6G	11.6	4.1	10	1.68	10.5	6
NM	9.6	10.1	10	1.70	9.3	6
M	10.0	15.7	10	1.66	5.6	6

Acknowledgements

This work was supported by the Swedish Cancer Society (1910-B88-01V). Samples of codeine metabolites were kindly provided by the National Institute of Drug Abuse, USA.

References

[1] L. Lasagna and T.J. de Kornfeld, J. Pharmacol., 124 (1958) 260.
[2] R. Osborne, S. Joel, D. Trew and M. Slevin, Lancet, i (1988) 828.
[3] Q.Y. Yue, J.O. Svensson, C. Alm, F. Sjöqvist and J. Säwe, Br. J. Clin. Pharmacol., 28 (1989) 639.
[4] J. Desmeules, M.P. Gascon, P. Dayer and M. Magistris, Eur. J. Clin. Pharmacol., 41 (1991) 23.
[5] S.H. Sindrup, K. Brøsen, P. Bjerring, L. Arendt-Nielsen, U. Larsen, H.R. Angelo and L.F. Gram, Clin. Pharmacol. Ther., 48 (1990) 686.
[6] Q.Y. Yue, J.O. Svensson, C. Alm, F. Sjöqvist and J. Säwe, Br. J. Clin. Pharmacol., 28 (1989) 629.
[7] Q.Y. Yue, J. Hasselström, J.O. Svensson and J. Säwe, Br. J. Clin. Pharmacol., 31 (1991) 635.
[8] J.O. Svensson, A. Rane, J. Säwe and F. Sjöqvist, J. Chromatogr., 230 (1982) 427.
[9] J.O. Svensson, J. Chromatogr., 375 (1986) 174.
[10] J. Säwe, J.O. Svensson and A. Rane, Br. J. Clin. Pharmacol., 16 (1983) 85.
[11] J. Hasselström and S. Säwe, Clin. Pharmacokinet., 24 (1993) 344.
[12] Q.Y. Yue, J.O. Svensson, F. Sjöqvist and J. Säwe, Br. J. Clin. Pharmacol., 31 (1991) 643.
[13] Q.Y. Yue, C. von Bahr and J. Säwe, Pharmacol. Toxicol., 66 (1990) 221.
[14] Q.Y. Yue, I. Odar-Cederlöf, J.O. Svensson and J. Säwe, Pharmacol. Toxicol., 63 (1988) 337.