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A rapid liquid chromatographic method for the determination of lamotrigine in plasma

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A rapid liquid chromatographic method for the determination of lamotrigine in plasma

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Abstract

A rapid, sensitive and simple high-performance liquid chromatographic (HPLC) method for the determination of lamotrigine in plasma is described. The drug was extracted from 100 μl of plasma with chloroform:isopropanol (95:5% v/v) in the presence of 100 μl of phosphate buffer (10 mM). The extract was evaporated and the residue was reconstituted with mobile phase and injected onto the HPLC system. The drug and the internal standard (chloramphenicol) were eluted from a Symmetry C_{18} stainless steel column at ambient temperature with a mobile phase consisting of 0.01 M potassium phosphate–acetonitrile–methanol (70:20:10% v/v/v), adjusted to pH 6.7, at a flow rate of 1.3 ml min^{-1} and the detector was monitored at 214 nm. Quantitation was achieved by measurement of the peak-area ratio of the drug to the internal standard and the lower limit of detection for lamotrigine in plasma was 20 ng ml^{-1}. The intraday precision ranged from 3.34 to 6.12% coefficient of variation (CV) and the interday precision ranged from 2.15 to 8.34% CV. The absolute and relative recoveries of lamotrigine ranged from 86.93 to 90.71% and from 95.18 to 107.13%, respectively. The method was applied in studying the pharmacokinetics of lamotrigine administered orally to rabbits. This reliable micro-method would have application in pharmacokinetic studies of lamotrigine where only small sample sizes are available, e.g. paediatric patients.

Keywords: Lamotrigine; Liquid chromatography; Plasma; Pharmacokinetic studies

1. Introduction

Lamotrigine [3,5 - diamino - 6(2,3 - dichloroph - enyl)-1,2,4-triazine] is a novel antiepileptic drug, chemically unrelated to antiepileptic agents in current use. Its pharmacological action is similar to that of phenytoin and carbamazepine [1,2]. Lamotrigine is effective as an add-on therapy in the management of simple and complex partial seizures and secondarily generalised tonic-clonic seizures resistant to multiple-drug therapy [3].

In humans, lamotrigine is rapidly and completely absorbed with an oral bioavailability of about 98% [4]. The drug has an elimination half-life of about 24 h [5] and a plasma protein binding of 55% [6]. Of the administered dose, 70% can be

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recovered in the urine, 90% of which is in the form of a glucuronide conjugate [6].

A number of high-performance liquid chromatographic (HPLC) assays are presently available for the measurement of lamotrigine in biological fluids [5,7–11]. However, there are a number of disadvantages associated with some of these methods. These include lengthy extraction procedures[11], relatively large volumes of organic solvents [5,8] and poor recovery [11].

This report presents a simple, rapid, selective, reproducible reversed-phase HPLC micro-assay for the determination of lamotrigine in human, rabbit and dog plasma and describes its potential application in pharmacokinetic.

2. Materials and methods

2.1. Apparatus

The high-performance liquid chromatographic system consisted of a system controller (M-721), a data module (M-730), a solvent delivery pump (M-501), an autosampler (WISP-712) and a variable wavelength UV detector (M-481). Chromatographic separations were performed using a Symmetry C_{18} stainless steel column (150 × 3.9 mm i.d., 5 μm). All of the above were supplied by Waters Associates (Milford, MA, USA).

2.2. Reagents

Lamotrigine was obtained from Glaxo–Wellcome (UK). Chloramphenicol (internal standard) was obtained from BDH biochemicals (Poole, UK). Solvents used were of HPLC grade and all other chemicals and reagents were of analytical grade.

2.3. Preparation of standard solutions

Lamotrigine stock solution was made up in methanol to a concentration of 1.0 mg ml^{-1}. This solution was further diluted in methanol to give a working standard solution of 200 μg ml^{-1}. A stock solution of chloramphenicol as internal standard was made up in methanol to a concentration of 1.0 mg ml^{-1}. Further dilution was made in methanol to give a working internal standard solution of 10 μg ml^{-1}.

2.4. Chromatographic conditions

A mobile phase consisting of 0.01 M potassium phosphate–acetonitrile–methanol (70:20:10% v/v/v) at a pH adjusted to 6.7 was used. It was freshly prepared and degassed daily by passing it through a 0.22 μm Millipore membrane filter (Millipore, Bedford, MA, USA). Chromatography was performed at ambient temperature using a flow rate of 1.3 ml min^{-1}. The column eluate was monitored at 214 nm with a sensitivity of 0.01 absorbance units full scale (AUFS) and a chart speed of 0.5 cm min^{-1}.

2.5. Extraction procedure

To 100 μl of plasma in a 1.5 ml microcentrifuge tube (LIP, Yorkshire, UK), 30 μl of the internal standard (10 μg ml^{-1}), 100 μl of potassium phosphate buffer (0.01 M) and 1 ml of chloroform in isopropanol (95:5% v/v) were added. The tube was vortex-mixed for 30 s, shaken on a rotary mixer for 10 min and then centrifuged at 4000 rpm for 10 min. After centrifugation, the organic layer was transferred to a 3-ml glass centrifuge tube (John Pulten, Essex, UK) and evaporated to dryness at 40°C under a stream of purified nitrogen. The residue was reconstituted in 100 μl of the mobile phase and 20 μl was injected into the chromatograph.

2.6. Application

Six healthy male New Zealand rabbits, weighing between 1.4 and 2.0 kg were used in the study. The animals were fasted overnight (water given ad libitum) and then given a single oral dose of (18.6 mg kg^{-1}) lamotrigine suspended in normal saline with the aid of Tween 80. Blood samples were collected into small plastic centrifuge tubes through a cannula inserted into the marginal ear vein just before dosing and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after lamotrigine administration. After each sample withdrawal, the cannula was fl
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3. Results and d

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cannula was flushed with an equal volume of heparinised saline. The blood samples were then immediately centrifuged and 100 μl of plasma samples were stored at −20°C until analysis.

2.7. Pharmacokinetic analysis

The maximum plasma concentration (C_{max}) and time needed to attain this concentration (T_{max}) were observed directly from the plasma concentration–time profiles. The first order disposition rate constant (K_d) was determined from the best log-linear fit of the terminal phase by least-squares linear regression analysis and then the half-life was calculated as $0.693/K_d$.

The area under the plasma concentration–time curve (AUC) and the area under the first moment of plasma concentration–time curve (AUMC) were calculated by the trapezoidal method. Mean residence time (MRT) of the drug in the body was estimated as $MRT = AUMC_{0-\infty}/AUC_{0=\infty}$.

Oral body clearance (Cl/F) was calculated as $Cl/F = D/AUC_{0-\infty}$. The volume of distribution (V_d/F) was calculated as $V_d/F = (Cl/F)/K_d$.

3. Results and discussion

The mobile phase at pH 6.7 and the flow rate used for the assay achieved optimum resolution of lamotrigine and the internal standard with no interference from other commonly prescribed antiepileptic agents or endogenous components in plasma. It was also observed that adjusting the detector wavelength at 214 nm gave maximum sensitivity of lamotrigine compared to that of 305 nm (Fig. 1).

A variety of extraction solvents, including chloroform, chlorobutane, dichloromethane, ethyl acetate, diethyl ether, with and without 5% isopropanol or 10% acetonitrile were tried. The best extracting solvents were 5% isopropanol in chloroform and 10% acetonitrile in chlorobutane. Although the latter gave cleaner chromatograms, it yielded poor lamotrigine recovery (68%). Therefore, the extraction solvent of 5% isopropanol in chloroform was selected, because it gave cleaner chromatograms and better recovery of lamotrigine.

In the previously published methods [5,8–10], the internal standard used was 3,5-diamino-6-(2-methoxyphenyl)-1,24-triazine. This compound is structurally related to lamotrigine, but in our HPLC assay it eluted very quickly with the endogenous plasma components at a retention time of 1.6 min. Rather than changing the optimal conditions of lamotrigine resolution and separation, we selected chloramphenicol as the internal standard.

Fig. 2 shows representative chromatograms of drug-free human plasma, the plasma sample of an epileptic child taking lamotrigine (5 mg per day), drug-free dog plasma, a plasma sample collected 9.0 h after oral administration of 100 mg lamotrigine tablet to a male dog, drug-free rabbit plasma and a plasma sample taken at 12 h from a rabbit taking lamotrigine (18.6 mg kg^{-1}, PO) using the described procedure. Retention times of lamotrigine and the internal standard were 4.00 and 6.65 min, respectively.

3.1. Quantitation

The quantitation of the chromatograms was achieved by the peak-area ratios of the drug to the internal standard. To determine the linearity of the assay, various human plasma standards were prepared by spiking drug-free human plasma samples with known quantities of the drug at eight non-zero concentrations over the range of

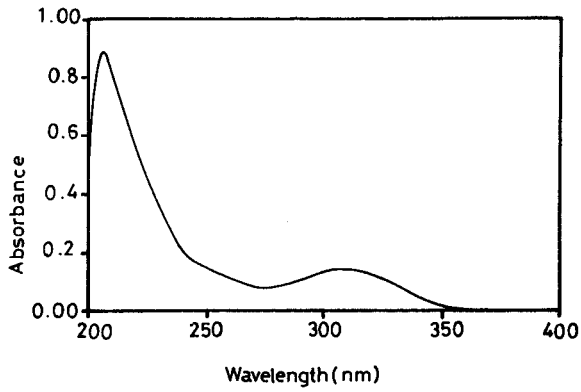

Fig. 1. UV absorption spectrum of lamotrigine.

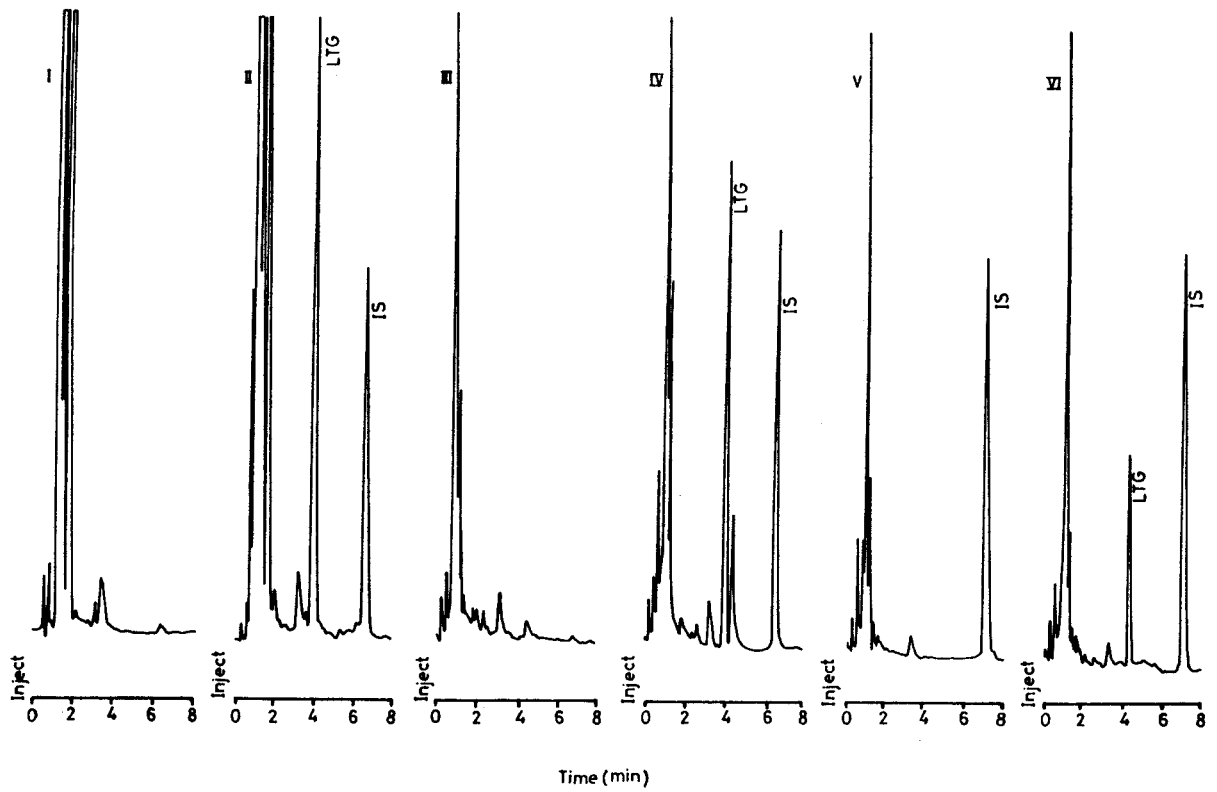

Fig. 2. Typical chromatograms of: (I) a drug-free human plasma; (II) a plasma extract following a daily oral administration of lamotrigine (5 mg) to an epileptic child; (III) a drug-free dog plasma; (IV) a plasma extract taken 9 h following oral administration of a 100 mg lamotrigine tablet to a male dog; (V) a drug-free rabbit plasma; and (VI) a plasma extract taken 12 h after oral administration of lamotrigine (18.6 mg kg^{-1}) to a rabbit. LTG, lamotrigine; IS, internal standard.

0.05–10 μg ml^{-1}. Standards were analysed in replicates of nine, analysed at concentrations 0.05, 0.2, 0.5, 1.0, 2.0, 3.0, 5.0 and 10 μg ml^{-1}. The peak area ratios of D/I (drug/internal standard) were plotted against the concentrations. The slope, intercept and correlation coefficient were determined by the method of least-squares linear regression analysis.

Standard curves of lamotrigine in human plasma were constructed on nine different days to determine the variability of the slopes and intercepts. Table 1 shows the results from the linearity study. The linear regression analysis of the data was characterized as having a slope of 1.110 and an intercept of −0.020 (correlation coefficient = 0.998). The results showed little day-to-day variability of slopes and intercepts, as well as good linearity over the plasma concentration range studied.

3.2. Sensitivity

The lower limit of quantitation (LOQ) for lamotrigine was established by injecting nine different human plasma samples containing 0.05 μg ml^{-1} (the lowest concentration on the standard curve). The CV was 8.6%. Therefore, the LOQ for lamotrigine was 0.05 μg ml^{-1}. The detection limit was considered as a concentration of lamotrigine giving a signal to noise ratio greater than 3:1. The detection limit for lamotrigine in human plasma was found to be 20 ng ml^{-1}, because this concentration resulted in a detectable peak of approximately three times the noise level.

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	−0.020
	−0.030
	−0.022
	−0.018
	−0.012
	−0.015
	−0.008
Mean	−0.020
SD	0.008
%CV	—

3.3. Specificity

The specificity of the method was established by analysing six independent sources of the drug-free human plasma. All the tested blanks were free from endogenous plasma components at the retention times of the drug and the internal standard.

3.4. Selectivity

Commonly administered antiepileptic drugs were tested for possible interference in the HPLC assay. Table 2 lists the retention times of the drugs and the metabolites tested.

3.5. Precision

The intraday precision was determined from replicate analysis of pooled human plasma samples containing lamotrigine at three different concentrations (0.4, 4.0 and 8.0 μg ml^{-1}) covering the low, medium and high ranges of the calibration curve. Precision is expressed as the percent coefficient of variation (%CV) for the concentrations back-calculated from the regression analysis. Accuracy is expressed as a percentage (observed concentration × 100/theoretical concentration). The intraday precision ranged from 3.34 to 6.12% CV. Accuracy ranged from 95.2 to 107.3% (Table 3).

The interday precision was similarly determined over a period of 4 weeks. The interday precision ranged from 2.15 to 8.34% CV. The accuracy ranged from 100.7 to 106.0% (Table 3).

Table 1
Lamotrigine standard curve summary

	Intercept	Slope	Correlation coefficient
	−0.020	1.209	0.9948
	−0.033	1.204	0.9967
	−0.020	1.192	0.9987
	−0.030	1.000	0.9984
	−0.022	1.065	0.9990
	−0.018	1.100	0.9983
	−0.012	1.101	0.9995
	−0.015	1.078	0.993
	−0.008	1.040	0.9991
Mean	−0.020	1.110	0.9982
SD	0.008	0.075	0.0015
%CV	—	6.80%	0.15%

Table 2
Retention times of some tested drugs and metabolites

Drug	Retention time (min)
Hexobarbital	14.68
Pentobarbital	17.70
Butabarbital	9.07
Cyclobarbital	9.02
Secobarbital	26.2
Amylobarbital	18.52
Phenobarbital	6.12
P-hydroxyphenobarbital	2.53
Phenytoin	19.05
5-(P-hydroxyphenyl)-5-phenylhydantoin	5.87
Ethosuximide	2.48
Carbamazepine	17.74
Carbamazepine-10,11-epoxide	7.16
Oxcarbazepine	9.56
10,11-dihydro-10-hydroxycarbamazepine	5.01
Primidone	2.84
Clonazepam	ND
Diazepam	ND
Valproic acid	ND

ND, Not detected within 30 min from injection.

3.6. Recovery

Absolute recoveries for lamotrigine and the internal standard were determined by spiking drug-free human plasma with known amounts of the drug and the internal standard to achieve the lamotrigine concentrations of 0.4, 4.0 and 8 μg ml^{-1}. The samples were extracted and analysed with the developed procedure. The absolute recoveries were calculated by comparing the resultant peak areas with those obtained from pure standards, in mobile phase, of the drug and the internal standard at the same concentrations. The absolute recoveries of lamotrigine ranged from 86.93 to 90.71%, while the absolute recovery for the internal standard was 96.30% (Table 4).

Table 3
Intraday and Interday precision of lamotrigine in human plasma

Intraday[a]			Interday[b]		
Added concentration (μg ml^{-1})	Measured concentration (μg ml^{-1})	Accuracy[c] (%)	Added conc. (μg ml^{-1})	Measured concentration (μg ml^{-1})	Accuracy[c] (%)
0.4			0.4		
Mean	0.429	107.3	Mean	0.424	106.0
SD	0.019		SD	0.035	
CV%	4.48		CV%	8.34	
30.4					
4.0			4.0		
Mean	3.807	95.2	Mean	4.070	101.8
SD	0.233		SD	0.282	
CV%	6.12		CV%	6.92	
8.0			8.0		
Mean	8.131	101.6	Mean	8.059	100.7
SD	0.272		SD	0.173	
CV%	3.34		CV%	2.15	

[a] Mean values represent eight different plasma samples for each concentration.
[b] Interday was determined from nine different runs over a 4-week period. The concentration of each run was determined from a single calibration curve run on the first day of the study.
[c] Accuracy = 100 (observed concentration/theoretical concentration).

The relative recovery of lamotrigine was calculated by comparing the concentrations of the drug-spiked plasma with the actual added concentrations. The relative recoveries of the lamotrigine ranged from 95.18 to 107.13% (Table 4).

3.7. Stability

Stability of lamotrigine in human plasma was determined through seven freeze–thaw cycles (-20 ± 5°C to room temperature). After thawing, samples were allowed to stand on the bench top, under room lighting, until 2 h had elapsed since their removal from the freezer. The results showed that lamotrigine was stable after seven cycles of freeze–thaw (Table 5).

3.8. Application

The mean plasma concentration–time profile after a single lamotrigine oral dose (18.6 mg kg^{-1}) to six healthy male New Zealand rabbits is shown in Fig. 3. The absorption of lamotrigine in rabbits is rapid, reaching peak plasma concentration in about 1.0 h. The computed pharmacokinetic parameters are shown in Table 6.

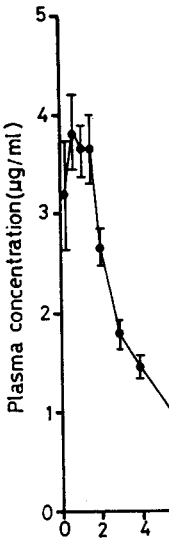
Table 4
Absolute and relative recoveries of lamotrigine and internal standard from human plasma

Concentration (μg ml^{-1})	Absolute recovery (% mean ± SD)	Relative recovery (% mean ± SD)
0.4	87.89 ± 4.11	107.13 ± 4.80
4.0	90.71 ± 6.04	95.18 ± 5.83
8.0	86.93 ± 2.22	101.64 ± 3.40
Internal standard	96.30 ± 2.20	—

Table 5
Effect of freeze–thaw on the stability of lamotrigine in human plasma

Concentration (μg ml^{-1})	Mean ± SD, CV%
0.4	0.435 ± 0.042, 9.67
4.0	4.346 ± 0.369, 8.49
8.0	7.943 ± 0.365, 4.59

The mean values represent seven cycles.



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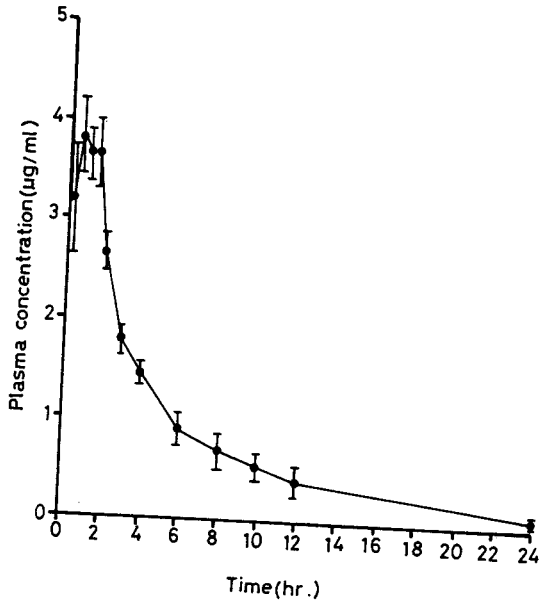

Fig. 3. Mean plasma concentration–time profile of lamotrigine following oral administration of 18.6 mg kg^{-1} to six rabbits.

4. Conclusion

The HPLC method described for the measurement of lamotrigine in plasma is sensitive, selective, simple, reproducible, rapid and precise, making it valuable in many applications, particularly in pharmacokinetic studies including paediatric patients (small sample size required, 100 μl), therapeutic drug monitoring and bioequivalency studies. Moreover, the method can be adapted to simultaneously measure the plasma concentrations of other antiepileptics and their active metabolites.

Acknowledgements

The authors gratefully acknowledge Glaxo–Wellcome Company for providing the lamotrigine used in this study.

Table 6
Pharmacokinetic parameters (mean ± SD) of lamotrigine after an oral administration of lamotrigine (18.6 mg kg^{-1}) to six rabbits

Parameter	Mean ± SD
C_{max} (μg ml^{-1})	3.81 ± 0.39
T_{max} (h)	0.94 ± 0.48
$t_{1/2}$ (h)	5.50 ± 1.65
$AUC_{0-\infty}$ (μg h ml^{-1})	23.29 ± 6.05
MRT (h)	4.82 ± 1.24
V_d/F (l kg^{-1})	6.40 ± 1.56
Cl/F (l h^{-1} kg^{-1})	0.84 ± 0.22

References

[1] A.A. Miller, D.A. Sawyer, B. Roth, P. L Wheatley, M.J. Leach, R.J. Lamb, Epilepsia 25 (1984) 655–656.
[2] A.A. Miller, P.L. Wheatley, D.A. Sawyer, M.G. Baxter, B. Roth, Epilepsia 27 (1986) 483–489.
[3] Anon, Drug Ther. Bull. 30 (1992) 75–76.
[4] W.C. Yuen, A.W. Peck, Br. J. Clin. Pharmacol. 26 (1988) 242.
[5] A.F. Cohen, G.S. Land, D.D. Breimer, W.C. Yuen, C. Winton, A. Peck, Clin. Pharmacol. Ther. 42 (1987) 535–541.
[6] A.W. Peck, Epilepsia (Suppl.) 32 (1991) S9–S12.
[7] A.D. Fraser, W. MacNeil, A.F. Isner, P.R. Camfield, Ther. Drug Monit. 17 (1995) 174–178.
[8] M. Meyler, M.T. Kelly, M.R. Smyth, Chromatographia 36 (1993) 27–32.
[9] A. Fazio, C. Artesic, M. Russo, R. Trio, G. Oteri, F. Pisani, Ther. Drug Monit. 14 (1992) 509–512.
[10] S. Ramachandran, S. Underhill, S.R. Jones, Ther. Drug Monit. 16 (1994) 75–82.
[11] M.W. Sinz, R. Remmel, J. Chromatogr. 571 (1991) 217–230.

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