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Determination of zolpidem in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats

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Abstract

A single-solvent extraction step high-performance liquid chromatographic method is described for quantitating zolpidem in rat serum microsamples (50 μl). The separation used a 2.1 mm I.D. reversed-phase OD-5-100 C_{18} column, 5 μm particle size with an isocratic mobile phase consisting of methanol–acetonitrile–26 m*M* sodium acetate buffer (adjusted to pH 2.0 with 40% phosphoric acid) containing 0.26 m*M* tetrabutylammonium phosphate (13:10:77, v/v/v). The detection limit was 3 ng/ml for zolpidem using an ultraviolet detector operated at 240 nm. The recovery was greater than 87% with analysis performed in 12 min. The method is simple, rapid, and applicable to pharmacokinetic studies of zolpidem after administering two intravenous bolus doses (1 and 4 mg/kg) in rats.

Keywords: Pharmacokinetics; Zolpidem

1. Introduction

Zolpidem, an imidazopyridine derivative, is a nonbenzodiazepine hypnotic agent which binds selectively to the benzodiazepine ω_1-subtype in the central nervous system [1,2]. It has been shown to be effective in inducing and maintaining sleep in adults [3,4]. Zolpidem has been extensively studied in animal research using various behavioral paradigms for a better understanding of its pharmacological effects; however, the analysis was limited to dose–response, rather than concentration–effect relations. Dose–response relations are influenced by the relation of dose to the resulting serum or plasma concentrations (i.e., pharmacokinetics) and by the relation between concentration and effect (i.e., pharmacodynamics). Integration of pharmacokinetics and pharmacodynamics permits the partition of the pharmacokinetic and pharmacodynamic components in drug action. To implement this approach, it is essential to use a sensitive and precise analytical method to determine the concentration of zolpidem in biological fluids.

A number of gas chromatographic and high-performance liquid chromatographic (HPLC) methods have been described for zolpidem in serum or plasma

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[5–13]. Of the HPLC methods, the sensitivity of zolpidem is higher with fluorimetric detection than with UV detection [5,7,11–13]. The aim of the present study was to develop a rapid, simple, and sensitive microsample (50 μl) HPLC-UV method for determination of the serum concentration–time profile of zolpidem in rats. Sample size is critical when the animal species used is small, especially when repeated blood sampling is necessary to trace temporal changes in drug levels in individual animals. No attempt was made to analyze metabolites of zolpidem because they were not pharmacologically active [14]. The convenience of our method is facilitated by use of a single-solvent extraction procedure and the commercially available 2.1 mm I.D. column. The major advantage is the approximately fivefold increase in sensitivity using the 2.1 mm I.D. column in comparison with that for a 4.6 mm I.D. column at constant injection volume. An added advantage is the reduction in solvent consumption by up to 80% compared to that of the 4.6 mm I.D. column. This method is hereby applied to evaluate the pharmacokinetics of zolpidem following intravenous (i.v.) zolpidem administration.

2. Materials and methods

2.1. Instrumentation

The HPLC system consisted of a Perkin Elmer 250 binary LC pump, and a 785A programmable absorbance UV detector with a detector cell volume of 12 μl, operated at 240 nm (Applied Biosystems, Foster City, CA, USA). The separation was performed at room temperature on an OD-5-100 C_{18} column, 100 mm×2.1 mm I.D., 5 μm particle size (Separation Methods Technologies, Newark, DE, USA) with a 2 μm Rheodyne precolumn filter (Cotati, CA, USA). The data were collected using a PE Nelson 900 series interface, Turbochrom 4.1 software (Perkin Elmer) and an IBM-type pentium microcomputer workstation.

2.2. Reagents and standards

Zolpidem hemitartrate, metabolites I (SL84.0589-10) and II (SL84.0853-10) were obtained from Synthelabo Recherche (Bagneux, France); their chemical structures are shown in Fig. 1. Mazindol was supplied by Hoffmann-La Roche (Nutley, NJ, USA). HPLC-grade methanol, acetonitrile, chloroform, and sodium acetate were purchased from Fisher (Springfield, NJ, USA). The 1 *M* borate–sodium carbonate–potassium chloride buffer (pH 9.0) was prepared by the method of de Silva and Puglisi [15]. All other chemicals were reagent grade.

Zolpidem hemitartrate and its two metabolites (I and II) as well as mazindol were dissolved in methanol individually to make 1 mg/ml stock base solution. Dilutions of the 1 mg/ml zolpidem standard were used to make the working standards (0.025, 0.05, 0.1, 0.25, 0.5, 1.0. and 2.0 μg/ml). The internal standard, mazindol, was diluted and used at a concentration of 2 μg/ml.

The HPLC analyses were performed using an isocratic mobile phase consisting of methanol–acetonitrile–26 m*M* sodium acetate buffer (adjusted to pH 2.0 with 40% phosphoric acid) containing 0.26 m*M* tetrabutylammonium phosphate (13:10:77, v/v/v). Mobile phases were degassed and filtered through a Solvent Filtration Apparatus (Alltech, Deerfield, IL, USA). The flow-rate was set at 0.3 ml/min and normally operated at a pressure of 101 bar (1450 p.s.i.).

2.3. Sample preparation

Standards and serum samples were prepared as previously described [16,17]. Briefly, a 25 μl volume of the internal standard (mazindol, 2 μg/ml) and 50 μl working serum standard were added to a 15 ml conical centrifuge tube. Borate buffer (1 *M*, pH 9.0, 100 μl) was added and the solution was mixed well. One ml of chloroform was added, and the sample mixture was vortex-mixed for 1 min and centrifuged for 5 min at 1100 *g*. To ensure vigorous mixing for extraction of alkalized zolpidem and mazindol to the organic solvent, the 1.15 ml sample mixture rose to 2 cm below the rim of the 15 ml conical centrifuge tube during vortex mixing. The organic layer was carefully transferred to a 5 ml conical centrifuge tube and evaporated to dryness in an evaporator (Pierce, Rockford, IL, USA) at 40°C under nitrogen. The residue was resuspended in 50 μl of mobile phase, and 20 μl was injected onto the column. Samples for

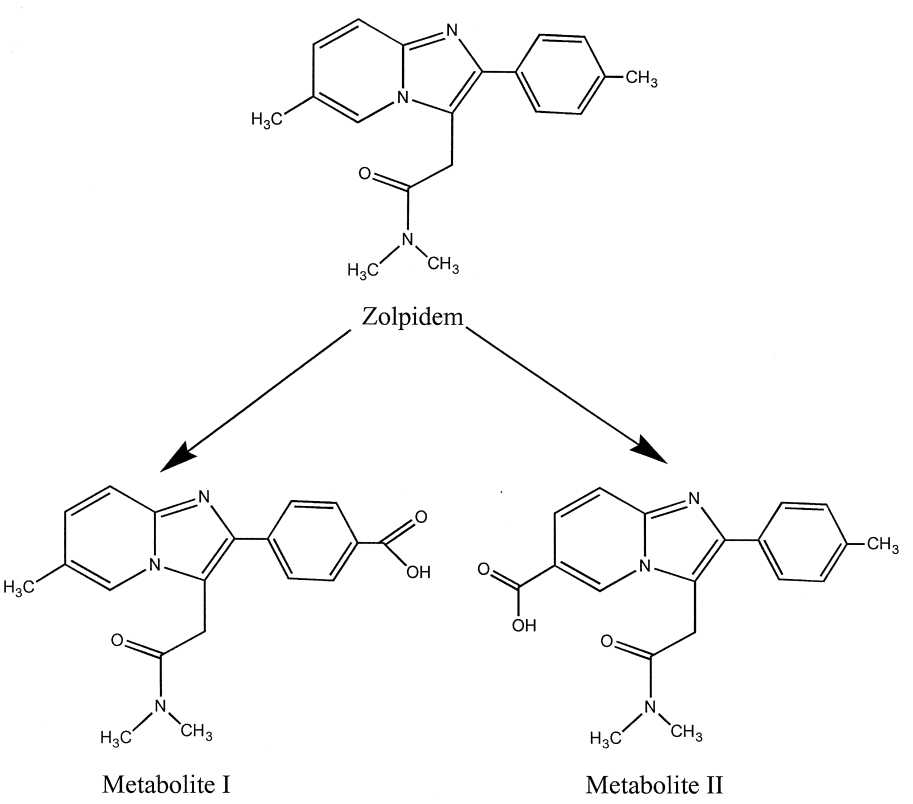

Fig. 1. Chemical structures of zolpidem and its two metabolites (metabolite I and II).

serum drug analysis were prepared identically except that standards were not added.

2.4. Extraction recovery

The assay recoveries of zolpidem were assessed at concentrations of 0.025, 0.5, and 1.0 μg/ml. Six replicates of each zolpidem concentration were extracted according to the method described above and were computed using the following equation: Recovery=(peak height extract)/(mean peak height direct injection)×100%.

2.5. Zolpidem administration and blood sampling

Five male, albino, virus-free Sprague-Dawley rats from HSD (Indianapolis, IN, USA), held to 80% of its normal, adult starting weight, 382 g, were used. Right jugular vein cannulation and blood sampling have been described previously [18]. The catheter was flushed with 0.9% saline containing 30 units of heparin per ml and sealed with fishing line when not in use.

The animals were allowed to recover for at least 2 days from the jugular vein catheterization prior to the drug administration series. Zolpidem hemitartrate was dissolved in 0.9% NaCl solution. Animals received i.v. bolus doses of zolpidem (1, and 4 mg/kg) via the jugular vein catheter. Drug doses of zolpidem were expressed in terms of the salt and were corrected to zolpidem base for the calculation of the pharmacokinetic parameters. Each drug dose was separated by 3–5 days in random order. All injections were given in a volume of 1 ml/kg body weight; zolpidem solution was delivered in 15 s. To ensure drug solution was completely administered, 0.3 ml of 0.9% saline was delivered in 15 s to wash out the catheter. Serial blood samples (100 μl) were individually collected in microcentrifuge tubes (0.6 ml) following i.v. 1 mg/kg zolpidem administration

at 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min postinjection. An additional blood sample at 360 min was obtained for the 4 mg/kg dose. Blood samples were centrifuged for 10 min at 13 700 *g*. The clear supernatant (i.e., serum) was separated and transferred to a clean tube, and stored frozen until analysis. Experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institute of Health Publ. No. 85-23, revised 1985).

2.6. Pharmacokinetic analysis

Pharmacokinetic analysis was performed using the SAAM II software system [19] (SAAM, Seattle, WA, 1997). The data were described by an open two-compartment model for zolpidem and fit to the following equation:

$$C_p = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

where C_p is the total serum drug concentration at time t; the terms A and B are the extrapolated zero intercepts; and α and β represent the apparent first-order distribution and elimination rate constants, respectively. The half-life ($t_{1/2}$) for the distribution or elimination phase and volume of distribution for the central compartment (V_c) were calculated by the equations: $t_{1/2}=0.693/\alpha$ or β and $V_c=\text{Dose}/(A+B)$. The area under the serum drug concentration–time curve ($AUC_{0-\infty}$) was calculated by the following equation: $AUC_{0-\infty}=A/\alpha+B/\beta$. Total clearance (Cl) was then defined as $\text{Dose}/AUC_{0-\infty}$.

3. Results and discussion

3.1. Method evaluation

Fig. 2 shows chromatograms of a serum blank containing no interfering peaks, a spiked serum sample containing a working standard (0.1 μg/ml), and a representative rat serum sample (50 μl) obtained 90 min following 4 mg/kg zolpidem administration. Zolpidem is known to be stable in biological samples when stored long-term at −20°C, or short-term at higher temperatures (e.g., room, 37°C), and during the liquid–liquid extraction procedure [5,6,12]. An unknown metabolite (peak 3)

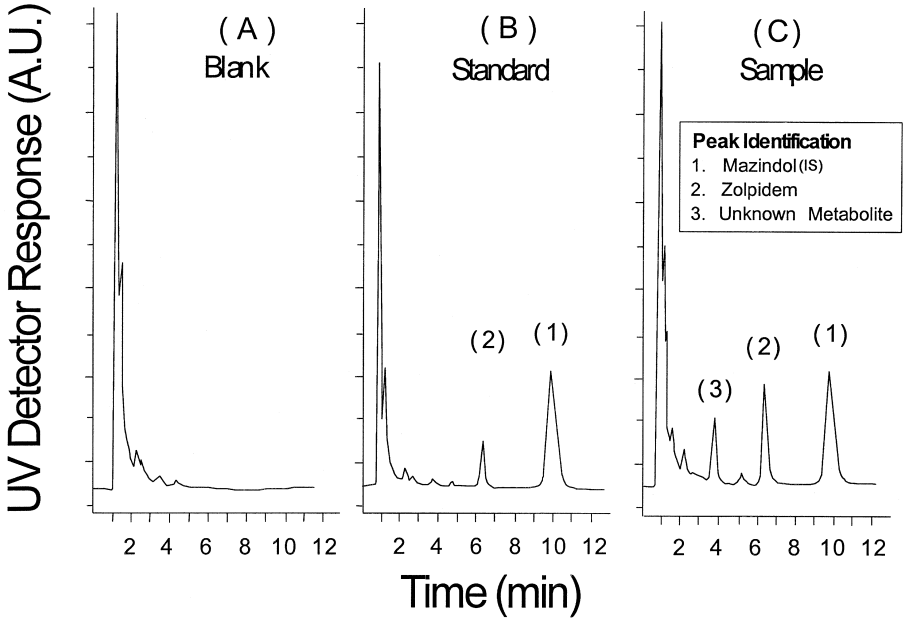

Fig. 2. Chromatograms of (A) serum blank, (B) serum containing 0.1 μg/ml zolpidem and 2 μg/ml mazindol, (C) a 50 μl rat serum sample obtained 90 min after 4 mg/kg zolpidem administration.

was identified in the serum sample. The analysis is performed in 12 min.

We used the internal standard method to calibrate and evaluate the unknown samples. Both within-day and between-day precisions established at three different concentrations (0.025, 0.5, and 1.0 μg/ml) for zolpidem were high as indicated by the coefficients of variation, which ranged from 2.43% to 2.85% and from 3.12% to 7.09%, respectively (Table 1). Table 1 also shows the accuracy of zolpidem, defined as the deviation between the true and measured values expressed in percent.

Calibration curves for zolpidem are linear within the ranges (0.025, 0.05, 0.1, 0.5, 1.0, and 2.0 μg/ml) examined. The linear regression equation was: $y = 3.172(\pm 0.038)x - 0.133(\pm 0.025)$. The correlation coefficient and the coefficient of variation of the slopes for the regression lines were 0.999 and 0.98%, respectively, with the intercept close to zero. The limit of detection for zolpidem was 3 ng/ml with signal-to-noise ratio of 3:1. The mean extraction recoveries for the internal standard was 87.89(±1.97) and for zolpidem at 0.025, 0.5, and 1.0 μg/ml were 90.37(±1.90), 91.32(±6.84), and 87.44(±2.78), indicating that a small volume of extraction solvent, 1 ml of chloroform, was sufficient for recovery of zolpidem from serum samples.

All the compounds listed in Table 2 did not interfere with the determination of zolpidem. Although metabolites I and II of zolpidem were eluted (Table 2), our liquid–liquid extraction procedure was unable to extract these two metabolites from serum samples. A column-switching HPLC method, which does not require any sample preparation, has been reported for the determination of these two metabolites in biological fluids [12]. Chlordiazepoxide or cocaethylene could be used as an alternative internal standard for zolpidem (Table 2). Inasmuch as the metabolites of zolpidem are more polar than the parent drug, the retention times of those metabolites were shorter than that of the parent drug using reversed-phase HPLC systems [12]. Therefore, we chose to use a compound that was eluted later than zolpidem as an internal standard such as mazindol to

Table 2
Relative retention times (k') of other drugs for possible interference with zolpidem

Compound	k'
Theophylline	0.76
β-Hydroxyethyltheophylline	0.99
Caffeine	1.44
Metabolite I of zolpidem	1.49
N-Desmethylclozapine	1.56
Clozapine	2.05
Benzoylecgonine	2.39
Benzoylnorecgonine	2.70
Unknown zolpidem metabolite	3.22
Oxazepam	3.69
Cocaine	4.15
Norcocaine	5.32
Metabolite II of zolpidem	5.90
Zolpidem	6.93
Chlordiazepoxide	7.46
Cocaethylene	9.59
Alprazolam	10.79
Clonazepam	10.94
Mazindol(internal standard)	11.17
Midazolam	11.22
α-Hydroxylmidazolam	16.26
Flurazepam	26.22
Barbital	N.D.
Diazepam	N.D.
Pentobarbital	N.D.
Hexobarbital	N.D.

N.D. = peak not observed within 30 min.

Table 1
Precision and accuracy data for zolpidem in serum by HPLC

Within-day ($n=6$)		Accuracy (%)	Between-day ($n=5$)		Accuracy (%)
Concentration (mean±SD) (μg/ml)	C.V.[a] (%)		Concentration (mean±SD) (μg/ml)	C.V. (%)	
0.025±0.001	2.43	100.0	0.025±0.001	4.75	100.0
0.500±0.014	2.85	100.0	0.501±0.036	7.09	100.2
1.002±0.025	2.48	100.2	0.992±0.031	3.12	99.2

[a] C.V.: coefficient of variation.

avoid any possible interference for the determination of zolpidem resulting from the interaction of the polar metabolites with an internal standard that was eluted earlier than zolpidem.

3.2. Zolpidem pharmacokinetics

Fig. 3A shows the mean serum zolpidem concentration–time profiles after i.v. 1 and 4 mg/kg zolpidem administration. Zolpidem decreased biexponentially following drug administration. One set of pharmacokinetic parameter values described zolpidem concentration–time profiles well for the two doses, indicating linear kinetics in the dose range used (Table 3). The observed (filled symbols) and predicted (solid lines) serum zolpidem concentration–time profiles for a representative animal are shown in Fig. 3B. The distribution and terminal elimination half-lives for zolpidem herein reported were consonant with data obtained in rats by a HPLC method with fluorimetric detection [14]. We detected a peak at the relative retention time of 3.22 min in all

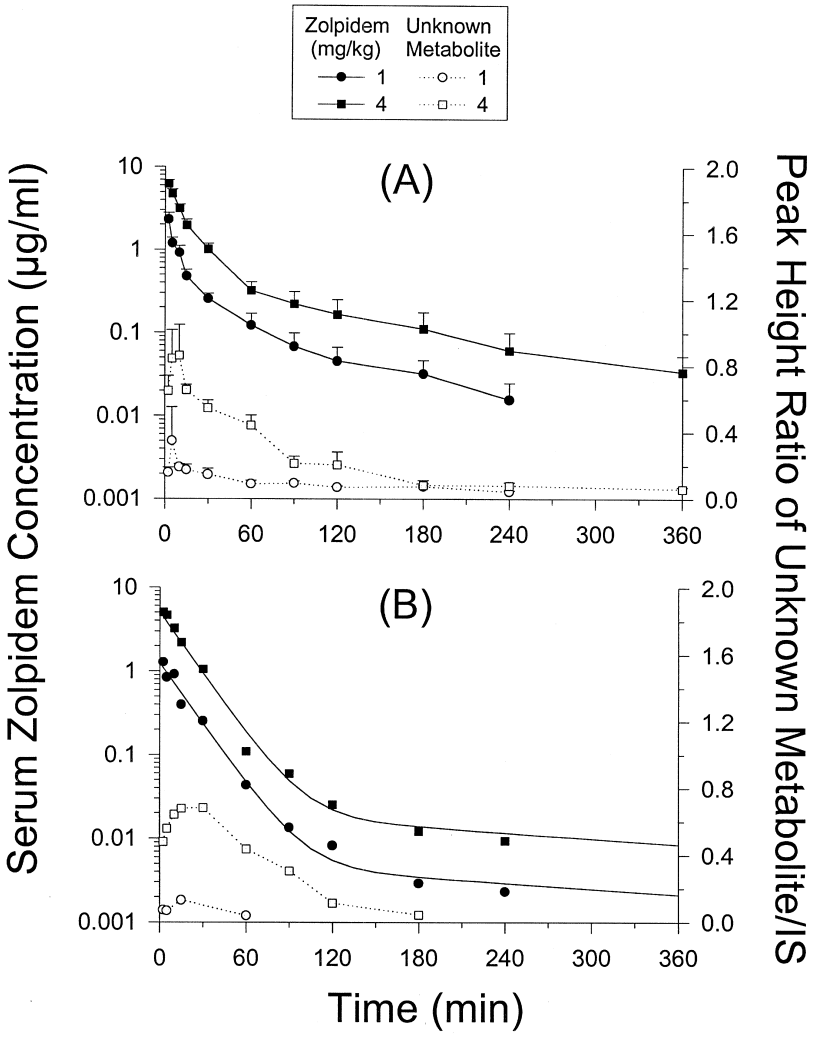

Fig. 3. Serum zolpidem concentration–time profiles (left *y*-axis) and profiles of peak-height ratios of unknown metabolite and internal standard (right *y*-axis) after i.v. bolus 1 and 4 mg/kg zolpidem administration: (A) mean(±S.E.) for the five animals; (B) observed (filled symbols) and predicted (solid lines) values of zolpidem for a representative animal.

Table 3
Mean pharmacokinetic parameters (±S.E.) for zolpidem after i.v. route of administration in rats ($n=5$)

V_c (l/kg)	0.56±0.10
V_{ss} (l/kg)	2.05±0.30
Cl (l/h/kg)	2.18±0.54
α (min^{-1})	0.072±0.015
β (min^{-1})	0.0086±0.002
$t_{1/2\alpha}$ (min)	9.57
$t_{1/2\beta}$ (min)	80.2
Zolpidem (mg/kg)	$AUC_{(0-\infty)}$ (μg×min/ml)
1	33.54±8.03
4	134.15±32.13

the serum samples following the administration of zolpidem regardless of dose (Fig. 2C and Table 2). However, we could not quantify it because the standard was unavailable to us. Thus, the peak height ratios of this unknown peak and the internal standard were used to describe its profiles across time for the two intravenous doses. The unknown peak was named as unknown metabolite because it exhibited the prototypic profile of a metabolite. The formation of unknown metabolite was not only time dependent (i.e., it increased to a peak value, then decreased subsequently) but was also dose dependent (Fig. 3A and B).

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References

[1] S. Arbilla, H. Depoortere, P. George, S.Z. Langer, Naunyn-Schmiedebergs Arch. Pharmacol. 330 (1985) 248.
[2] J. Benavides, H. Depoortere, D. Sanger, G. Perrault, S. Arbilla, S.Z. Langer, B. Zivkovic, B. Scatton B, L'Encephale 16 (1990) 13.
[3] J.D. Hoehns, P.J. Perry, Clin. Pharm. 12 (1993) 814.
[4] H.D. Langtrey, P. Benfield, Drugs 40 (1990) 291.
[5] A.L.B. Durol, D.J. Greenblatt, J Anal. Toxicol. 21 (1997) 388.
[6] P. Guinebault, C. Dubruc, P. Hermann, J.P. Thenot, J. Chromatogr. 383 (1986) 206.
[7] G. Debailleul, F.A. Khalil, P. Lheureux, J. Anal. Toxicol. 15 (1991) 35.
[8] D. Debruyne, J. Lacotte, B. Hurault de Ligny, M. Moulin, J. Pharm. Sci. 80 (1991) 71.
[9] Y. Gaillard, J.P. Gay-Montchamp, M. Ollagnier, J. Chromatogr. 622 (1993) 197.
[10] F. Stanke, N. Jourdil, J. Bessard, G. Bessard, J. Chromatogr. B 675 (1996) 43.
[11] A. Tracqui, P. Kintz, P. Mangin, J. Chromatogr. 616 (1993) 197.
[12] V. Ascalone, L. Flaminio, P. Guinebault, J.P. Thenot, P.L. Morselli, J. Chromatogr. 581 (1992) 237.
[13] P. Ptacek, J. Macek, J. Klima, J. Chromatogr. B. 694 (1997) 409.
[14] J.P. Thenot, P. Hermann, A. Durand, J.T. Burke, J. Allen, in: J.P. Sauvanet, S.Z. Langer, P.L. Morselli (Eds.), Imidazopyridines in sleep disorders: a novel experimental approach, Raven Press, New York, 1988, p. 139.
[15] J.A.F. de Silva, C.V. Puglisi, Anal. Chem. 42 (1970) 1725.
[16] C.E. Lau, F. Ma, J.L. Falk, J. Chromatogr. 532 (1990) 95.
[17] F. Ma, C.E. Lau, J. Chromatogr. B 712 (1998) 193.
[18] C.E. Lau, F. Ma, Y. Wang, C. Smith, Psychopharmacology 126 (1996) 241.
[19] SAAM II, User Guide, SAAM Institute, University of Washington, Seattle, WA, 1997.