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Development and validation of a high-performance liquid chromatography method using diode array detection for the simultaneous quantification of aripiprazole and dehydro-aripiprazole in human plasma

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ABSTRACT

A high-performance liquid chromatography method with diode array detection (HPLC-DAD) was developed for quantification of aripiprazole and dehydro-aripiprazole, in human plasma. After a simple liquid–liquid extraction, chromatographic separation was carried out on a C18 reversed-phase column, using an ammonium buffer–acetonitrile mobile phase (40:60, v/v). The total run time was only 7 min at a flow-rate of 1.0 ml/min. The precision values were less than 12% and the accuracy values were ranging from 98 to 113% and the lower limit of quantification was 2 ng/ml for both compounds. Calibration curves were linear over a range of 2–1000 ng/ml. The mean trough plasma concentrations in patients treated with aripiprazole were 157 and 29 ng/ml for aripiprazole and dehydro-aripiprazole, respectively.

1. Introduction

Aripiprazole is a novel antipsychotic indicated for the treatment of schizophrenia [1]. It is the first member of a new class of antipsychotic agents called "dopamine system stabilizers" [2]. Aripiprazole acts as a potent partial agonist at dopamine D_2 receptors and serotonin 5-HT_{1A} receptors [3,4]. The drug is rapidly absorbed after oral administration. The peak plasma concentration is reached approximately 3–5 h following oral administration and the bioavailability of the drug is 87% [5]. Aripiprazole is extensively metabolized by the liver through the cytochrome P 450 system (CYP 3A4 and CYP 2D6) [6] and its major active metabolite is the dehydro-aripiprazole [7]. The mean elimination half-life following multiple oral doses of aripiprazole is ranging from 47 to 68 h [5]. The steady-state plasma drug concentrations are achieved after 14 days of treatment for both aripiprazole and dehydro-aripiprazole [5].

Drug plasma concentration monitoring in patients treated with psychotropic drugs is helpful either to control compliance, to improve efficacy when the patient does not respond to therapeutic doses, or to detect an overdosage [8]. Few analytical methods are described in the literature for the quantification of both aripiprazole and dehydro-arpiprazole in human plasma. These assays were based on chromatographic methods using mass spectrometric detection [9,10]. Two methods using the spectrophotometric detection had been reported for the measurement of aripiprazole alone [11,12]. To our knowledge, no HPLC method using UV detection allowing the simultaneous quantification of aripiprazole and dehydro-aripiprazole in human plasma has been reported.

The aim of this study was to develop and validate a rapid, simple and specific method by HPLC with UV detection, to quantify the concentration of both aripiprazole and dehydro-aripiprazole in human plasma for the therapeutic drug monitoring of aripiprazole-treated patients. The method validation was performed according to the Food and Drug Administration (FDA) guidelines for the analytical methods [13].

2. Experimental

2.1. Chemicals and reagents

The pure compounds aripiprazole and dehydro-aripiprazole were supplied by Bristol-Myers Squibb (Rueil-Malmaison, France) and chlorohaloperidol, used as internal standard, by Janssen Cilag (Issy les Moulineaux, France). Ammonium hydrogen carbonate and

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anhydrous sodium carbonate were purchased from Merck (Darmstardt, Germany) and Carlo Erba (Val de Reuil, France), respectively. Ammonium buffer 10 mM was prepared in deionized and osmosed water (purification system Aquadem, Le Plessis Robinson, France); the pH was adjusted to 8.35 with sodium hydroxide 6 mol/l. Acetonitrile and orthophosphoric acid were obtained from VWR (Fontenay sous Bois, France). All chemicals used in this study were of the high purity "HPLC grade".

2.2. Instrumentation and chromatographic conditions

The liquid chromatographic system was carried out using a 600 controller pump (Waters, Saint-Quentin en Yvelines, France) with in-line degasser coupled to a 2996 photodiode array detector (Waters).

The Empower™ software was used for system control, data acquisition and processing.

The HPLC chromatographic separations of compounds were carried out on a C18 column X Bridge® C18 3.5-μm particle size, 100 mm × 4.6 mm I.D. (Waters). Compounds were eluted isocratically using a mobile phase consisting of acetonitrile:ammonium buffer (10 mM; pH 8.35) (60:40, v/v) with a flow rate of 1 ml/min. Before use, the mobile phase was filtered through a 0.22 μm filter (Millipore Corporation, Belfast, MA). The aripiprazole, dehydro-aripiprazole and internal standard were detected at 217 nm. Acquisition from 200 to 350 nm was performed to allow the drug identification by its ultraviolet absorption spectrum and for the study of the peaks' purity.

2.3. Calibration and quality control samples

Methanol stock solutions (200 μg/ml) of aripiprazole and dehydro-aripiprazole were diluted in methanol to obtain the working solutions of 10 and 1 μg/ml. Working solution of internal standard was prepared by diluting the stock solution (10 mg/ml) in methanol to yield a 10 μg/ml chlorohaloperidol concentration. All stock and working solutions were stored at 4 °C for a maximum of 4 weeks and 1 week, respectively.

Routine daily calibration curves were prepared by spiking 1 ml of human drug-free serum Lyphochek® (Bio-Rad, Marnes-la-Coquette, France) with an appropriate volume of working solutions (2–50 μl) to yield concentrations of 0, 2, 5, 30, 50, 100, 200 and 500 ng/ml, and with 5 μl of stock solution to yield 1000 ng/ml. Calibration curves were used to calculate the aripiprazole or dehydro-aripiprazole concentrations according to the peak height ratios (aripiprazole/internal standard or dehydro-aripiprazole/internal standard).

To prepare the in-house quality control samples (QCs), an appropriate amount of aripiprazole and dehydro-aripiprazole working or stock solutions were added to human drug-free serum to achieve final concentrations of 2, 5, 100 and 1000 ng/ml. The QCs were aliquoted and stored at −20 °C until use.

2.4. Extraction preparation

20 μl of working I.S. solution, 500 μl of sodium carbonate (2 mol/l) and 10 ml of the extracting solvent heptane and isopropanol in a ratio of 98:2 (v/v) were added to 1 ml of plasma sample (calibrators, QCs or patient samples). After a 20 min shaking with a horizontal agitator (Agitelec SL200, J.Toulemonde, Paris, France) and a 10 min centrifugation at 1800 × *g* at 4 °C (CR 412, Jouan, Saint-Herblain, France), the organic layer was removed and 150 μl of orthophosphoric acid (0.05 mol/l) was added. After a further 30 s shaking, 50 μl of the aqueous phase was injected in the chromatographic system.

2.5. Patients samples

Samples for the titration of aripiprazole and dehydro-aripiprazole were sent to our laboratory from various psychiatric units in a routine therapeutic drug monitoring setting. The decision to request therapeutic drug monitoring was made by the patients' psychiatrists on the basis of the clinical evaluations (either to control compliance, or when overdosage is suspected).

We received 50 samples, corresponding to 34 out or inpatients [15 females and 19 males aged from 16 to 60 years (mean: 33 years) and from 25 to 59 years (mean: 36 years) respectively] over a 14-months period. These patients were treated with an aripiprazole daily dose of 10–30 mg, together with one or several other psychotropic drugs.

Blood samples were drawn in the morning, before the oral intake. The steady state conditions could not be ascertained for some samples.

The blood samples, collected into heparinized tubes, were centrifuged at 1800 × *g* for 10 min and the plasma was stored at −20 °C until analyzed.

All the procedures that we followed were in accordance with the Helsinki declaration, as revised in 2000.

2.6. Method validation

The within-day precision was assessed in 10 replicate at three concentrations (5, 100 and 1000 ng/ml), and the coefficients of variation (CVs) were calculated.

The QCs containing 2, 5, 100 and 1000 ng/ml of both aripiprazole and dehydro-aripiprazole were measured for 10 days to evaluate the between-days precision and accuracy of the method. Accuracy was determined according to the equation:

$$\text{Accuracy}\,(\%) = \left(\frac{\text{measured concentration}}{\text{theoretical concentration}}\right) \times 100$$

The lowest limit of quantification (LLOQ) was defined as the lowest measurable concentration with a between-days coefficient of variation (CV) below 20%.

The linearity was assessed using a linear regression method between 2 and 1000 ng/ml.

The recovery of the extraction procedure was assessed at four different concentrations (2, 5, 100 and 1000 ng/ml) in replicate over 5 days. The peak height obtained from the samples after extraction was compared to those obtained by the direct injection of the same amount of pure compounds (aripiprazole and dehydro-aripiprazole) dissolved in orthophosphoric acid (0.05 mol/l).

The specificity was evaluated by comparing the retention time of different pure unextracted drugs with those of the aripiprazole, dehydro-aripiprazole and internal standard. These compounds included amisulpride, amitriptyline, amoxapine, chlorpromazine, clomipramine, desmethylclomipramine, clozapine, norclozapine, cyamemazine, desipramine, diazepam, escitalopram, desmethyeslcitalopram, fluoxetine, norfluoxetine, fluphenazine, fluvoxamine, haloperidol, hydroxyhaloperidol, imipramine, levomepromazine, loxapine, nortriptyline, paroxetine, pipothiazine, risperidone, 9-hydroxyrisperidone, sertraline, venlafaxine and *O*-desmethylvenlafaxine. Furthermore, potential interference of psychotropic drugs commonly administrated with aripiprazole treatment was studied in plasma samples from patients treated with aripiprazole. Medications commonly administrated to these patients consisted in oxazepam, clonazepam, prazepam, zolpidem, zopiclone, alimemazine, alprazolam, hydroxyzine fluoxetine, paroxetine, tianeptine, escitalopram, venlafaxine, valproate, olanzapine, clozapine, risperidone, amisulpride,

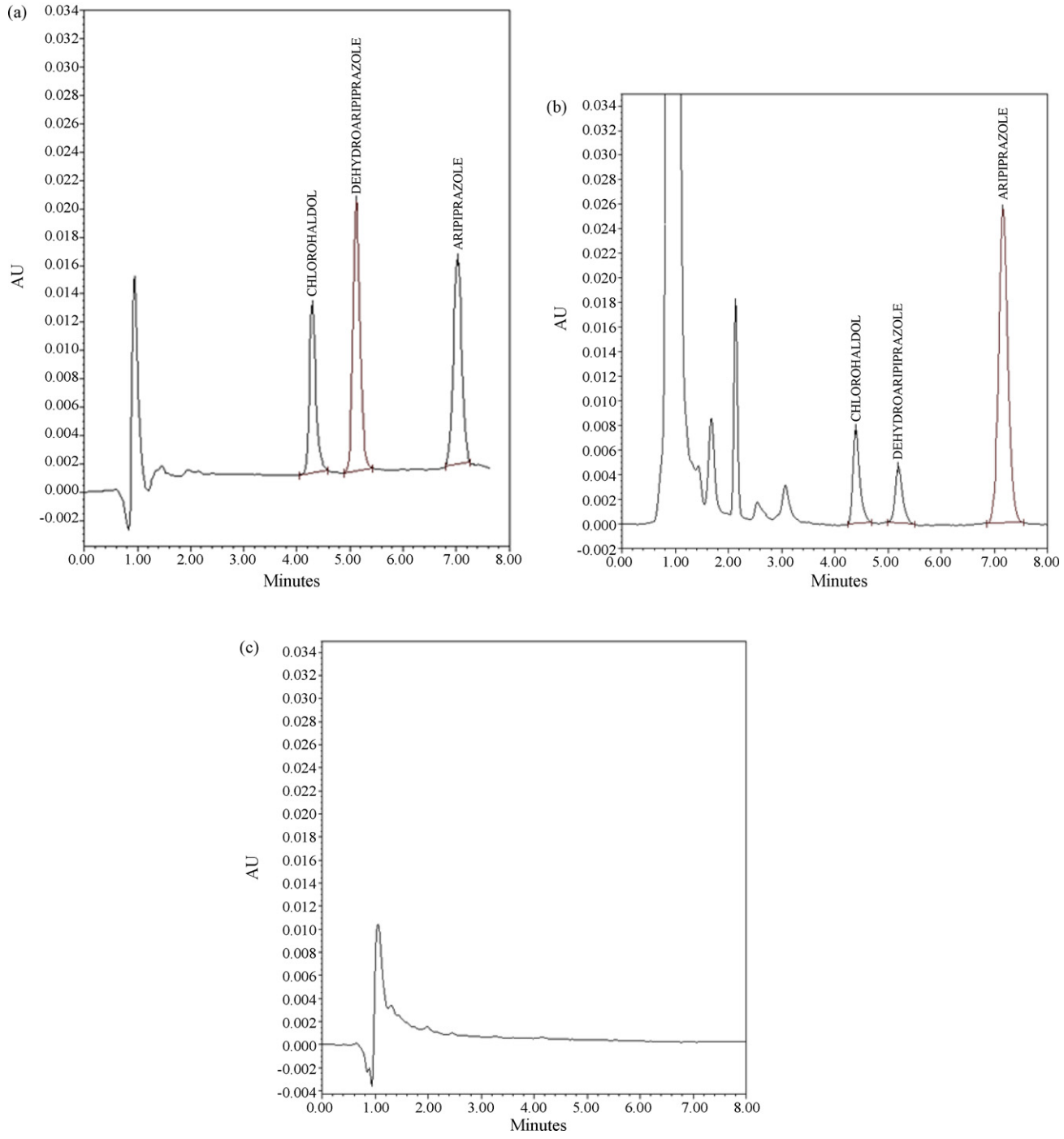

Fig. 1. Typical chromatograms obtained after extraction of a calibration sample at a concentration of 50 ng/ml (a), a plasma from a patient treated with aripiprazole at a dose of 10 mg/day (measured concentrations were 272 and 41 ng/ml for aripiprazole and dehydro-aripiprazole, respectively) (b) and a human drug free plasma (c).

haloperidol, levomepromazine, cyamemazine, chlorpromazine or loxapine.

3. Results

3.1. Chromatography

Under the described chromatographic conditions, the retention times of the internal standard, dehydro-aripiprazole and aripiprazole were 4.2, 5 and 6.8 min, respectively (Fig. 1a and b). No interfering peak was observed in the extracted blank serum (Fig. 1c) or in plasma from "drug-free" patients (data not shown).

3.2. Precision, accuracy and sensitivity

Precision and accuracy are reported in Tables 1 and 2. The LLOQ of the method was defined at 2 ng/ml for both aripiprazole and dehydro-aripiprazole. The within-day and between-days CVs were less than 18.5% at 2 ng/ml and less than 11.8% for the other QCs (5, 100 and 1000 ng/ml) for both aripiprazole and dehydro-aripiprazole. The method was accurate: the deviation from the

Table 1
Accuracy and precision of the aripiprazole quantification method

Theoretical concentrations (ng/ml)	Measured concentrations (ng/ml)[a]	Accuracy (n = 10) (%)	CV (%)[b]	
			Within-day (n = 10)	Between-days (n = 10)
2 (LLOQ)	2.23 (±0.4)	111.5	ND[c]	18.5
5 (low)	4.99 (±0.6)	99.8	7.4	11.8
100 (medium)	100.0 (±5.7)	100.0	1.5	5.7
1000 (high)	984.9 (±54.3)	98.5	2.4	5.5

[a] Mean (±standard derivation) of concentrations obtained on 10 different days.
[b] Coefficient of variation.
[c] Not done.

Table 2
Accuracy and precision of the dehydro-aripiprazole quantification method

Theoretical concentrations (ng/ml)	Measured[a] concentrations (ng/ml)	Accuracy (n = 10) (%)	CV[b] (%)	
			Within-day (n = 10)	Between-days (n = 10)
2 (LLOQ)	2.26 (±0.4)	113.0	*ND*[c]	17.8
5 (low)	5.30 (±0.3)	106.0	7.2	5.5
100 (medium)	102.5 (±5.3)	102.5	1.6	5.1
1000 (high)	980.6 (±66.3)	98.1	2.1	5.8

[a] Mean (±standard derivation) of concentrations obtained on 10 different days.
[b] Coefficient of variation.
[c] Not done.

theoretical concentration (bias) was less than 20% for the LLOQ level and less than 15% for the QCs (low, medium and high).

3.3. Linearity

The peak height ratio (aripiprazole or dehydro-aripiprazole/internal standard) as a function of the aripiprazole or the dehydro-aripiprazole concentration in the range of 2–1000 ng/ml, gave regression coefficients r^2 higher than 0.999. The linear regression equations were

$Y = 0.0122X - 0.0646$ (aripiprazole) and

$Y = 0.0158X - 0.0577$ (dehydro-aripiprazole)

3.4. Extraction efficiency

The mean extraction recoveries for LLOQ, low, medium and high concentrations were respectively 82, 73, 74 and 78% for aripiprazole and 84, 69, 70 and 69% for dehydro-aripiprazole.

3.5. Specificity

Among the 30 drugs tested to evaluate potential interference, only two potentially interfered with this method: fluphenazine and amitriptyline had retention times similar to that of internal standard and aripiprazole, respectively (Table 3).

We also tested patients co-treated with other psychotropic drugs. Acquisition from 200 to 350 nm allowed identification of drugs by their ultraviolet absorption spectrum and verification of their purity. No interference with the aripiprazole, dehydro-aripiprazole or the internal standard peak was observed in the chromatograms of these patients.

3.6. Clinical application of the method

The mean (±standard deviation) plasma concentrations obtained from 50 patient samples were 157 ± 90 (range: <2–391 ng/ml) and 29 ± 20 ng/ml (range: <2–108 ng/ml) for aripiprazole and dehydro-aripiprazole, respectively. Twenty of these samples could be controlled as collected in steady-state conditions: their plasma concentrations ranged from 101 to 391 ng/ml (mean = 208 ng/ml) and from 12 to 71 ng/ml (mean = 35 ng/ml) for aripiprazole and dehydro-aripiprazole, respectively.

4. Discussion and conclusion

To the best of our knowledge, no HPLC method using diode array detection for the simultaneous measurement of both aripiprazole and dehydro-aripiprazole has been described until now.

Table 3
Psychotropic drugs assayed for potential interference

Drugs	Retention time (min)
Amisulpride	1.2
O-Desmethylvenlafaxine	1.5
9-Hydroxyrisperidone	1.5
Risperidone	1.7
Desmethylescitalopram	1.8
Norclozapine	1.9
Amoxapine	2.3
Hydroxyhaloperidol	2.3
Paroxetine	2.4
Escitalopram	2.4
Fluvoxamine	2.6
Diazepam	2.6
Venlafaxine	2.7
Desipramine	2.7
Norfluoxetine	2.8
Haloperidol	2.9
Pipothiazine	2.9
Clozapine	3.0
Nortriptyline	3.1
Fluoxetine	3.3
Desmethylclomipramine	3.7
Internal standard	**4.2**
Fluphenazine	4.2
Loxapine	4.5
Dehydro-aripiprazole	**5**
Amitriptyline	6.6
Aripiprazole	**6.8**
Sertraline	7.4
Cyamemazine	8.0
Levomepromazine	8.2
Imipramine	8.5
Clomipramine	8.6
Chlorpromazine	9.2

The method validated in this study is a rapid, precise, accurate and specific quantification of aripiprazole and dehydro-aripiprazole in human plasma by isocratic HPLC coupled with diode array detection.

The liquid–liquid extraction procedure is convenient and efficient, since evaluated recoveries were higher than 69%. During assay development, two volumes of heptane were assayed for the extraction procedure. Extraction efficiency obtained when 10 ml of heptane was added to the plasma was higher than that obtained with 5 ml of heptane (mean extraction recoveries were averaging 57% with 5 ml, versus 75% with 10 ml, for both aripiprazole and dehydro-aripiprazole).

The volumes of the working solutions used for the preparation of calibration and QC samples were small enough not to observe any dilution effect.

The time required for the chromatographic separation of the three compounds is short, averaging approximately 6–7 min. In a previous method described by Kirschbaum [11] the complete separation (aripiprazole and internal standard, dehydro-aripiprazole was not included) was obtained within 20 min.

This method presents a good selectivity. Only two of the pure drugs tested, fluphenazine and amitriptyline, interfered with the method. These interferences should be taken into account when patients are treated with combinations of one of these two drugs. The retention time of loxapine was between that of the internal standard and dehydro-aripiprazole peaks. Nevertheless the three peaks were separated enough to quantify the aripiprazole and dehydro-aripiprazole in the plasma of patients treated with aripiprazole and loxapine. None of the samples from patients treated with any other commonly used psychiatric drug showed potentially interfering peaks with same retention time that the compounds of interest.

Aripiprazole and dehydro-aripiprazole can be measured in plasma over a wide range of concentrations from 2 to 1000 ng/ml with satisfactory precision. This range is wide enough to quantify plasma levels of aripiprazole and dehydro-aripiprazole in the therapeutic drug monitoring. In the present study, the trough steady state concentrations interquantile ranges (25–75th) were 150–265 ng/ml for aripiprazole and 185–310 ng/ml for the active moiety (aripiprazole and dehydro-aripiprazole). These results were similar to those previously reported (an interquantile ranging from 146 to 254 ng/ml for aripiprazole) [11]. A recent study found trough concentrations ranging between 102 and 425 ng/ml [14] for aripiprazole and between 146 and 536 ng/ml for the active moiety [14]. This study was performed in a larger patient group ($n = 118$) nevertheless the steady state conditions were not controlled.

We conclude that this method is a rapid, reproducible and accurate method to quantify both aripiprazole and dehydro-aripiprazole. It is easy and less expensive than those based on a mass spectrometry detection, which is not available in many laboratories. It represents therefore an alternative procedure for routine therapeutic drug monitoring of patients treated with aripiprazole.

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References

[1] S. Gupta, P. Masand, Ann. Clin. Psychiatry 16 (2004) 155.
[2] S.M. Stahl, J. Clin. Psychiatry 62 (2001) 841.
[3] J.A. Lieberman, CNS Drugs 18 (2004) 251.
[4] S. Jordan, V. Koprivica, R. Chen, K. Tottori, T. Kikuchi, C.A. Altar, Eur. J. Pharmacol. 441 (2002) 137.
[5] S. Mallikaarjun, D.E. Salazar, S.L. Bramer, J. Clin. Pharmacol. 44 (2004) 179.
[6] M. Kubo, T. Koue, A. Inaba, H. Takeda, H. Maune, T. Fukuda, J. Azuma, Drug Metab. Pharmacokinet. 20 (2005) 55.
[7] Bristol-Myers squibb company/OtsuDk of America Pharmaceutical Inc. Abilify™ (aripiprazole) tablets prescribing information, Revised November 2006.
[8] P. Baumann, C. Hiemke, S. Ulrich, I. Gaertner, M.L. Rao, G. Eckermann, M. Gerlach, H.J. Kuss, G. Laux, B. Müller-Oerlinghausen, P. Riederer, G. Zernig, Ther. Drug Monit. 26 (2004) 167.
[9] M. Kubo, Y. Mizooku, Y. Hirao, T. Osumi, J. Chromatogr. B 822 (2005) 294.
[10] H.C. Huang, C.H. Liu, T.H. Lan, T.M. Hu, H.J. Chiu, Y.C. Wu, Y.L. Tseng, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 856 (2007) 57.
[11] K.M. Kirschbaum, M.J. Müller, G. Zernig, A. Saria, A. Mobascher, J. Malevani, C. Hiemke, Clin. Chem. 51 (2005) 1718.
[12] Y. Shimokawa, H. Akiyama, E. Kashiyama, T. Koga, G. Miyamoto, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 821 (2005) 8.
[13] Center for Drug Evaluation and Research (FDA), Guidance for industry: Bioanalytical Method Validation, May 2001 (http://www.fda.gov/cder/guidance/4252fnl.pdf).
[14] E. Molden, H. Lunde, N. Lunder, H. Refsum, Ther. Drug Monit. 28 (2006) 744.