Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 794 (2003) 35–47
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: a simple tool for therapeutic drug monitoring

Christine Frahnert, Marie Luise Rao*, Katja Grasmäder

Department of Psychiatry, University of Bonn, Sigmund-Freud-Strasse 25, D-53105 Bonn, Germany

Received 27 February 2003; received in revised form 22 April 2003; accepted 28 April 2003

Abstract

Therapeutic drug monitoring necessitates efficient, fast and reliable analytical methods validated by external quality control. We therefore devised an isocratic reversed-phase HPLC method with ultraviolet detection and optimised this to quantify mirtazapine, reboxetine, moclobemide, venlafaxine, O-desmethylvenlafaxine, paroxetine, fluvoxamine, fluoxetine, norfluoxetine, sertraline, citalopram, amitriptyline, nortriptyline, imipramine, desipramine, doxepin, nordoxepin, clomipramine, norclomipramine, trimipramine, mianserine, maprotiline, normaprotiline, amisulpride, clozapine, norclozapine, quetiapine, risperidone and 9-OH-risperidone in human serum. After solid-phase extraction of the drugs and metabolites, the chromatographic separation was achieved on a Nucleosil 100-Protect 1 column with acetonitrile–potassium dihydrogenphosphate buffer as mobile phase. The method was validated for therapeutic and toxic serum ranges. A linear relationship ($r>0.998$) was obtained between the concentration and the detector signal. Recoveries were between 75 and 99% for the drugs and metabolites. The accuracy of the quality control samples, expressed as percent recovery, ranged from 91 to 118%; intra- and inter-assay-relative standard deviations were 0.9–10.2% and 0.9–9.7%, respectively. Additional external quality control is carried out since 3 years. This method is applicable to rapidly and effectively analyze serum or plasma samples for therapeutic drug monitoring of about 30 antidepressants and atypical antipsychotics.

Keywords: Therapeutic drug monitoring; Antidepressants; Antipsychotics, atypical

1. Introduction

Therapeutic drug monitoring (TDM) of psychotropic drugs is an established tool to optimise dosing regimen of drugs with a narrow therapeutic range, such as tricyclic antidepressants, butyrophenones or clozapine. There is evidence of therapeutic and economic benefit of monitoring these drugs to avoid adverse events, intoxication, nonresponse or noncompliance [1–5]. Over the last decade a new generation of antidepressants and antipsychotics has been developed which are rather safe with respect to severe side effects and overdose and thus are increasingly administered to treat depression or schizophrenia. However, there is still limited information on therapeutic ranges for drug monitoring of these compounds. For venlafaxine, the ratio between venlafaxine and its desmethyl metabolite O-desmethylvenlafaxine has been shown to be significantly

*Corresponding author. Fax: +49-228-287-6383.
E-mail address: m.l.rao@uni-bonn.de (M.L. Rao).

1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00393-3

higher in responders of antidepressive drug therapy than nonresponders [6]. As regards selective serotonin reuptake inhibitors (SSRIs), there is evidence for an economic benefit of TDM of citalopram, sertraline and paroxetine although serum concentration–effect relationships still remain unclear [7]. Concerning atypical antipsychotics, high doses of risperidone, olanzapine and amisulpride are suspected to pose an increased risk for extrapyramidal side effects [8,9]. As most of these drugs are extensively metabolised by the liver and their metabolic products excreted by the kidney, therapeutic drug monitoring may be useful in cases of hepatic and renal impairment, for poor metabolisers of cytochrome P450 isoenzymes or comedication with inhibitors or inducers of these isoenzymes [6,10–13]. Furthermore, TDM is a tool to control noncompliance.

Analytical procedures that allow the rapid quantification of several psychotropic drugs in one step are useful for the clinical routine to provide clinicians with the individuals' serum level. Methods to determine several antidepressants published so far focus on tri- and tetracyclic antidepressants (TCAs) or SSRIs [14–23], while for reboxetine, mirtazapine, nefazodone, venlafaxine and moclobemide separate methods have been described [24–28]. TDM of antipsychotics to quantify either phenothiazines, antipsychotics with high dopamine-D2 receptor affinity or clozapine in combination with olanzapine have been established [29–32], whereas single component assays exist for the determination of amisulpride, quetiapine or risperidone via high-performance liquid chromatography (HPLC) [33–35]. The radioreceptor assay detects all dopamine-D2 receptor antagonists in serum [36], but lacks distinction between different antipsychotic drugs or their active metabolites. The most common technique is HPLC with ultraviolet (UV), fluorimetric or electrochemical detection. Sample preparation is predominantly performed by liquid–liquid extraction but solid-phase extraction (SPE) as a two-stage or online approach is increasingly applied and reported as sensitive, robust and fast [16,31,34].

This paper presents an isocratic HPLC method with UV detection preceded by SPE to cost- and time-effectively analyse 22 psychotropic drugs, seven of them also including their active metabolites. This method was developed for therapeutic drug monitoring and validated by internal (recovery, linearity, accuracy, precision, interferences) and external quality control.

2. Experimental

2.1. Reagents

Acetonitrile, methanol, 2-propanol and water (Baker, Deventer, The Netherlands) were HPLC grade, potassium dihydrogenphosphate, *n*-hexane, ethyl acetate, acetic acid dichloromethane and ammonia solution (25%) were analytical grade obtained from Merck (Darmstadt, Germany). The antidepressants and atypical antipsychotics were kindly provided by the following companies: doxepin (Boehringer, Mannheim, Germany); clomipramine, norclomipramine, maprotiline, normaprotiline, imipramine (Ciba Geigy, Wehr, Germany); fluoxetine, norfluoxetine (Eli Lilly, Indianapolis, IN, USA); amisulpride (Finorga, Mourenx, France); moclobemide (Hoffmann La Roche, Grenzach-Wyhlen, Germany); risperidone, 9-OH-risperidone (Janssen-Cilag, Beerse, Belgium); citalopram, nortriptyline (Lundbeck, Copenhagen, Denmark); melperone (Knoll, Ludwigshafen, Germany); mianserine, mirtazapine (Organon, Oss, The Netherlands); nordoxepin, sertraline (Pfizer, Groton, USA); reboxetine (Pharmacia and Upjohn, Kalamazoo, MI, USA); trimipramine (Rhône-Poulenc Pharma, Köln, Germany); clozapine, norclozapine (Sandoz, Nürnberg, Germany); amitriptyline (MSD Sharp and Dohme, Haar, Germany); desipramine (Sigma, Taufkirchen, Germany); paroxetine (SmithKline Beecham, München, Germany); fluvoxamine (Solvay Duphar, Hannover, Germany); venlafaxine, O-desmethylvenlafaxine (Wyeth-Pharma, Münster, Germany); quetiapine (Zeneca, Schwetzingen, Germany).

2.2. Standard preparation

The stock solutions for calibration standards and quality control were prepared by dissolving 10 mg of the respective drug in 10 ml methanol. Drug-free serum from healthy volunteers, provided by the Institute of Hematology of the University of Bonn,

was spiked with stock solution of the drug in water (HPLC grade, 1:10) to achieve the following calibration standard concentrations: amisulpride, clozapine, norclozapine: 10, 25, 125, 500, 800, 1000 ng/ml; quetiapine: 5, 10, 125, 500, 800, 1000 ng/ml; risperidone, 9-OH-risperidone: 5, 10, 25, 75, 150, 200 ng/ml; moclobemide: 50, 75, 200, 500, 1000, 1500 ng/ml; mirtazapine, citalopram, sertraline: 5, 10, 50, 100, 200, 300 ng/ml; fluvoxamine, paroxetine: 5, 10, 50, 150, 400, 500 ng/ml; venlafaxine, O-desmethylvenlafaxine: 10, 25, 50, 150, 400, 500 ng/ml; reboxetine: 5, 10, 100, 400, 800, 1000 ng/ml; fluoxetine, norfluoxetine, trimipramine: 10, 25, 125, 200, 400, 500 ng/ml; maprotiline, normaprotiline: 10, 25, 100, 200, 400, 500 ng/ml; mianserine: 5, 10, 50, 100, 250, 300 ng/ml; amitriptyline, nortriptyline, imipramine, desipramine: 10, 30, 75, 200, 400, 500 ng/ml; doxepin, nordoxepin: 5, 25, 75, 200, 400, 500 ng/ml; clomipramine, norclomipramine: 10, 25, 75, 300, 600, 750 ng/ml. Quality control samples that were run in each assay, were prepared in the same way. All serum standards, quality control samples and stock solutions were stored in aliquots at −20 °C and were stable for at least 3 months as inferred from the chromatograms. The aliquots were never refrozen or rethawed for a second time. The internal standard melperone was diluted with serum to a concentration of 3000 ng/ml. External quality control was carried out every month for amitriptyline, nortriptyline, imipramine, desipramine, clomipramine, norclomipramine, clozapine and norclozapine and every 3 months for maprotiline, normaprotiline, doxepin, nordoxepin, trimipramine, fluoxetine, norfluoxetine, fluvoxamine, paroxetine, sertraline and citalopram in cooperation with Heath Control (Cardiff, UK).

2.3. Solid-phase extraction

Blood samples were transfered to the laboratory (as a rule within 4 h) and centrifuged at 2000 *g* for 10 min at 4 °C. Serum samples were frozen in aliquots of 1.1 ml. For the SPE 1 ml of serum was centrifuged at 13 000 *g* for 10 min at 4 °C in an Eppendorf centrifuge. We used 3-ml 3M-Empore high-performance extraction disk cartridges (Varian, Darmstadt, Germany) and a Baker spe-12G vacuum instrument. The mixed-phase sorbent was conditioned with 1 ml methanol followed by 1 ml water. Then 0.9 ml supernatant, 0.1 ml melperone (3000 ng/ml) as internal standard and 2.0 ml 0.1 *M* potassium dihydrogenphosphate buffer (pH 6.0) were mixed in 100×16 mm polypropylene tubes (Sarstedt, Nymbrecht, Germany). The sample was transfered and passed through the extraction disk cartridge. To eliminate interferences, we washed the cartridge with 1 ml water, 1 ml 1 *M* acetic acid, 1 ml *n*-hexane, 2 ml *n*-hexane–ethyl acetate (1:1) and 1 ml methanol. The antidepressants and atypical antipsychotics were eluted with 1 ml 2-propanol–ammonia solution (25%)–dichloromethane (20:2:78). The sample was evaporated to dryness, the residue dissolved in 250 μl acetonitrile–water (3:7) and 100 μl was injected.

2.4. Instrumentation and chromatographic conditions

The HPLC system consisted of a Bischoff 2200 high-performance liquid chromatography pump (Bischoff, Leonberg, Germany), a solvent degasser unit SDU 2003 (Bischoff) and a Waters Intelligent Sample Processor (WISP 717) equipped with a cooling module at 4 °C (Millipore-Waters, Eschborn, Germany). The analytical column (250×4.6 mm I.D.) containing Nucleosil 100-5-Protect 1 (endcapped), particle size 5 μm (Macherey and Nagel, Düren, Germany) was kept in a column oven (EchoTherm CO30, Torrey Pines Scientific LLC, Solana Beach, USA) maintained at 25 °C. The mobile phase consisted of 25 m*M* potassium dihydrogenphosphate (pH 7.0)–acetonitrile (60:40) at a flow-rate of 1 ml/min. The eluted substances were detected by a Shimadzu SPD-10AVP UV detector (Shimadzu, Duisburg, Germany) at 230 nm. The acquisition and integration was performed by McDacq32 Software, version 1.51 (Bischoff).

2.5. Validation

Calibration was performed by linear regression of the peak-height ratios of the drugs to the internal standard (melperone) versus the respective standard concentration. To evaluate linearity, six calibration curves for every drug were separately prepared. After comparison of residuals and correlation co-

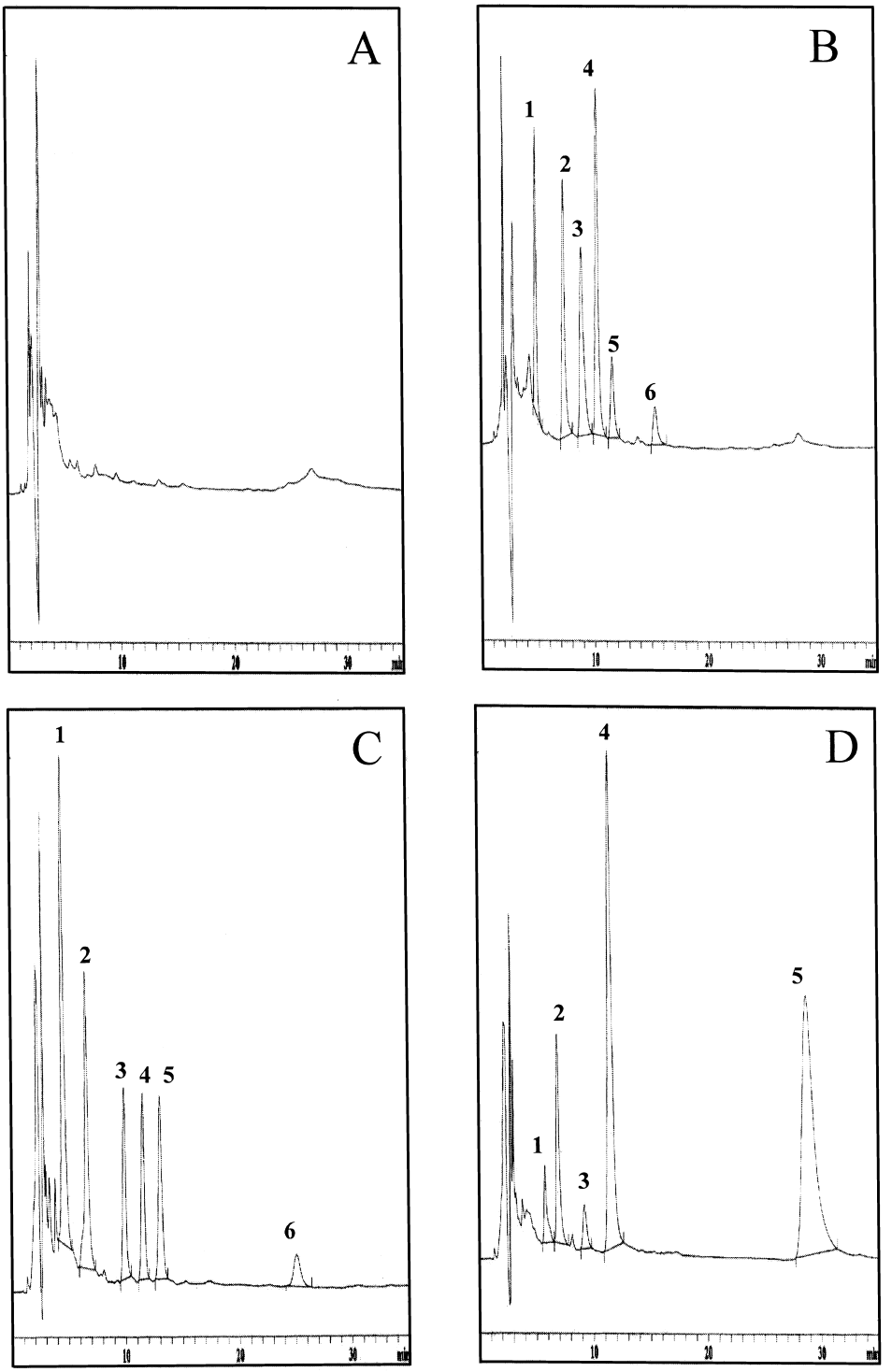
A
B
1
2
3
4
5
6
C
1
2
3 4 5
6
D
1
2
3
4
5
10
20
30

Table 1
Retention times of psychotropic drugs and metabolites analysed for interferences

Drug	Retention time (min)	Drug	Retention time (min)
Sulpiride	4.1	Mirtazapine	16.6
O-Desmethylvenlafaxine	4.8	Fluoxetine	17.8
Moclobemide	5.6	Doxepin	18.3
Amisulpride	6.1	Norclomipramine	19.2
9-OH-Risperidone	6.6	Imipramine	20.6
Venlafaxine	7.3	Trifluperidol	20.8**
m-Chlorophenylpiperazine	8.0	Olanzapine	21.0**
Normirtazapine	8.3**	Trimipramine	21.5
Melperone	8.8	Amitriptyline	23.4
Reboxetine	10.2	Ziprasidone	26.4
Zolpidem	10.2**	Promethazine	28.1**
Nordoxepin	10.9	Mianserine	29.0
Diazepam	11.0	Clomipramine	30.8
Risperidone	11.1	Clozapine	30.9
Benperidol	11.5**	Flupenazine	31.0**
Normaprotiline	11.5	Nefazodone	32.5
Dibenzepine	11.5	Sertraline	33.6
Opipramol	11.6	Chlorprothixene	36.4**
Fluvoxamine	11.6	Thioridazine	43.2**
Quetiapine	11.7	Pimozide	44.1**
Desipramine	12.8	Carbamazepine	n.d.*
Citalopram	13.3	Perazine	n.d.*
Norfluoxetine	13.4	Zotepine	n.d.*
Norclozapine	14.4	Valproate	n.d.*
Nortriptyline	14.5	Zopiclone	n.d.*
Haloperidol	15.3**	Buspirone	n.d.*
Paroxetine	15.3	Lorazepam	n.d.*
Maprotiline	15.3	Biperidene	n.d.*

The analyses comprises solid-phase extraction for all drugs prior to HPLC.
n.d.*=Not detectable using the above mentioned conditions.
** Not detectable under therapeutic concentrations.

efficients (r), the best fit was obtained with a weighting factor of 1/concentration for all drugs except for risperidone, 9-OH-risperidone, mianserine, moclobemide, clomipramine, norclomipramine and nortriptyline; the latter were best fitted with a weighting factor of 1/concentration^2. The putative interferences during the analysis were evaluated by processing spiked serum samples that contained the relevant drugs in therapeutic concentrations. The extraction recoveries were determined by comparing peak-height ratios of the extracts of spiked serum with those obtained by direct injection

Fig. 1. Chromatograms of (A) blank serum; (B) spiked serum with (1) 150 ng/ml O-desmethylvenlafaxine, (2) 150 ng/ml venlafaxine, (3) 300 ng/ml melperone, (4) 400 ng/ml reboxetine, (5) 150 ng/ml fluvoxamine and (6) 150 ng/ml paroxetine; (C) spiked serum with (1) 125 ng/ml amisulpride, (2) 300 ng/ml melperone, (3) 125 ng/ml norfluoxetine, (4) 125 ng/ml quetiapine, (5) 125 ng/ml fluoxetine and (6) 50 ng/ml sertraline; (D) spiked serum with (1) 25 ng/ml 9-hydroxyrisperidone, (2) 300 ng/ml melperone, (3) 25 ng/ml risperidone, (4) 500 ng/ml norclozapine, (5) 500 ng/ml clozapine. (E) Chromatograms of spiked serum with (1) 300 ng/ml melperone, (2) 50 ng/ml citalopram and (3) 50 ng/ml mirtazapine; (F) spiked serum with (1) 200 ng/ml moclobemide, (2) 300 ng/ml melperone, (3) 100 ng/ml normaprotiline, (4) 100 ng/ml maprotiline, (5) 125 ng/ml trimipramine and (6) 50 ng/ml mianserine; (G) chromatogram of spiked serum with (1) 300 ng/ml melperone, (2) 75 ng/ml nordoxepin, (3) 75 ng/ml desipramine, (4) 75 ng/ml nortriptyline, (5) 75 ng/ml doxepin, (6) 75 ng/ml imipramine and (7) 75 ng/ml amitriptyline; (H) spiked serum with (1) 300 ng/ml melperone, (2) 300 ng/ml norclomipramine and (3) 300 ng/ml clomipramine.

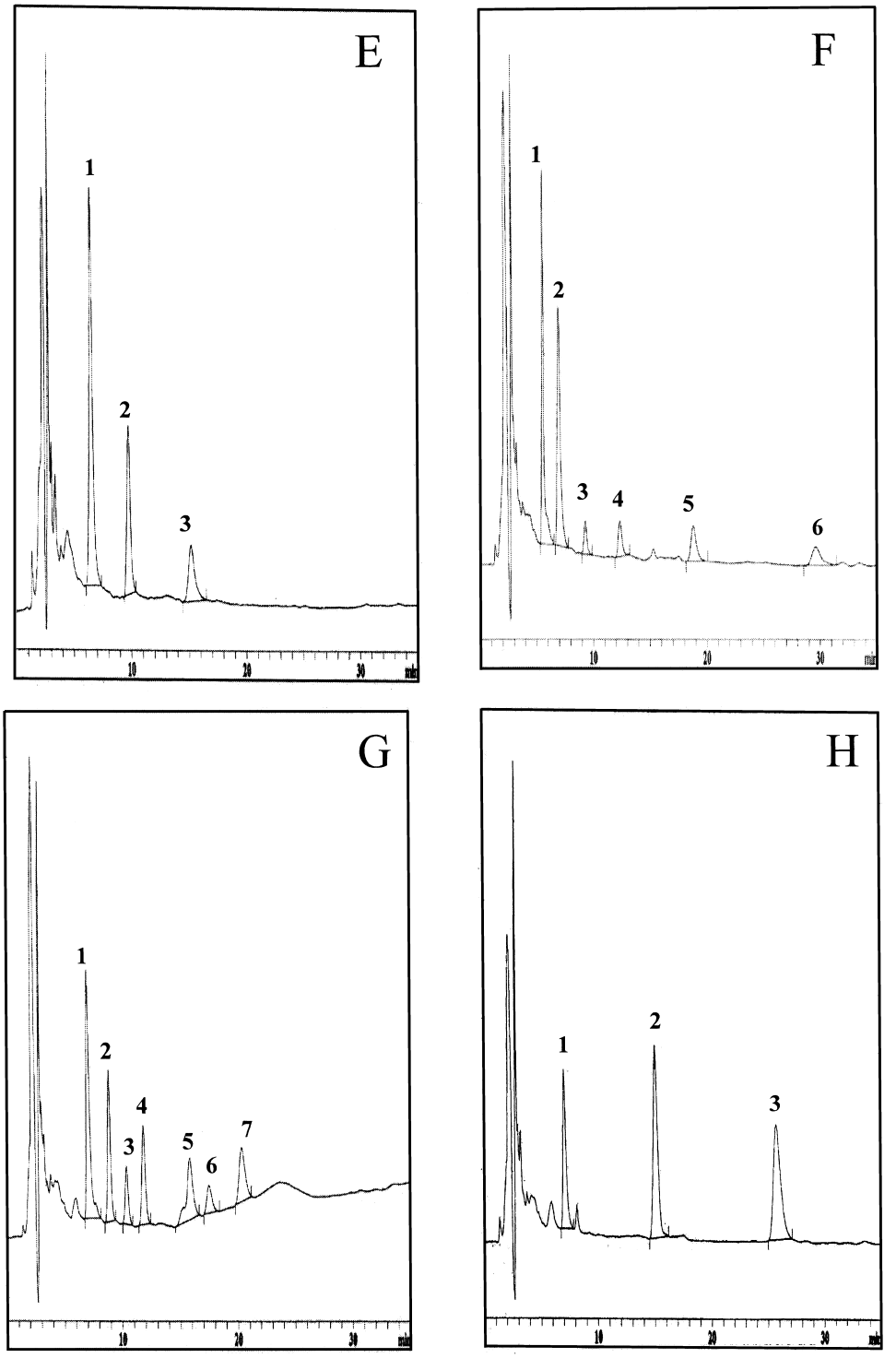

Fig. 1. (*continued*)

of an aqueous solution of the drugs. We repeatedly tested the accuracy, intra- and inter-day precision of every drug at low, middle and high concentrations (n=10–61). Accuracy was expressed as percent recovery after analysing drug-spiked serum and comparing this to the added amounts; precision was expressed as intra- and inter-assay relative standard deviations (RSDs) of the determined concentrations. The acceptance criteria to fulfill the requirements for therapeutic drug monitoring were: ±10% for accuracy and an intra- and inter-assay RSD≤10%; for the lowest concentration on the calibration curve the RSD was ≤20%, which is in accordance with US Food and Drug Administration (FDA) and ICH requirements [37,38].

3. Results and discussion

Chromatographic separation was satisfactory as can be seen by chromatograms of spiked and drug-free serum samples (Fig. 1). No interfering peaks from endogenous components and psychotropic drugs were detected in pooled drug-free human serum. The retention times of drugs that potentially interfered are specified in Table 1. These interferences are controlled for when comedication was specified in our request form for TDM.

SPE yielded reproducible recoveries from 75 to 99% over the entire concentration range and thus resulted in improved recovery compared to liquid–liquid extraction ([16,18], own data, not shown).

Table 2
Regression parameters of calibration curves

Compound	Concentration range (ng/ml)	Correlation coefficient (r)	Intercept (peak-height ratio)* (a)	Slope (b)
Amitriptyline	10–500	0.9993	−0.0120	0.0032
Nortriptyline	10–500	0.9991	−0.0061	0.0053
Imipramine	10–500	0.9993	−0.0041	0.0015
Desipramine	10–500	0.9987	0.0026	0.0028
Doxepin	5–500	0.9995	−0.0045	0.0036
Nordoxepin	5–500	0.9994	−0.0087	0.0083
Clomipramine	10–750	0.9987	−0.0072	0.0023
Norclomipramine	10–750	0.9990	−0.0043	0.0036
Trimipramine	10–500	0.9990	−0.0016	0.0013
Maprotiline	10–500	0.9997	0.0023	0.0014
Normaprotiline	10–500	0.9994	0.0025	0.0013
Mianserine	5–300	0.9979	−0.0020	0.0018
Paroxetine	5–500	0.9996	0.0038	0.0012
Fluvoxamine	5–500	0.9994	−0.0006	0.0026
Fluoxetine	10–500	0.9991	−0.0005	0.0054
Norfluoxetine	10–500	0.9988	−0.0084	0.0060
Sertraline	5–300	0.9995	−0.0016	0.0023
Citalopram	5–300	0.9992	0.0042	0.0045
Venlafaxine	10–500	0.9995	−0.0216	0.0089
O-Desmethylvenlafaxine	10–500	0.9991	−0.0143	0.0103
Mirtazapine	5–300	0.9994	−0.0015	0.0015
Moclobemide	50–1500	0.9998	−0.0175	0.0098
Reboxetine	5–1000	0.9992	−0.0018	0.0043
Quetiapine	5–1000	0.9994	0.0086	0.0079
Amisulpride	10–1000	0.9995	0.0008	0.0159
Clozapine	10–1000	0.9995	−0.0150	0.0032
Norclozapine	10–1000	0.9997	−0.0348	0.0053
Risperidone	5–200	0.9974	−0.0052	0.0032
9-OH-Risperidone	5–200	0.9962	0.0033	0.0059

* Peak-height ratio of the analyte to the internal standard; for details refer to Section 2.5.

Table 3
Accuracy, inter- and intra-assay precision for quality control samples at different concentrations*

Compound	Concentration (ng/ml)	Recovery (n=10) (%)	Intra-assay RSD (n=10) (%)	Inter-assay RSD (n>10) (%)
Amitriptyline	75	101.9	1.7	1.9
	100	102.6	2.6	7.2
	200	103.4	2.6	6.3
Nortriptyline	75	97.9	2.0	1.7
	100	103.5	3.9	8.1
	200	103.9	3.2	7.2
Imipramine	75	100.5	1.6	3.1
	100	103.8	2.6	8.7
	200	104.2	5.4	7.8
Desipramine	75	100.1	4.2	2.5
	100	97.1	4.6	8.7
	200	99.6	2.1	6.5
Doxepin	75	100.8	2.2	1.9
	100	102.0	2.7	8.3
	200	98.7	3.0	7.3
Nordoxepin	75	100.5	3.0	2.5
	100	107.0	2.8	7.2
	200	100.9	2.3	7.2
Clomipramine	75	97.2	3.1	2.9
	100	99.6	5.2	7.6
	300	99.2	4.6	5.9
Norclomipramine	75	98.8	3.2	3.7
	100	102.1	5.7	5.8
	300	99.7	1.5	3.7
Trimipramine	25	97.6	5.9	7.6
	150	100.6	2.9	5.2
	200	101.3	3.6	2.9
Maprotiline	50	95.4	7.2	7.6
	100	97.5	2.4	2.3
	200	97.8	2.2	2.3
Normaprotiline	50	94.4	4.4	4.3
	100	98.3	4.3	4.4
	200	98.6	4.0	2.4
Mianserine	10	101	9.4	9.7
	50	101.3	2.1	2.3
	100	99.4	1.9	2.1
Paroxetine	10	98.6	4.7	6.3
	50	107.0	6.2	4.5
	150	102.5	4.3	3.2
Fluvoxamine	10	111.7	8.7	5.8
	50	107.2	4.9	4.8
	150	106.8	3.6	3.3
Fluoxetine	75	105.6	1.4	1.9
	125	99.4	2.7	1.8
	250	95.2	3.2	3.4
Norfluoxetine	75	100.1	3.6	3.8
	125	100.1	3.9	2.8
	250	100.2	3.6	3.6

Table 3. Continued

Compound	Concentration (ng/ml)	Recovery (n=10) (%)	Intra-assay RSD (n=10) (%)	Inter-assay RSD (n>10) (%)
Sertraline	10	111.1	1.4	7.4
	50	100.6	3.6	1.6
	100	98.6	1.9	4.2
Citalopram	10	118.7	5.9	8.7
	50	97.8	1.5	1.3
	100	100.4	1.3	2.6
Venlafaxine	50	94.2	4.1	3.9
	150	99.5	4.8	3.1
	250	98.9	2.7	2.8
O-Desmethylvenlafaxine	50	100.0	3.4	3.0
	150	103.2	4.1	2.2
	250	100.3	6.2	3.9
Mirtazapine	10	101.0	5.6	7.7
	50	98.0	4.6	2.5
	100	94.7	5.2	3.9
Moclobemide	250	98.8	2.8	4.1
	500	99.0	1.1	0.9
	1000	98.6	2.2	2.2
Reboxetine	50	100.6	7.0	7.0
	100	100.3	6.4	2.9
	250	93.4	3.8	3.9
Quetiapine	25	104.8	3.0	2.6
	100	96.3	2.0	2.1
	125	98.9	3.8	4.2
Amisulpride	50	100.6	3.9	4.4
	250	98.9	3.4	3.7
	500	99.3	3.0	5.6
Clozapine	125	101.4	5.5	2.0
	150	101.1	3.6	6.0
	500	96.4	3.1	1.5
Norclozapine	125	104.0	4.5	6.0
	150	102.4	2.6	2.7
	500	96.6	1.8	3.7
Risperidone	10	109.3	4.9	3.5
	25	94.0	4.5	6.5
	100	91.2	5.5	4.3
9-OH-Risperidone	10	105.9	5.4	4.6
	25	95.6	5.3	5.0
	100	99.4	4.8	1.7

* For details refer to Section 2.5.

Linearity of the calibration curves was tested according to Mandel and by examining the residuals for all drugs [39]. Correlation coefficients were >0.997 and intercepts did not differ significantly from zero (Table 2).

Precision and accuracy were evaluated for three concentrations (n=10 for each concentration), resulting in percent recovery between 91.2 and 107.2% and intra-assay RSDs ranging from 1.1 to 9.4% (Table 3). The inter-assay RSDs were 0.9 to 9.7%. At the lower end of the calibration curves recoveries were 95.7 to 118.7% and RSDs 1.5 to 10.2%. The low-concentration quality controls of citalopram, fluvoxamine and sertraline were close to the lower end of the calibration curve and are therefore included here.

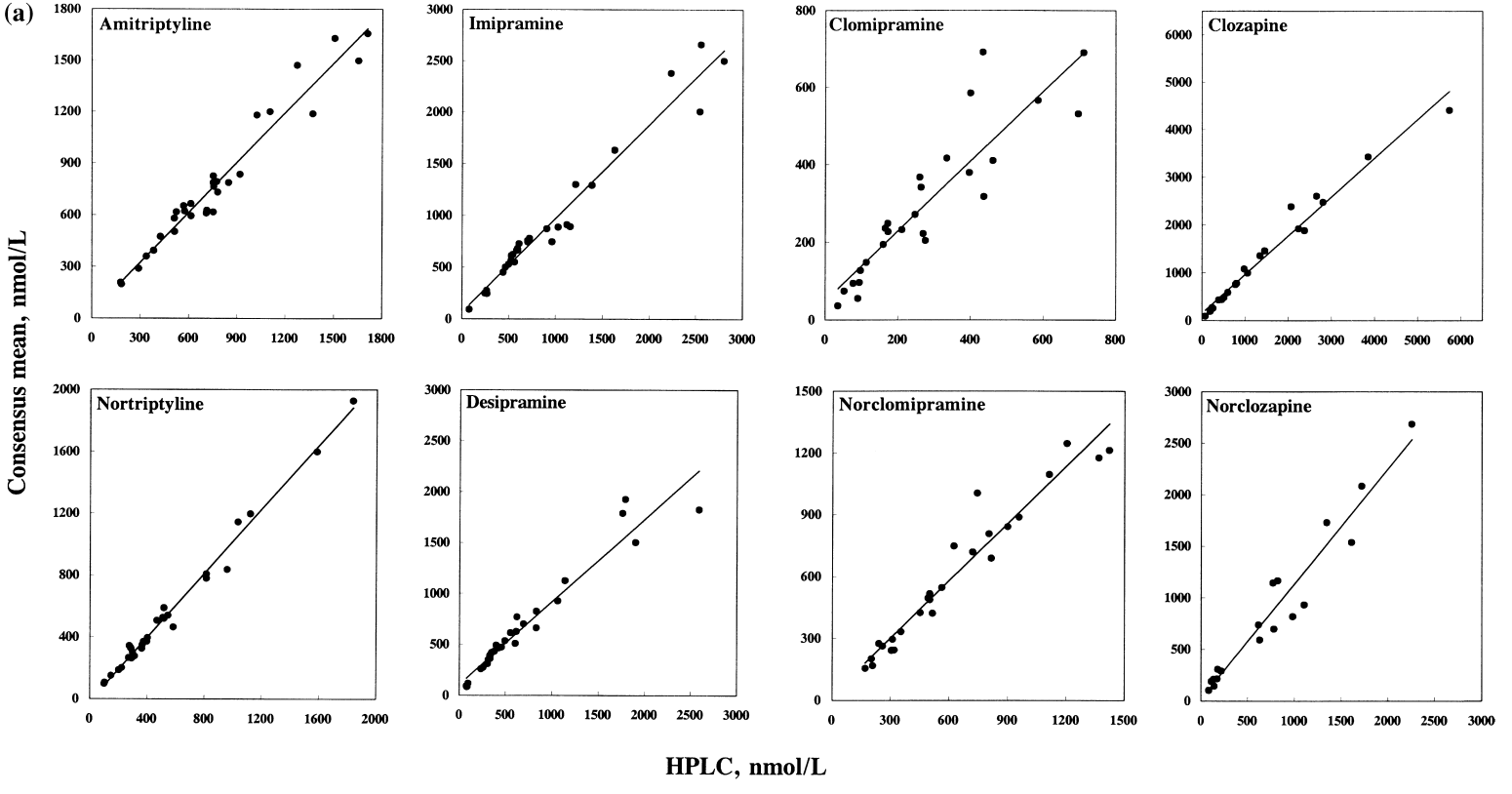

Fig. 2. External quality control consensus means of all participating laboratories using HPLC methods plotted against results of our laboratory: (a) drugs evaluated monthly; (b) drugs evaluated 3-monthly.

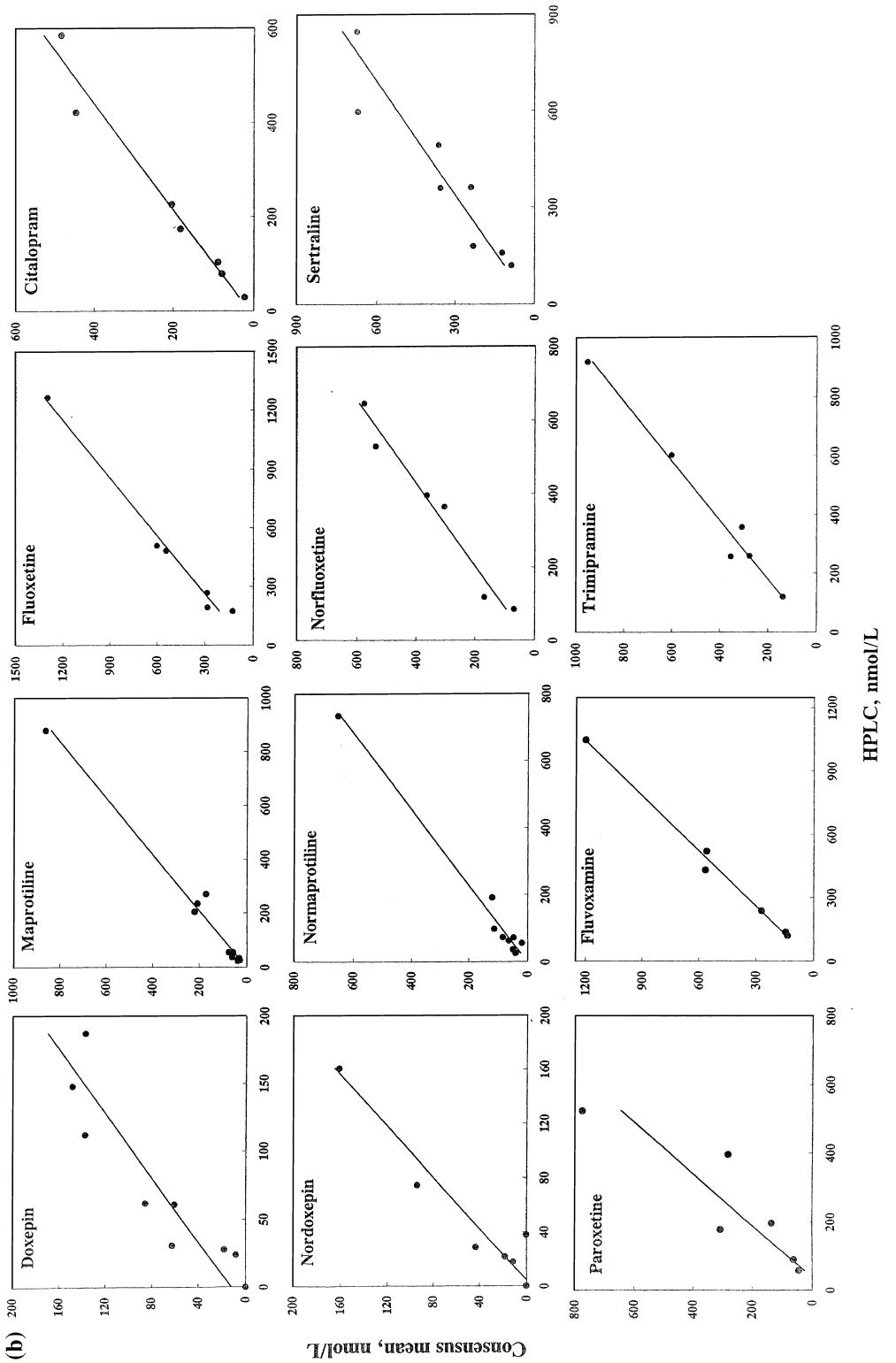

Fig. 2. (*continued*)

Table 4
Linear regression analysis of the external quality control*

Drug	*n*	Slope	Intercept (nmol/l)	Correlation coefficient (*r*)
Amitriptyline	30	0.9672	32.659	0.975
Nortriptyline	30	1.0367	−20.911	0.994
Imipramine	30	0.9067	64.403	0.980
Desipramine	30	0.8111	102.99	0.968
Clomipramine	26	0.8985	49.021	0.898
Norclomipramine	26	0.9256	23.547	0.970
Clozapine	21	0.986	153.73	0.986
Norclozapine	18	1.1193	16.524	0.971
Doxepin	9	0.8423	11.661	0.919
Nordoxepin	7	1.0413	−1.4538	0.977
Maprotiline	10	0.9497	2.0484	0.991
Normaprotiline	9	0.8781	−0.491	0.992
Trimipramine	6	0.9926	20.092	0.986
Fluoxetine	6	1.0132	33.641	0.992
Norfluoxetine	6	0.8873	19.384	0.983
Fluvoxamine	6	1.1474	−0.2626	0.996
Paroxetine	6	1.3318	−53.244	0.892
Sertraline	8	0.8495	11.16	0.936
Citalopram	7	0.8855	10.012	0.984

* For details refer to Section 3.

Results of external quality controls obtained within three years were retrospectively evaluated. Consensus means of all participating laboratories using HPLC methods are plotted against our results (Fig. 2). Regression parameters of all drugs that were included in external quality controls are displayed in Table 4. Intercepts are small and slopes are close to unity except for high levels of clomipramine and amitriptyline; the latter finding was also described by other laboratories [17].

4. Conclusion

This paper presents a simple and accurate HPLC method to analyse 22 commonly prescribed psychotropic drugs including seven metabolites. Among these 29 compounds are new psychotropic drugs for which so far only single-component assays are described in the literature. This method allows an efficient and rapid analysis of serum concentrations within 24 h with a single system, thus reducing the time for apparatus preparation and system instabilities linked to this process.

Acknowledgements

The study was kindly supported by the Competence Network Depression obtained from the BMBF.

References

[1] E. Spina, A. Avenoso, G. Facciola, M.G. Scordo, M. Ancione, A.G. Madia, A. Ventimiglia, E. Perucca, Psychopharmacology 148 (2000) 83.
[2] A.S. Freeman, F.K. Weddige, J.L. Lipinski Jr., Eur. J. Pharmacol. 9 (2001) 43.
[3] S.H. Preskorn, G.A. Fast, J. Clin. Psychiatry 52 (Suppl.) (1991) 23.
[4] P.J. Perry, B.M. Pfohl, S.G. Holstad, Clin. Pharmacokinet. 13 (1987) 381.
[5] T. Van Putten, S.R. Marder, J. Mintz, R.E. Poland, Am. J. Psychiatry 149 (1992) 500.
[6] A.H. Veefkind, P.M. Haffmans, E. Hoencamp, Ther. Drug Monit. 22 (2000) 202.
[7] J. Lundmark, F. Bengtsson, C. Nordin, M. Reis, J. Walinder, Acta Psychiatr. Scand. 101 (2000) 354.
[8] R. Yoshimura, N. Ueda, J. Nakamura, Neuropsychobiology 44 (2001) 129.
[9] J. Gerlach, Ann. Clin. Psychiatry 14 (2002) 47.
[10] B.B. Rasmussen, K. Brøsen, Ther. Drug Monit. 22 (2000) 143.
[11] M.D. Kohnke, E.U. Griese, D. Stosser, I. Gaertner, G. Barth, Pharmacopsychiatry 35 (2002) 116.
[12] E. Spina, A. Avenoso, G. Facciola, M.G. Scordo, M. Ancione, A. Madia, Ther. Drug Monit. 23 (2001) 223.
[13] M.L. Rao, C. Hiemke, K. Grasmäder, P. Baumann, Fortschr. Neurol. Psychiatr. 69 (2001) 510.
[14] E. Tanaka, M. Terada, T. Nakamura, S. Misawa, C. Wakasugi, J. Chromatogr. B 692 (1997) 405.
[15] G. Aymard, P. Livi, Y.T. Pham, B. Diquet, J. Chromatogr. B 700 (1997) 183.
[16] L. Kristoffersen, A. Bugge, E. Lundanes, L. Slordal, J. Chromatogr. B 734 (1999) 229.
[17] R. Theurillat, W. Thormann, J. Pharm. Biomed. Anal. 18 (1998) 751.
[18] E. Lacassie, J.M. Gaulier, P. Marquet, J.F. Rabatel, G. Lachatre, J. Chromatogr. B 742 (2000) 229.
[19] W.N. Lin, P.D. Frade, Ther. Drug Monit. 9 (1987) 448.
[20] G. Tournel, N. Houdret, V. Hedouin, M. Deveau, D. Gosset, M. Lhermitte, J. Chromatogr. B 761 (2001) 147.
[21] S. Joron, H. Robert, Biomed. Chromatogr. 8 (1994) 158.
[22] R.H. Queiroz, V.L. Lanchote, P.S. Bonato, D. de Carvalho, Pharm. Acta Helv. 70 (1995) 181.
[23] M.P. Segatti, G. Nisi, F. Grossi, M. Mangiarotti, C. Lucarelli, J. Chromatogr. 536 (1991) 319.
[24] F.A. Maris, E. Dingler, S. Niehues, J. Chromatogr. B 721 (1999) 309.
[25] S. Härtter, H. Weigmann, C. Hiemke, J. Chromatogr. B 740 (2000) 135.

[26] S. Dodd, A. Buist, G.D. Burrows, K.P. Maguire, T.R. Norman, J. Chromatogr. B 730 (1999) 249.
[27] J.M. Hoskins, A.S. Gross, G.M. Shenfield, L.P. Rivory, J. Chromatogr. B 754 (2001) 319.
[28] M. Matoga, F. Pehourcq, K. Titier, F. Dumora, C. Jarry, J. Chromatogr. B 760 (2001) 213.
[29] S. Walter, S. Bauer, I. Roots, J. Brockmöller, J. Chromatogr. B 720 (1998) 231.
[30] M. Bagli, M.L. Rao, G. Höflich, J. Chromatogr. B 657 (1994) 141.
[31] H. Weigmann, S. Härtter, S. Maehrlein, W. Kiefer, G. Kramer, G. Dannhardt, C. Hiemke, J. Chromatogr. B 759 (2001) 63.
[32] S. Ulrich, J. Chromatogr. B 668 (1995) 31.
[33] J. Sachse, S. Härtter, H. Weigmann, C. Hiemke, J. Chromatogr. B 784 (2003) 405.
[34] P.C. Davis, J. Wong, O. Gefvert, J. Pharm. Biomed. Anal. 20 (1999) 271.
[35] A. Avenoso, G. Facciola, M. Salemi, E. Spina, J. Chromatogr. B 746 (2000) 173.
[36] M.L. Rao, Psychopharmacology 90 (1986) 548.
[37] FDA Guidance for Industry on Biomedical Method Validation, 2001, http://www.fda.gov/cder/guidance/index.htm.
[38] ICH Harmonised Tripartite Guideline. Validation of Analytical Procedures: Methodology, 1996, ICHQ2B, CPMP/ICH281/95.
[39] S. Kromidas (Ed.), Handbuch Validierung in der Analytik, Wiley–VCH, Weinheim, 2000.