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Liquid chromatography–tandem mass spectrometry method for determination of five antidepressants and four atypical antipsychotics and their main metabolites in human serum

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ABSTRACT

The rapid and simple ultra performance liquid chromatography–tandem mass spectrometry method was developed and validated for simultaneous determination parent drugs: sertraline, fluoxetine, citalopram, paroxetine, venlafaxine, clozapine, olanzapine, quetiapine, risperidone, and their active and nonactive metabolites N-desmethylsertraline, norfluoxetine, desmethylcitalopram, didemethylcitalopram, N-desmethylvenlafaxine, O-desmethylvenlafaxine, N-desmethylclozapine, N-desmethylolanzapine, 2-hydroxyolanzapine and 9-hydroxyrisperidone in human serum. Precipitation of serum proteins was performed with a precipitation reagent consisting of 0.05% solution of $ZnSO_4 \cdot 7H_2O$ in acetonitrile/methanol (40:60, v/v). Alprenolol was used as an internal standard. Chromatographic separation was carried out on a BEH C18 column using gradient elution mobile phase A (2 mmol/L ammonium acetate, 0.1% formic acid in 5% acetonitrile, v/v/v) and B (2 mmol/L ammonium acetate, 0.1% formic acid in 95% acetonitrile, v/v/v). Electrospray in positive mode was used for ionization. Detection was performed on a triple–quadrupole tandem mass spectrometer by multiple reaction monitoring. Analysis time was 5 min. Drugs were separated into three groups with low, medium and high levels. Correlation coefficients of calibration curves were in the range 0.995–1.000. Coefficients of variation were 4.2–9.5% for intra-assay and 3.0–11.9% for inter-assay. Recoveries were 87.1–110% for intra-assay and 88.1–108.2% for inter-assay. The method was fully validated and can be successfully applied for routine analyses.

1. Introduction

Therapeutic drug monitoring is an important tool for the clinical management of patients receiving pharmacotherapy, particularly in psychiatry. There is evidence of therapeutic and economic benefits of monitoring these drugs to avoid adverse effects, intoxication, no response or non-compliance. Baumann et al. [1] worked out guidelines for the routine use of TDM of psychoactive drugs as follows: (a) strongly recommended for clozapine and olanzapine; (b) recommended for venlafaxine plus O-desmethylvenlafaxine and risperidone plus 9-hydroxyrisperidone; (c) useful for citalopram, fluoxetine plus norfluoxetine, paroxetine, sertraline and quetiapine; (d) probably useful for fluvoxamin and escitalopram; (e) not recommended for clomethiazol and zolpidem.

Psychoactive drugs can be classified according to their chemical structure or mechanism of action. Clozapine, olanzapine and risperidone represent atypical antipsychotics. Olanzapine and clozapine are mainly metabolized into N-desmethylolanzapine and N-desmethylclozapine, respectively. Since the ongoing usage of clozapine can cause agranulocytosis, it should primarily be used in schizophrenic patients who are resistant to, or intolerant of, conventional antipsychotic medication. 9-Hydroxyrisperidone is a major active metabolite of risperidone and its pharmacological activity is almost similar to the parent drug [2,3]. Venlafaxine (VEN) is a non-tricyclic antidepressant, which inhibits the reuptake of serotonin, noradrenaline and, to a lesser extent, dopamine. In humans, VEN is metabolized into two minor metabolites (N-desmethylvenlafaxine, N,O-didesmethylvenlafaxine) and one major active metabolite (O-desmethylvenlafaxine) [4]. Fluoxetine, paroxetine, citalopram and sertraline belong to selective

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serotonin reuptake inhibitors. Sertaline and fluoxetine are metabolized into active metabolite N-desmethylsertraline and N-desmethylfluoxetine, respectively. Paroxetine and citalopram have no active metabolites [5].

To monitor psychoactive drugs, the analytical methods have to be highly sensitive and selective for accurate and precise quantification. Generally, the plasma concentrations of the drugs are low and patients are frequently co-medicated with other drugs, which may interfere with the assay. HPLC with fluorimetric detection [6–8], with coulometric detection [3], or UV detection [9–13] and HPLC/MS [14–21] were applied in analysis of one or a group of selected antidepressants or antipsychotics.

The first implementation of HPLC/MS in clinical routine laboratories started about fifteen years ago with the first therapeutic drug monitoring [22]. The technique has been used in particular for a new generation of antipsychotic drugs. In the beginning, it was applied for the determination of only one drug [23], subsequently, a few additional drugs and their metabolites have been quantified in one chromatographic run [14,15,17,24].

The presented work is aimed at the development and validation of a new analytical method for simultaneous separation and determination of the most important psychoactive drugs belonging to groups' a–d of Baumann's guidelines [1]. In routine laboratories, such a method allows the analysis of all clinical samples containing some of the studied drugs with the same instrumental and experimental set-up.

2. Materials and methods

2.1. Chemicals and solution

Acetonitrile and methanol of HPLC gradient grade, amonium acetate fractopur, and formic acid extra pure were obtained from Merck (Darmstand, Germany). Water of HPLC grade and zinc sulphate (≥99%) was obtained from Sigma–Aldrich (Prague, Czech Republic). The following reference standards of antidepressants and antipsychotics were purchased from TRC (Toronto Research Chemicals Inc., Canada): fluoxetine, norfluoxetine, citalopram, desmethylcitalopram, didesmethylcitalopram, paroxetine, sertraline, desmethylsertraline, venlafaxin, O-desmethylvenlafaxine, N-desmethylvenlafaxine, olanzapine, 2-hydroxy olanzapine, desmethylolanzapine, risperidone, 9-hydroxyrisperidone. Quetiapine was obtained from JS Research Chemicals Trading (Wedel, Germany). Clozapine, N-desmethylclozapine and alprenolol hydrochloride were purchased from Sigma–Aldrich (Prague, Czech Republic). The drug-free serum of healthy volunteers was provided by The Blood Centre University Hospital, Ostrava. Quality control samples were obtained from Chromsystems (Munchen, Germany).

2.2. Preparation of calibration standards and patient samples

Standard stock solutions of 100 mg/L were prepared by dissolving single drugs in methanol. Three standard mixtures were prepared. Their concentration was related to the therapeutic range of drugs. A low level standard mixture (400 ng/mL) was prepared for risperidon and hydroxyrisperidon, a medium level mixture (4000 ng/mL) for fluoxetine, norfluoxtine, citalopram, desmethylcitalopram, didesmethylcitalopram, paroxetine, sertraline, demethylsertralin, olanzapine, 2-hydroxyolanzapine, demethylolanzapine and quetiapine, and a high level mixture (8000 ng/mL) for clozapine, N-desmethylclozapine, venlafaxin, O-desmethylvenlafaxine and N-desmethylvenlafaxine. Calibration standards were prepared in concentrations as follows: 0.5, 1, 2.5, 5, 10, 25, 50 and 100 ng/mL for low level drugs, 2.5, 10, 25, 50, 100, 250, 500 and 1000 ng/mL for medium level drugs, and 5, 50, 100, 250, 500, 1000 and 2000 ng/mL for high level drugs. Alprenolol was used as an internal standard at concentration 20,000 ng/mL and was stored at 4 °C. Standard stock solutions and calibration standards were stored at −20 °C. Solution 0.05% $ZnSO_4 \cdot 7H_2O$ in acetonitrile/methanol (40:60, v/v) was utilized for protein precipitation.

The drug-free serum (0.2 mL) was spiked with 0.05 mL internal standard and 0.05 mL calibration standard. 0.5 mL precipitation solution and 0.2 mL water was added. The mixture was vortex-mixed for 30 s and was left for 5 min at 4 °C. After centrifugation for 10 min at 1370 × *g* and 4 °C, the upper layer was transferred into vials and 10 μl was injected into a chromatographic column. Patient samples and quality control samples were prepared in the same manner.

2.3. Chromatographic and mass spectrometric conditions

HPLC/MS/MS analysis was carried out using a Waters Acquity UPLC system (Waters, Milford, MA, USA) connected to a Quattro Micro API triple quadrupole (Micromass, Manchester, UK) with a Acquity UPLC RP BEH C18, 1.7 μm; 2.1 mm × 50 mm column. The gradient elution was performed using mobile phase A (2 mmol/L ammonium acetate, 0.1% formic acid in 5% acetonitrile, v/v/v) and mobile phase B (2 mmol/L ammonium acetate, 0.1% formic acid in 95% acetonitrile, v/v/v) with the time program: 0 min A:B = 80:20 (v/v), 3 min 5:95, 3.1 min 80:20, 5 min 80:20. The flow rate was 0.4 mL/min. The temperature of the column was maintained at 30 °C. The injection interval of samples was 5 min. Both positive ion electrospray ionization and positive atmospheric pressure chemical ionization were tested in the method development. The final optimized conditions for ESI^+ were: capillary voltage 1.5 kV, source temperature 100 °C, desolvation temperature 420 °C; for $APCI^+$: corona current 3 μA, source temperature 100 °C, desolvation temperature 350 °C. Cone voltage and collision energy were optimized for both ionization techniques and for each drug individually. Finally, the positive ion electrospray ionization mode was chosen for routine analysis. High purity argon was used as collision gas and multiple reaction monitoring (MRM) was applied to follow the analytes. All data were evaluated using MassLynx 4.1software (Waters, Milford, MA, USA).

2.4. Matrix effect

Matrix effects were evaluated using post-column infusion experiments [25]. A precipitation solution with 0.2 ml water (A) and a precipitation solution with 0.2 ml drug-free serum (B), respectively, were injected into a chromatographic column and then, separately, all drugs and metabolites were infused post-column in concentration 100 ng/mL, Samples A and B were enriched with olanzapine and desmethylolanzapine in concentration 100 ng/ml and were repeatedly injected on column. Peak areas of serum samples were correlated with corresponding peaks in a reference samples prepared from water and precipitating reagent.

2.5. Validation of method

The method was validated for linearity, accuracy and precision using FDA criteria [26]. Calibration curves for serum standard samples were constructed by plotting ratios of the peak area of each drug to peak area of internal standard versus standard concentrations. Linearity of calibration curves were chosen to cover the therapeutic range of individual drugs.

Table 1
Correlation of ionization sources ESI and APCI.

	ESI+				APCI+				R_t (min)
Drug	Q1 (m/z)	Q3 (m/z)	CE	Area ± SD	Q1 (m/z)	Q3 (m/z)	CE	Area ± SD	
Risperidone	411.3	191.1	30	55748.2 ± 1914.9	411.2	191.1	34	3011.5 ± 54.7	0.84
9-Hydroxyrisperidone	427.2	206.9	30	44151.8 ± 1383.1	427.1	207.1	29	1608.1 ± 60.6	0.78
Sertraline	306	158.6	24	48701.7 ± 1959.2	306	158.9	27	1367.2 ± 102.8	1.36
N-desmethylsertraline	292.2	158.9	24	21989.9 ± 528.9	291.9	158.9	23	3656.9 ± 272.7	1.33
Fluoxetine	310.2	43.6	11	38780.8 ± 1009.7	310.1	43.5	29	1084.7 ± 199.6	1.36
Norfluoxetine	296.2	133.7	7	20863.9 ± 487.1	295.9	134	6	1511.7 ± 131.6	1.28
Citalopram	325.2	109.2	27	149130.2 ± 8757.7	325.2	108.9	25	12290.2 ± 828.1	1.08
Desmethylcitalopram	311.2	108.8	25	136648 ± 4202.2	312.9	192	41	6270.1 ± 507.7	1.05
Didesmethylcitalopram	297.2	108.9	25	53994.6 ± 1051.8	296.9	108.9	19	7987.9 ± 244.1	1.03
Paroxetine	330	191.7	30	11313.9 ± 242.9	330.1	69.8	26	3489.3 ± 226.2	1.15
Quetiapine	384.2	252.8	20	663495.3 ± 20454.3	384.4	253.2	21	5703.3 ± 171.8	1.00
Clozapine	327	269.6	25	1167626.1 ± 31951.2	327.1	269.8	25	11320.8 ± 439.3	0.94
N-desmethylclozapine	313.2	270.2	24	98364.2 ± 4579.3	312.9	269.9	25	2635.6 ± 131.8	0.85
Venlafaxine	278.6	57.6	17	44168.6 ± 2731.2	278.1	57.6	18	9495.7 ± 257.7	0.87
O-desmethylvenlafaxine	264.3	57.7	18	171314.1 ± 6779.3	264	57.6	17	27608.1 ± 546.8	0.58
N-desmethylvenlafaxine	264.2	120.8	29	83740.0 ± 690.3	264.2	121	27	1755.8 ± 123.3	0.83
Olanzapine	313.3	212.8	30	60422.9 ± 1630.7	313.1	255.9	23	77121.3 ± 3688.4	0.52
N-desmethylolanzapine	299.2	197.8	40	185190.6 ± 3063.9	299	198	38	8787.0 ± 680.8	0.47
2-Hydroxyolanzapine	329.2	271.9	23	1447401.2 ± 33374.7	329	271.9	25	73516.1 ± 5637.1	0.42
Alprenolol (IS)	250.2	91	34	66567.4 ± 2377.8	250.1	90.9	40	2402.7 ± 256.8	0.99

ESI+, electrospray ionization; APCI+, atmospheric-pressure chemical ionization; Q1, parent ion mass; Q3, daugther ion mass; CE, collision energy.

2.5.1. Limit of quantification

The LOQs in the serum, defined as the lowest concentration with acceptable precision and accuracy (coefficient of variations less than 20%) were defined as the first point of reference curves.

2.5.2. Accuracy, precision and recovery

The assays were repeated ten times within the same day to obtain repeatability (intraday precision) and ten times over different days to obtained inter-day precision. Intra-assay and inter-assay precision, accuracy and recovery for each drug were evaluated by analyses of the three various concentrations: (5, 25, 100 ng/mL for the low level mixture, 10, 100, 500 ng/mL for the medium level mixture, and 50, 250, 1000 ng/mL for the high level mixture). The precision of the method was determination by coefficient of variation (%CV) which was expected to be within ±15.0%. Similarly, the accuracy should not deviate by ±15.0% of nominal concentration. For recovery, the analytes' responses from extracted samples at known concentration were compared with responses un-extracted standards that represent 100% recovery.

2.6. Analysis of patient samples

Serum samples were obtained from in-patients treated with antidepressants and antipsychotics in the psychiatric department of University Hospital Ostrava. Serum samples from patients were measured on the day of the admission to hospital, then on 3rd or 4th days of their hospitalization before and after drug administration. Some patients were admitted to hospital repeatedly and their serum samples were collected and measured by the same manner as in the first stay. Patients receiving citalopram, venlafaxine, clozapine, quetiapine and risperidone were sampled 2 h after dose, patients receiving fluoxetine, paroxetine, sertraline and olanzapine, 6 h after dose (in accordance with drug pharmacokinetic) [27]. All samples were sent to our laboratory immediately after being taken from the patient, centrifuged and analyzed the same day.

3. Results

Chromatographic analysis of the highest concentration of standards from each group of drugs (low, medium and high) was performed five times to compare the ionization efficiency of ESI and APCI (Table 1). The method was further validated using +ESI as it offers better response in comparison with APCI for all drugs, except olanzapine (Table 1).

3.1. Matrix effect

A negligible matrix effect was observed between 0.45 and 1.75 min, but more significantly evaluated in the time window where elution of olanzapine and desmethylolanzapine occurs (typical response is seen in Fig. 1). The possible matrix effect for olanzapine and desmethylolanzapine was further tested as follows. The mean peak areas of olanzapine and desmethylolanzapine in serum samples were compared with corresponding peaks in a reference sample and the ratios were 0.92 and 0.83, respectively.

3.2. Validation of method

Chromatograms of all analyzed drugs are shown in Fig. 2. Linearity of calibration curves were chosen to cover over therapeutic range of individual drugs. Correlation coefficients of calibration curves so as and concentration range for each drug are summarized in Table 2. The limit of quantification in the serum was determined

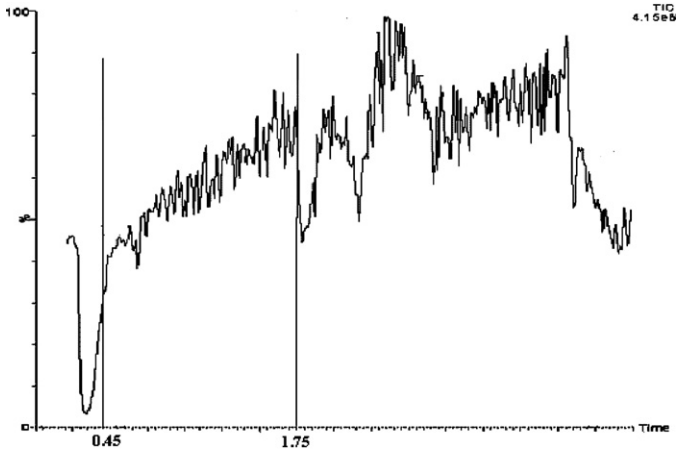

Fig. 1. Chromatogram of sample prepared from drug free serum with post column infusion of olanzapine. Verticals indicate a part of chromatogram without matrix effects.

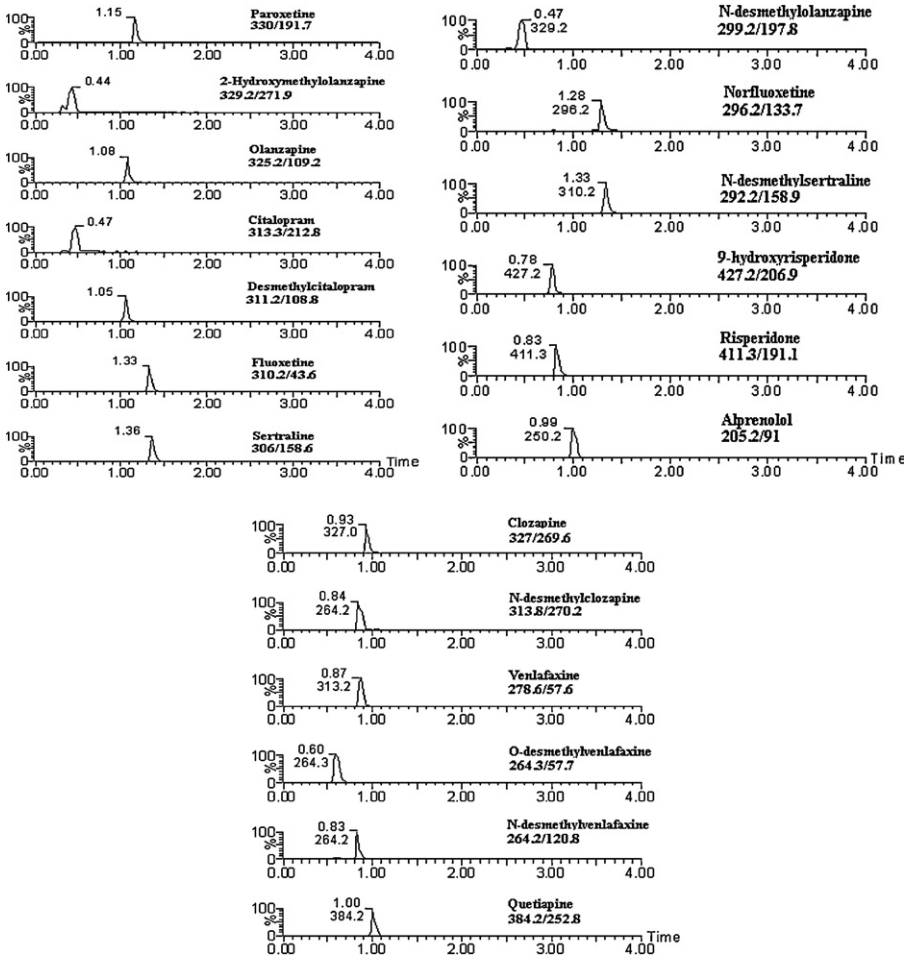

Fig. 2. Chromatograms of single drugs with retention time and mass transition.

as the lowest concentration of calibration curves. The parameters of intra-assay and inter-assay precision, accuracy and recovery met validation requirements and are stated in Table 2.

3.2.1. Quality control

QC samples at two concentration levels (low and high) with declared concentrations were used as an independent source of data and were not included in the validation protocol. Quality control samples were prepared and measured in each run with patient samples. Results of quality control were compared with declared range.

3.3. Analysis of patient samples

Overall, 379 samples from 228 patients (63 male and 165 female) were measured and some of the patients were examined repeatedly. The average age of the patients was 35.4 ± 7.4 years (19–81) for males and 38.9 ± 10.3 years (17–88) for females. The average weight of the patients was 90.1 ± 9.6 kg for males and 69.8 ± 2.8 kg for females. The concentration of the drugs and their main measured metabolites, together with therapeutic range, number of patients in and out of therapeutic range and average daily dosage for single drugs, are given in Table 3. Since co-medication with antiepileptic drugs such as carbamazepine, lamotrigine, valproic acid and clonazepam has often occurred in this group of patients, these drugs were tested for possible interference. None of them influence analysis of the target compounds as they elute in a different retention time.

4. Discussion

Although HPLC methods for determination of psychoactive drugs have been gradually developed, clinical significance of TDM was confirmed only when Baumann et al. published their guidelines [1]. Our method was developed and validated for simultaneous analysis of most antidepressants and antipsychotics, including their main metabolites, which were recommended for TDM.

HPLC method with mass spectrometric detection was applied to one, or a selected group of drugs, using either ESI or APCI [28–31]. Concerning psychoactive drugs, APCI is used less often, most authors preferring ESI. Our results confirmed that ESI offers a more efficient ionization for the determination of analyzed psychoactive drugs (except olanzapine). Berna et al. [28] and Bogusz et al. [29] employed APCI in the analysis of olanzapine, but ESI were not tested. Precipitation of proteins by acetonitrile/methanol (40:60, v/v), with a small amount of zinc sulphate, was confirmed as a useful procedure. This procedure is widely adopted in analysis of immunosuppressive drugs and has been described in detail elsewhere [32–35].

The quantitative analysis of biological samples, using mass spectrometry with atmospheric pressure ionization, can be complicated by the presence of matrix components, e.g. lipids and phospholipids that can co-elute with analytes and influence their response [36]. Chin et al. [37] investigated the matrix effect of a commonly used anticoagulant and lipemia. Their results indicate that sodium heparin and K_3EDTA can complicate determination and are not useful

Table 2
Parameters of validation.

Drug	Correlation coefficient	Concentration range (ng/ml)	Concentration (ng/ml)	Intra-assay ($n = 10$)			Inter-assay ($n = 10$)		
				Found concentration Mean ± SD (ng/mL)	CV (%)	R (%)	Found concentration Mean ± SD (ng/mL)	CV (%)	R (%)
Risperidone	0.998	1–100	5	4.9 ± 0.4	8.1	98.4	5 ± 0.4	8.4	99.4
			25	25 ± 0.6	2.2	100.1	26 ± 0.7	2.6	103.8
			100	98.7 ± 3.6	3.7	98.2	101.1 ± 3.2	3.1	101.1
9-Hydroxyrisperidone	0.998	1–100	5	4.9 ± 0.4	5.4	97.7	5 ± 0.3	6.5	100.0
			25	25.6 ± 0.8	3.3	102.3	26.5 ± 1.58	6.0	105.8
			100	98.3 ± 3.1	3.1	98.3	103.6 ± 3.6	3.5	103.6
Sertraline	0.998	5–500	10	9.8 ± 0.6	6.6	98.3	9.2 ± 0.8	9.0	91.8
			100	102.1 ± 5	5.0	102.1	100.5 ± 5	5.0	100.5
			500	487.5 ± 41.3	8.5	96.7	499.5 ± 38.3	7.7	99.9
N-desmethylsertraline	0.999	5–500	10	9.9 ± 0.4	4.2	99.0	10.3 ± 0.7	6.9	103.0
			100	105.5 ± 2.8	2.7	105.5	112.4 ± 3.8	3.4	112.4
			500	459.6 ± 17.5	3.8	91.9	476.7 ± 22.2	4.7	95.3
Fluoxetine	0.999	5–500	10	10.1 ± 0.9	8.9	100.5	10.4 ± 1.1	10.8	104.0
			100	91.1 ± 4.1	4.2	99.1	98 ± 8.2	8.4	98.0
			500	480.7 ± 30.3	6.3	96.1	487.7 ± 33.5	6.9	97.5
Norfluoxetine	1.0	5–500	10	10.3 ± 0.4	3.7	103.1	9.9 ± 0.6	6.1	98.7
			100	101.1 ± 5	5.0	101.1	104.5 ± 6.2	5.9	104.5
			500	445.7 ± 20.3	4.5	89.1	503.6 ± 32.4	6.4	100.7
Desmethylcitalopram	0.999	5–500	10	10.2 ± 0.3	2.9	100.6	9.7 ± 0.4	3.7	97.2
			100	101.8 ± 6.5	6.4	101.9	97.7 ± 6.4	6.6	97.7
			500	505.0 ± 22.6	4.5	101.1	505 ± 17.9	3.5	101
Drug	Correlation coefficient	Concentration range (ng/ml)	Concentration (ng/ml)	Intra-assay ($n > 10$)			Inter-assay ($n > 10$)		
				Found concentration Mean ± SD (ng/mL)	CV (%)	R (%)	Found concentration Mean ± SD (ng/mL)	CV (%)	R (%)
Didesmethylcitalopram	0.999	5–500	10	10.1 ± 0.3	3.2	101.3	10.8 ± 0.7	6.2	108
			100	108 ± 6.7	6.2	108.3	108.2 ± 5.4	5.0	108.2
			500	502.2 ± 3.6	0.7	100.4	477.9 ± 20.3	4.2	95.6
Paroxetine	0.995	5–500	10	10.1 ± 0.6	5.5	101	10 ± 0.8	8.3	99.9
			100	110.4 ± 6.1	5.5	110	102.4 ± 12.1	11.9	102.4
			500	521.9 ± 32.4	6.2	104.4	492.8 ± 14.5	2.9	98.6
Quetiapine	0.999	5–500	10	9.8 ± 0.8	7.9	98.4	10.4 ± 0.6	6.0	103.5
			100	97.9 ± 4.9	5.0	97.9	101.3 ± 5.7	5.6	101.3
			500	507.6 ± 10.7	2.1	101.5	491.7 ± 8.3	1.7	98.3
Clozapine	0.997	5–2000	50	44.2 ± 1.3	2.9	88.3	45.2 ± 1.4	3.0	90.5
			250	227.8 ± 7.1	3.1	91.1	225 ± 13	5.8	90.0
			1000	1012.8 ± 33.2	3.3	101.3	1008.9 ± 40.6	4.0	100.9
N-desmethylclozapine	0.998	50–1000	50	45.3 ± 2.6	5.7	90.6	49.9 ± 2.9	5.8	99.8
			250	240.3 ± 14	5.8	96.1	264.5 ± 17.6	6.7	105.8
			1000	1065 ± 47.1	4.4	106.5	996.6 ± 70.8	7.1	99.7
Venlafaxine	0.998	50–2000	50	54.4 ± 3	5.4	108.8	48.6 ± 4.6	9.5	97.1
			250	271.2 ± 12.2	4.5	108	225.8 ± 9.5	4.2	90.3
			1000	1051.1 ± 36.4	3.5	105.1	1062.7 ± 59.1	5.6	106.3
O-desmethylvenlafaxine	0.998	5–2000	50	48.7 ± 2.5	5.2	97.5	44.1 ± 1.6	3.7	88.1
			250	246 ± 10.4	4.2	98.4	231.1 ± 25.6	11.1	92.5
			1000	1018 ± 29.5	2.9	101.8	1031.9 ± 48.2	4.7	103.2
N-desmethylvenlafaxine	0.998	5–1000	50	45.3 ± 2.5	2.7	90.6	47.6 ± 3	6.2	95.2
			250	252 ± 10.8	4.3	100.8	245.9 ± 11	4.5	98.4
			1000	1049.4 ± 37.5	3.6	105	1036.7 ± 65.9	6.4	103.7
Olanzapine	0.999	5–500	10	10.2 ± 0.5	4.9	102.2	9.9 ± 0.6	6.1	99.4
			100	106.9 ± 8.2	7.7	106.9	97.6 ± 7.1	7.3	97.6
			500	512.1 ± 38.8	7.6	102.4	501.2 ± 17.2	3.4	100.2
N-desmethylolanzapine	0.996	5–500	10	10.7 ± 0.6	5.8	106.8	10 ± 09	8.9	99.7
			100	109.7 ± 4.2	3.8	109.7	100.6 ± 6.2	6.2	100.6
			500	495.4 ± 17.5	3.5	99.1	488.6 ± 17	3.5	97.7
2-hydroxyolanzapine	0.998	5–500	10	10.9 ± 0.7	6.1	108.6	10 ± 0.6	6.3	99.6
			100	107.7 ± 5.9	5.5	107.7	103.8 ± 7.7	7.4	103.8
			500	519.1 ± 41.4	8.0	103.4	495.4 ± 8.1	8.2	99.1

CV, coefficient of variation; R, recovery.

for the clinical study of drugs. It is better to analyze serum samples without additives. The matrix effect can also be caused by exogenous substances such as polymers contained in different brands of plastic tubes. Eeckhaut et al. [36] described the influence of matrix effects on APCI and ESI but the mechanism of these effects is still not fully understood. In our study matrix effects were evaluated using post-column infusion experiment and were found to be negligible between 0.45 and 1.75 min, where the most compounds were measured. Only olanzapine and des-methylolanzapine which had faster elution (RT below 0.45 min) could co-eluted with matrix components decreasing their response and ion suppression 8% and 17%, respectively, was observed. Finally, a matrix effect of about 20% is generally tolerated, as it does not significantly influence analytical results [38].

Table 3
Therapeutic range, range of measured concentrations and usual dosage range/average daily dosage.

Drug	Therapeutic range (ng/ml)	n/o	Range of measured concentrations (ng/ml)	UDR/ADD (mg/day)
Risperidone	20–60*	13/9	0.5–28.5	2–8/2.1 [27]
9-Hydroxyrisperidone		13	3.2–30.7	
Sertraline	10–50	56/30	3.4–111.9	100–150/115 [27]
N-desmethylsertraline		56	2.5–249.7	
Fluoxetine	120–300*	4/3	58.7–151.2	20–40/20 [27]
Norfluoxetine		4	165.6–203.8	
Citalopram	30–130	80/50	2.8–172.0	20–40/18 [27]
Desmethylcitalopram		46	2.7–18.8	
Didesmethylcitalopram		13	2.7–9.0	
Paroxetine	70–120	43/4	3.9–229.8	20–40/30 [27]
Quetiapine	70–170	76/10	3.1–344.5	200–900/144 [27]
Clozapine	350–600	6/1	62.9–484.3	200–900/350 [27]
N-desmethylclozapine		6	38.4–332.9	
Venlafaxine	195–400*	90/43	4.4–1017.3	75–225/174 [27]
O-desmethylvenlafaxine		89	3.8–1054.6	
N-desmethylvenlafaxine		87	3.5–781.1	
Olanzapine	20–80	11/7	4.9–46.6	7.5–30/10 [27]
N-desmethylolanzapine		6	3.3–12.9	
2-Hydroxyolanzapine		3	2.6–2.7	

n/o, number of samples/number of samples in therapeutic range. UDR/ADD, usual dosage range/average daily dosage.
* Drug plus metabolite.

Coefficients of correlation between 0.995 and 1.0 were obtained for calibration curves of all analytes. Because of significant differences in the therapeutic concentration of drugs in the serum, analytes were divided into three groups with low, medium and high concentrations, and validated separately. Validation criteria of precision and accuracy were evaluated in intra-assay and inter-assay conditions and were between 0.7–11.9 and 88.1–110, respectively, which met validation requirements. Analyses of quality control samples in two concentration levels (low and high with declared values) confirmed the validity of the method. The clinical significance of the method was verified using analyses of real patient samples, which were taken during the whole dosing interval for one or more given drugs.

Overall, 379 samples from 228 patients have so far been measured, which is relatively a small group to use to state any clinical conclusions. Nevertheless, some relationship between drug concentrations in the serum and daily dose can be described. Patients with the lowest concentration levels, which were significantly below the therapeutics range, had the lowest recommended daily dose. Metabolism of antidepressant and antipsychotic drugs is due to cytochrome P 450 and shows a high inter-individual variability in the concentrations of parent drugs and their main metabolites. The further flow of patients raised the question of their compliance, because many of these drugs are badly tolerated. When the drugs were analyzed during admission of the patients to the psychiatric department, concentrations were often lower in comparison with those measured later when regular dosing is guaranteed.

For all of the drugs, except venlafaxine, measured concentrations were in the therapeutic range or below the lower limit. For venlafaxine, the measured concentrations remained well above the upper range in spite of the fact that the patients received only an average daily dose of 300 mg/day.

As is indicated, therapeutic drug monitoring of antidepressants and antipsychotics might be important to optimize pharmacotherapy and thus improve care of psychiatric patients. The validated LC–MS/MS method for simultaneous analysis of the nine drugs and their main metabolites represents a significant tool which enables measurement and monitoring of frequently taken psychoactive drugs.

5. Concluding remarks

This method was developed and validated for analysis of nine psychoactive drugs and ten metabolites. Because of the different concentration ranges of single drugs observed in the serum, analytes were divided into three groups with low, medium and high levels. The method uses electrospray ionization (ESI) with a negligible matrix effect of co-eluting compounds. It allows determination of a new, so far unpublished, group of drugs and their metabolites, which are suitable for routine use, in a very short period of time. This may be important in terms of possible intoxication or drug interactions.

Conflict of interest

The authors have declared no conflict of interest.

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