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Quantitative determination of forty-eight antidepressants and antipsychotics in human serum by HPLC tandem mass spectrometry: A multi-level, single-sample approach

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Abstract

This method describes the simultaneous determination of amisulpride, amitriptyline, aripiprazole, benperidol, chlorpromazine, chlorprothixene, citalopram, clomipramine, clozapine, desipramine, doxepin, fluoxetine, flupentixol, fluphenazine, fluvoxamine, haloperidol, hydroxyrisperidone, imipramine, levomepromazine, maprotiline, mianserine, mirtazapine, moclobemide, norclomipramine, nordoxepin, norfluoxetine, nortriptyline, *O*-desmethylvenlafaxine, olanzapine, opipramol, paroxetine, perazine, perphenazine, pimozide, pipamperone, quetiapine, reboxetine, risperidone, sertraline, sulpiride, thioridazine, trazodone, trimipramine, venlafaxine, viloxazine, ziprasidone, zotepine and zuclopenthixol with a single sample/triple injection approach. Drugs were assigned to subgroups covering low, medium and high concentrations (overall range of therapeutic levels to be considered: 0.5–2000 ng/mL) by further dilution of the supernatant obtained after the first protein precipitation. Chromatographic separation was necessary for isobaric mass fragments and performed on a monolithic C18 column (50 mm × 4.6 mm) with methanol gradient and 5 mM acetate buffer at pH 3.9. The injection interval was 8 min. A set of three internal standards was used for quantification of drugs with widely varying hydrophobicity. After electrospray ionization positive ion fragments were detected in the multiple reaction monitoring (MRM) mode with an API 4000 tandem mass spectrometer. Regression parameters of calibration curves and limits of quantification showed good covering of therapeutic and subtherapeutic ranges with an average correlation coefficient of 0.9988. Imprecision and inaccuracy measures were prepared for intra- and inter-assay comparisons at three concentration ranges in all subgroups. Average coefficients of variation were 6.1% for intra-assay and 7.4% for inter-assay comparisons, while average deviations from spiked concentrations were 4.8% for intra-assay and 4.2% for inter-assay comparisons, respectively. Recovery rates, measured as the percent recoveries of spiked serum samples against standard solutions without serum matrix, varied between 92 and 111%, with an average of 101%. As the only exception, the olanzapine response was much higher (185%) in serum matrix than in matrix-free controls.
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1. Introduction

Therapeutic drug monitoring of antidepressants and antipsychotics is necessary for an optimal supervision of patients and their drug therapy to avoid medical complications, intoxication, nonresponsiveness or noncompliance. A lot of analytical procedures have been described for the determination with HPLC of several subgroups such as tricyclic antidepressants [1,2], selective serotonin reuptake inhibitors [3,4] or atypical antipsychotics [5–7]. Multi-drug methods for screening or quantification have been generated for HPLC, GC(MS) or LC–MS(MS) approaches [8–14], but these methods are either used for screening purposes only or suffer from the disadvantage that not all common antidepressants and antipsychotics are included for quantification. The novel method presented here incorporates all the antidepressants, antipsychotics and metabolites recommended for inclusion in therapeutic drug monitoring (TDM) by "The AGNP-TDM Expert Group Consensus Guidelines: Therapeutic Drug Monitoring in Psychiatry" [15]. In addition to this list of drugs opipramole, pipamperone and arip-

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iprazole are also accomodated. The determination of 48 drugs and metabolites with a homogeneous method is a great challenge in liquid chromatography. A particular difficulty results from the fact that compounds to be considered differ in therapeutic ranges by about 3 orders of magnitude. Ideal condition for some drugs is not generally transferable to all analytes in this study.

Therefore, a compromise must be found in mobile and stationary phase to get a usable method with sharp peak form, short run time and necessary chromatographic separation. It is emphasized that the method principally allows inclusion of further metabolites provided that chromatographic or mass fragment interferences do not form an obstacle. The advantage of such a multi-method is to minimize the expenditure for routine determination of a large set of individual and varying drug combinations and for a general toxicological screening of antidepressants and antipsychotics as well.

2. Experimental

2.1. Reagents

Acetonitrile and methanol (Sigma, Taufkirchen, Germany) were HPLC grade, acetic acid (100%), ammonia solution (25%) and ethanol were analytical grade obtained from VWR/Merck (Darmstadt, Germany). The following reference standards of antidepressants and antipsychotics or internal standards were purchased from Sigma (Taufkirchen, Germany): amitriptyline, chlorpromazine, chlorprothixene, clozapine, *N*-desmethylclozapine, doxepin, flupentixol, fluphenazine, haloperidol, maprotiline, nordoxepin, norfluoxetine, nortriptyline, thioridazine, trazodone and internal standard clonidine. Zuclopenthixol was from Promochem (Wesel, Germany) and internal standard methabenzthiazurone from Ehrensdorfer (Augsburg, Germany). Other antidepressants and antipsychotics reference standards were kindly provided by the following companies: aripiprazole (Otsuka/Bristol Myers Squibb, München, Germany), benperidol and levomepromazine (Tropon, Köln, Germany), citalopram, normaprotiline and perazine (Lundbeck, Copenhagen, Denmark), pimozide, pipamperone, risperidone, hydroxyrisperidone and internal standard dehydromethylrisperidone (Janssen, Beerse, Belgium), clomipramine, desipramine, imipramine and opipramol (Novartis, Nürnberg, Germany), fluoxetine and olanzapine (Lilly, Indianapolis, USA), amisulpride and sulpiride (Sanofi-Synthelabo, Berlin, Germany), fluvoxamine (Solvay Duphar, Hannover, Germany), mianserin and mirtazapine (Organon, Oss, The Netherlands), melperone (Nordmark, Uetersen, Germany), moclobemide (Roche, Grenzach-Wyhlen, Germany), quetiapine and viloxazine (Astra-Zeneca, Wedel, Germany), paroxetine (Smith Kline Beecham, München, Germany), reboxetine (Pharmacia Upjohn, Kalamazoo, USA), sertraline and ziprasidone (Pfizer, Groton, USA), trimipramine and nortrimipramine (Aventis, Bad Soden, Germany), venlafaxine, *O*-desmethylvenlafaxine and *N*-desmethylvenlafaxine (Wyeth, Münster, Germany), zotepine (Klinge, München, Germany).

2.2. Standard preparation

Standard stock solutions of 1000 mg/L were prepared by dissolving the equivalent of 10 mg of the respective drug related to its free base in 10 mL methanol, except ziprasidone. This drug is dissolved in ethanol instead of methanol. For preparing the low level standard mixture, stock solutions of benperidol, flupentixol, fluphenazine, haloperidol, perphenazine, pimozide, risperidone and zuclopenthixol were first diluted to a concentration of 10 mg/L with methanol. Then 0.2 mL of each of these diluted drug solutions were mixed with 2.4 mL of methanol ($8 \times 0.2\,\text{mL} + 2.4\,\text{mL} = 4.0\,\text{mL}$), resulting in a final concentration of 0.5 mg/L for each drug in this low level mixture (solution "L"). To prepare the medium level mixture, 0.1 mL each of 1000 mg/L stock solution of amitriptyline, nortriptyline, aripiprazole, chlorpromazine, chlorprothixene, citalopram, desipramine, doxepin, nordoxepin, fluoxetine, norfluoxetine, fluvoxamine, hydroxyrisperidone, imipramine, levomepromazine, mianserin, mirtazapine, olanzapine, opipramol, paroxetine, perazine, pipamperone, quetiapine, reboxetine, sertraline, viloxazine, ziprasidone and zotepine were mixed with 17.2 mL of methanol ($28 \times 0.1\,\text{mL} + 17.2\,\text{mL} = 20.0\,\text{mL}$). The concentration of the medium level mixture (solution "M") is now 5 mg/L per drug. For the high level mixture containing 50 mg/L of each drug (solution "H"), 0.2 mL each of 1000 mg/L stock solutions of amisulpride, clomipramine, norclomipramine, clozapine, maprotiline, melperone, moclobemide, sulpiride, thioridazine, trazodone, trimipramine, venlafaxine and *O*-desmethylvenlafaxine were mixed with 1.4 mL of methanol ($13 \times 0.2\,\text{mL} + 1.4\,\text{mL} = 4.0\,\text{mL}$). To prepare the final combined mixture of low level (0.1 mg/L), medium level (1 mg/L) and high level drugs (10 mg/L), 2.0 mL of L, 2.0 mL of M and 2.0 mL of H were mixed with 4.0 mL of methanol ($3 \times 2.0\,\text{mL} + 4.0\,\text{mL} = 10.0\,\text{mL}$) and stored frozen and aliquoted in dark-brown reaction vials. Stock solutions (1000 mg/L) of the internal standards clonidine, dehydromethylrisperidone and methabenzthiazurone were prepared by dissolving 10 mg of the analytes in 10 mL of methanol each. Clonidine was further diluted to 5 mg/L, and dehydromethylrisperidone and methabenzthiozurone were diluted to 1 mg/L with methanol. To prepare an internal standard mixture for sample preparation, 0.1 mL of clonidine, 0.1 mL of dehydromethylrisperidone and 0.1 mL of methabenzthiazurone solutions were dissolved with 0.7 mL methanol and 9.0 mL acetonitrile to obtain a final volume of 10.0 mL. Pool serum for calibration standards and quality control was provided by mixing of in-house human serum samples, and drug-free testing was performed by running the analysis prior to standard addition. Spiking of pool serum with standard mixture was performed by mixing nine parts of pool serum with one part of combined mixture of low level, medium level, and high level drugs to receive the following calibration standard concentrations: 1, 2, 5, 10, 20, 50 and 100 μg/L for low level drugs, 10, 20, 50, 100, 200, 500 and 1000 μg/L for medium level drugs, and 100, 200, 500, 1000, 2000, 5000 and 10,000 μg/L for high level drugs. Quality control samples for intra- and inter-assay comparisons were prepared in the way described above. For inter-assay

Table 1
Acquisition parameters

	Q1 (*m/z*)	Q3 (*m/z*)	DP (V)	CE (V)	CXP (V)	Rt (min)	ISTD
Low level drug							
Benperidol	382.4	165.1	101	35	10	3.0	M
Flupentixol	435.1	265.1	106	55	18	4.5	Z
Fluphenazine	438.3	171.2	96	37	10	4.4	Z
Haloperidol	376.1	165.1	61	37	10	3.4	M
Perphenazine	404.2	171.2	91	33	10	4.3	Z
Pimozide	462.2	109.1	76	81	6	4.0	Z
Risperidone	411.3	191.1	106	41	12	2.9	M
Hydroxyrisperidone	427.2	207.1	101	41	14	2.7	M
Zuclopenthixol	401.0	128.1	76	33	12	4.3	Z
Medium level drug							
Amitriptyline	278.2	233.0	76	25	16	3.8	M
Nortriptyline	264.1	232.9	61	21	22	3.9	M
Aripiprazole	448.1	284.9	131	37	10	4.0	M
Chlorpromazine	319.0	86.0	76	35	6	4.1	Z
Chlorprothixene	316.1	271.0	86	29	18	4.1	Z
Citalopram	325.1	109.1	81	37	6	3.3	M
Desipramine	267.1	72.0	76	29	4	3.8	M
Doxepin	280.3	107.1	61	31	6	3.4	M
Nordoxepin	266.1	107.0	71	29	10	3.4	M
Fluoxetine	310.1	148.1	56	13	10	3.9	M
Norfluoxetine	296.1	134.1	41	11	10	3.9	M
Fluvoxamine	319.1	71.0	46	31	6	3.9	M
Hydroxyrisperidone	427.2	207.1	101	41	14	2.7	M
Imipramine	281.3	86.1	61	25	6	3.7	M
Levomepromazine	329.2	100.0	71	31	6	3.8	M
Mianserin	265.1	208.0	91	31	14	3.4	M
Mirtazapine	266.1	195.1	81	37	14	2.7	M
Olanzapine	313.2	255.9	86	33	18	2.1	M
Opipramol	364.2	171.2	61	29	28	3.1	M
Paroxetine	330.2	192.2	86	31	26	3.7	M
Pipamperone	376.2	165.1	56	39	14	2.9	M
Quetiapine	384.2	253.0	66	33	12	3.5	M
Reboxetine	314.2	176.1	41	19	12	3.5	M
Sertraline	306.1	275.0	41	17	8	4.1	Z
Viloxazine	238.1	100.0	26	27	18	2.4	M
Ziprasidone	413.1	194.1	106	37	12	3.7	M
Zotepine	332.1	72.0	56	33	12	4.2	Z
High level drug							
Amisulpride	370.1	242.0	91	39	16	1.5	C
Clomipramine	315.1	86.1	61	27	4	4.1	Z
Norclomipramine	301.1	72.0	26	29	4	4.1	Z
Clozapine	327.1	270.0	76	33	8	3.5	M
Maprotiline	278.2	250.0	76	29	22	3.8	M
Melperone	264.1	165.2	76	29	10	2.6	M
Moclobemide	268.9	182.0	56	27	12	2.1	M
Perazine	340.2	141.1	71	31	26	4.0	Z
Sulpiride	342.1	112.1	76	37	6	0.8	C
Thioridazine	371.1	126.0	81	35	8	4.3	Z
Trazodone	372.1	176.1	96	35	12	3.1	M
Trimipramine	295.2	100.0	56	27	8	3.9	M
Venlafaxine	278.2	58.1	61	43	2	3.0	M
O-desmethylvenlafaxine	264.1	58.1	56	47	10	2.1	M
Internal standard (ISTD)							
Clonidine (C)	230.0	43.9	111	49	2	1.2	
Methylrisperidone (M)	421.2	201.1	96	39	14	3.2	
MBHZ (Z)	222.0	165.0	46	21	6	4.2	

Q1, parent ion mass; Q3, daugther ion mass; DP, declustering potential; CE, collision energy; CXP, collision cell exit potential.

Table 2
Linearity, recovery and limit of quantification

	Conentration range (ng/mL)	Correlation coefficient (*r*)	Recovery* (%)	LOQ (ng/mL)	Therapeutic range (ng/mL)[a]
Compound low level drugs					
Benperidol	1–20	0.9982	102	0.17	2–10
Flupentixol	1–100	0.9990	104	0.83	>2
Fluphenazine	1–100	0.9999	101	0.13	0.5–2
Haloperidol	1–20	0.9977	98	0.23	5–17
Perphenazine	1–20	0.9970	104	0.30	0.6–2.4
Pimozide	1–50	0.9996	92	0.47	15–20
Risperidone	1–50	0.9983	100	0.67	20–60[b]
Hydroxyrisperidone	5–200	0.9990	100	0.03	
Zuclopenthixol	1–100	0.9989	102	0.47	4–50
Medium level drugs					
Amitriptyline	10–1000	0.9966	104	1.17	80–200[b]
Nortriptyline	5–500	0.9988	102	0.73	
Aripiprazole	10–1000	0.9986	95	0.80	50–350[c]
Chlorpromazine	10–1000	0.9988	96	0.27	30–300
Chlorprothixene	5–500	0.9982	103	0.93	20–200
Citalopram	10–1000	0.9995	98	1.00	30–130
Desipramine	5–500	0.9990	104	2.73	100–300
Doxepin	10–1000	0.9993	101	4.43	50–150[b]
Nordoxepin	10–1000	0.9963	104	1.67	
Fluoxetine	10–1000	0.9988	95	2.17	120–300[b]
Norfluoxetine	10–1000	0.9986	98	1.37	
Fluvoxamine	10–1000	0.9974	100	1.17	150–300
Hydroxyrisperidone	5–500	0.9998	99	0.10	
Imipramine	10–1000	0.9993	98	0.23	175–300[b]
Levomepromazine	10–1000	0.9988	100	0.47	15–60
Mianserin	10–1000	0.9976	98	2.20	15–70
Mirtazapine	10–1000	0.9986	102	3.10	40–80
Olanzapine	10–1000	0.9991	185	1.83	20–80
Opipramol	10–1000	0.9991	98	1.83	100–500[d]
Paroxetine	5–500	0.9995	104	1.07	70–120
Pipamperone	10–1000	0.9991	106	1.03	100–400[d]
Quetiapine	10–1000	0.9995	99	0.17	70–170
Reboxetine	10–1000	0.9987	109	1.43	10–100
Sertraline	5–500	0.9982	103	0.70	10–50
Viloxazine	10–1000	0.9990	100	0.31	20–500
Ziprasidone	10–1000	0.9994	93	1.87	50–120
Zotepine	5–500	0.9995	99	1.03	12–120
High level drugs					
Amisulpride	100–10000	0.9992	106	28.3	100–400
Clomipramine	100–10000	0.9989	95	5.3	175–450[b]
Norclomipramine	100–10000	0.9982	107	8.7	
Clozapine	100–10000	0.9990	98	14.3	350–600
Maprotiline	100–10000	0.9989	95	6.7	125–200
Melperone	100–10000	0.9995	100	6.7	50–200[d]
Moclobemide	100–10000	0.9997	102	5.7	300–1000
Perazine	100–10000	0.9997	100	7.3	100–230
Sulpiride	100–10000	0.9989	105	8.3	200–1000
Thioridazine	100–10000	0.9990	103	2.7	200–2000
Trazodone	100–10000	0.9991	111	6.7	650–1500
Trimipramine	100–10000	0.9982	99	3.3	150–350
Venlafaxine	100–10000	0.9988	98	12.7	195–400[b]
O-desmethylvenlafaxine	100–10000	0.9997	102	11.0	

LOQ: limit of quantification as signal/noise = 10.
* Percent recovery pool serum + standard vs. standard solution.
[a] Ref. [15].
[b] Drug plus metabolite.
[c] Ref. [16].
[d] Ref. [17].

quality controls, spiked serum samples were stored in aliquots at −20 °C.

2.3. Sample preparation

To 0.1 mL of serum 0.3 mL internal standard mixture containing 50 μg/L clonidine, 10 μg/L dehydromethylrisperidon and 10 μg/L methabenzthiazurone in acetonitrile/methanol (9 + 1 by volume) was added and vortexed thoroughly. The mixture was allowed to stand for about 5 min at room temperature to complete protein precipitation. After centrifugation at 13,000 × *g* in an Eppendorf centrifuge, 100 μL of the supernantant were diluted with 100 μL of mobile phase, i.e., methanol/5 mM acetic acid, pH 3.9 (20:80 by volume) to obtain the solution for low level drug injection (total dilution factor 8). The medium level drug preparation was completed by pipetting and mixing 50 μL of the supernantant after protein precipitation, 50 μL of the internal standard mixture and 100 μL of mobile phase 20:80 (total dilution factor 16). For high level drugs 50 μL of the previous 1:16 preparation was mixed with 100 μL internal standard mixture and 350 μL of mobile phase 20:80 (total dilution factor 160). Ten microlitre of each mixture were injected onto the HPLC system.

2.4. Instrumentation and acquisition parameters

The HPLC system consisted of a 1100 series binary pump (Agilent, Waldbronn, Germany) and a HTC-PAL autosampler (CTC-Analytics, Zwingen, Switzerland). The analytical column was a Chromolith Speed ROD C18, 50 mm × 4.6 mm with particle size of 5 μm (VWR/Merck, Darmstadt, Germany). The mobile phase was generated by mixing of methanol and 5 mM

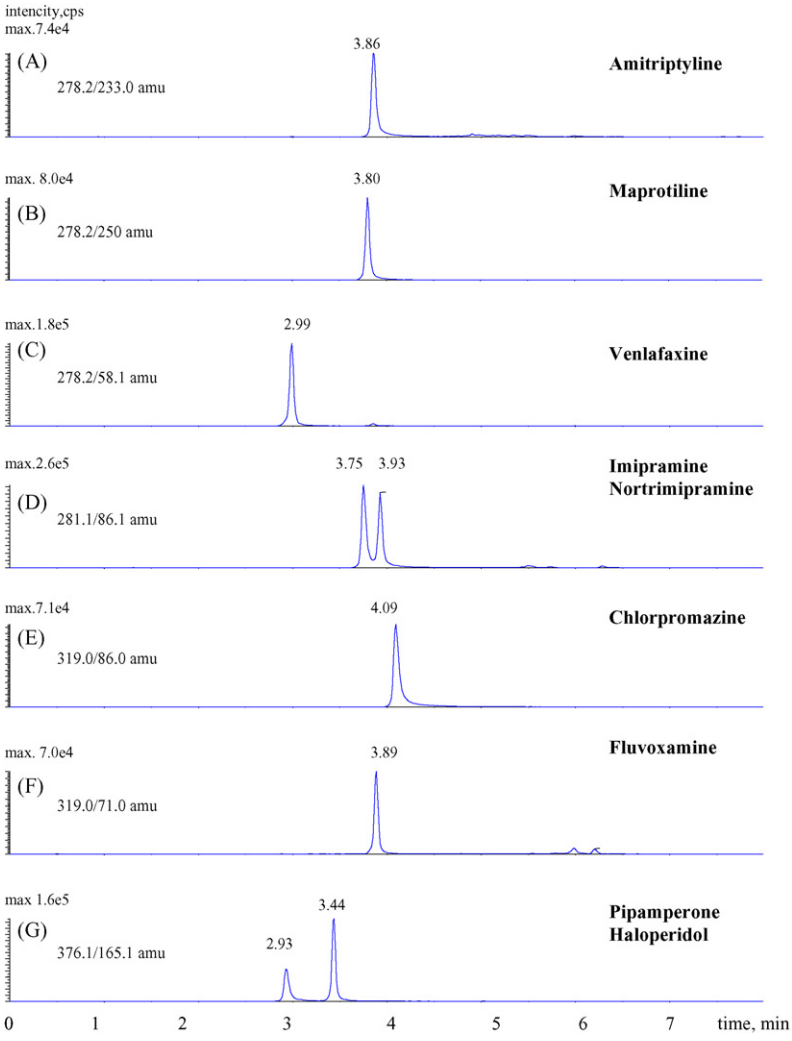

Fig. 1. Extracted ion current (XIC) chromatograms of isobaric drug pairs. Positive MRM mass transitions (MT) of a mixture of 10 ng/mL standard solution each. Chromatographic conditions as described in Section 2. (A) Amitripyline, retention time (Rt) 3.86 min, (MT) 278.2 to 233.0 *m/z*, (B) maprotiline, Rt 3.80 min, MT 278.2 to 250.0 *m/z*, (C) venlafaxine, Rt 2.99 min, MT 278.2 to 58.1 *m/z*, (D) imipramine, Rt 3.75 min, nortrimipramine, Rt 3.93 min, MT 281.1 to 86.1 *m/z*, (E) chlorpromazine, Rt 4.09 min, MT 319.0 to 86.0 *m/z*, (F) fluvoxamine, Rt 3.89 min, MT 319.1 to 71.0 *m/z*, (G) pipamperone, Rt 2.93 min, haloperidol, Rt 3.44 min, MT 376.1 to 165.1 *m/z*, (H) nortriptyline, Rt 3.90 min, MT 264.1 to 232.9 *m/z*, (I) melperone, Rt 2.61 min, MT 264.1 to 165.1 *m/z*, (K) *O*-desmethylvenlafaxine, Rt 2.17 min, MT 264.1 to 58.1 *m/z*, (L) *N*-desmethylvenlafaxine, Rt 3.03 min, MT 264.1 to 43.9 *m/z*, (M) normaprotiline, Rt 3.82 min, nortriptyline, Rt 3.89 min, MZ 264.0 to 117.0 *m/z*, (N) olanzapine, Rt 2.08 min, MT 313.2 to 255.9 *m/z* and (O) norclozapine, Rt 3.44 min, MT 313.1 to 192.1 *m/z*.

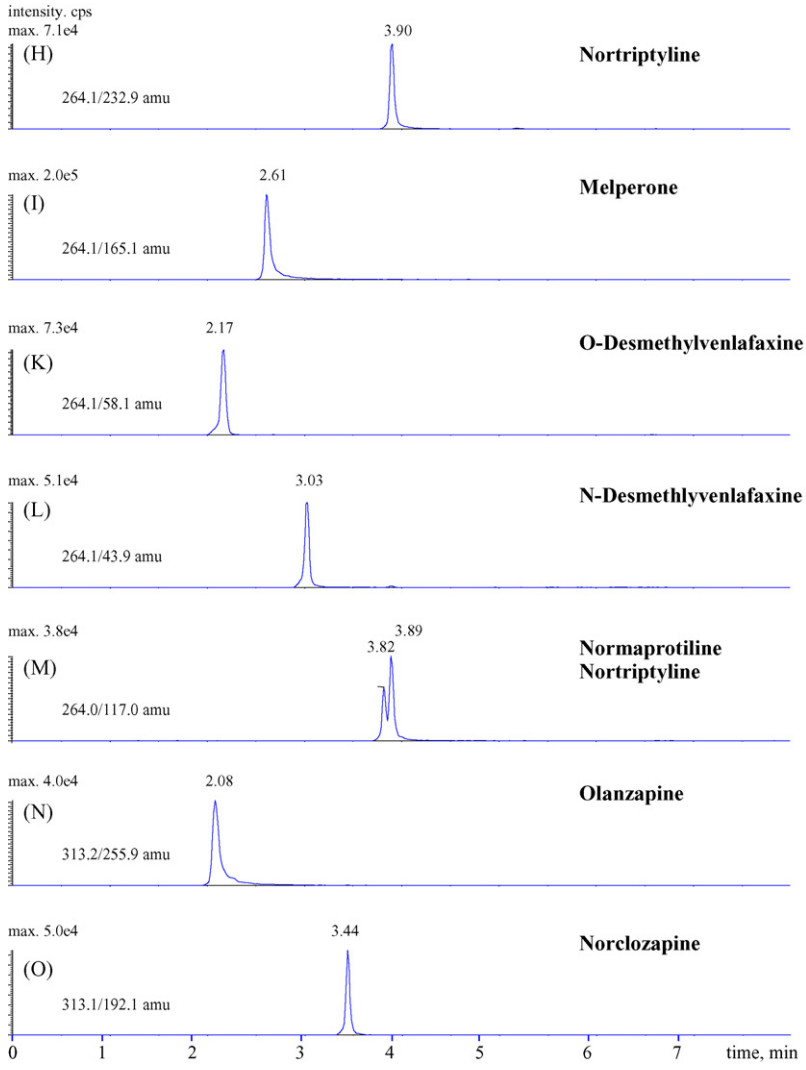

Fig. 1. (*Continued*).

acetic acid, pH 3.9 with ammonia solution in the binary HPLC pump. The flow rate was 1.0 mL/min at ambient (air-conditioned at 20–24 °C) temperature. Starting condition was 20% methanol and 80% buffer solution with linear gradient to 70% methanol in 4 min. This methanol content was held for 1 min before column re-equilibration started with 20% methanol and 80% buffer solution. Injection intervals of samples were 8 min. Detection of analytes was performed with an API 4000 tandem mass spectrometer (Applied Biosystems, Darmstadt, Germany) with coupled turbo ion spray interface in positive MRM mode. General adjustment of the instrument was as follows: Turbo spray temperature was set to 600 °C, ionization voltage was 4500 V, nitrogen gas adjustment was set to 40 and 50 units for gas 1 and 2. The curtain gas was set to 10 and the CAD gas was set to 4 units. Entrance potential was 10 V and other adjustments like declustering potential, collision energy and collision cell exit potential were optimized for each analyte. The dwell time of each drug was set to 50 ms for low level drugs, 25 ms for medium level drugs and 30 ms for high level drugs, respectively. The optimized parameters for each drug are listed together with the mass transitions in Table 1.

2.5. Validation

Assay linearity, inaccuracy, imprecision and detection limit were validated by adding various amounts of drugs to pooled human serum. To evaluate linearity, three calibration curves with seven concentration points for each drug were prepared separatety. Calibration was performed by linear regression of peak-area ratios of the drugs to the internal standard versus the respective standard concentration. Inaccuracy and imprecision were derived from intra- and inter-assay variations of 10 runs of each drug in the low, middle and high concentration ranges. Inaccuracy was expressed as percent deviation from the expected (added) amounts. Intra- and inter-assay imprecision was expressed as mean concentration found ± standard deviation and coefficient of variation in percent. Limits of quantification (LOQ) were calculated on the basis of the chromatograms and defined as a signal/noise ratio of 10. Signal quenching effects were examined by assessment of spiked pool serum versus standard solutions without biological matrix (this is termed the absolute recovery; cf. Table 2).

3. Results and discussion

The individual acquisition parameters of the tandem mass spectrometer for low, medium and high level drugs as well as the internal standards are summarized in Table 1. Q1 is the parent ion mass and Q3 the fragment (daughter) ion mass of interest. The declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) were optimized for each drug and can be transferred to another API 4000 instrument. Further, retention times of each drug and internal standard used for quantification are compiled. Even with detection by coupled tandem mass spectrometry there are still analytes with identical or indistinguishable molecular and fragmental masses due to the structural resemblance of some drugs and their metabolites. Chromatographic separations are still necessary in these cases. The extracted ion current chromatograms (XIC) of all isobaric drugs and metabolites to be found in this study are shown in Fig. 1. Amitriptyline, maprotiline and venlafaxine have the same ion mass (278 *m*/*z*) but can be distinguished by their different mass fragments (233, 250 and 58 *m*/*z*, respectively); discrimination of venlafaxine is additionally possible by the different retention time (A–C). Imipramine shows the same ion mass and mass fragment as nortrimipramine (281–86 *m*/*z*) and must be separeted chromatographically (D). Chlorpromazine and fluvoxamine, which have the same ion masses (319 *m*/*z*), differ from each other by their fragments (86 and 71 *m*/*z*) and retention times (E, F). Pipamperone and haloperidol, with indistinguishable ion masses (376 *m*/*z*) and fragments (165 *m*/*z*), must likewise be separated by the HPLC run (G). Nortripyline, melperone, *O*-desmethylvenlafaxine, *N*-desmethyvenlafaxine and normaprotiline with the same ion mass (264 *m*/*z*) can be distinguished by mass fragment and retention time (H–L), but normaprotiline must be separated from nortriptyline by chromatography (M). The isobaric drug pairs olanzapine and norclozapine (313 *m*/*z*) have different mass fragments (256 and 192 *m*/*z*) and retention times (N, O). Change of the HPLC column can result in small shifts of retention times, but, according to our present experience, the critical separations (i.e., imipramine/nortrimipramine, pipamperone/haloperidol, and normaprotiline/nortriptyline) are not critically affected. Despite the complexity of the method, this observation is a first indication to its robustness against methodological confounding factors.

Because of the very different concentration ranges to be observed in serum, analytes were subdivided into the three groups with low, medium and high level of drugs. Fig. 2 shows the total ion chromatogram (TIC) of the low level drugs, spiked with 10 ng/mL of each drug to pool serum and the internal standards dehydromethylrisperidone and MBHZ. Dilution factor during sample preparation was 8. Clonidine was not used in this run as internal standard. Pipamperone does not belong to this group, but appears in this chromatogram because of the ion mass and fragment resemblance with haloperidol. Hydroxyrisperidone was not shown in this chromatogram because of high signal intensity and illustration in the medium level drug figure. Fig. 3 contains the TIC of medium level drugs (100 ng/mL spiked to pool serum, dilution factor 16) and was separated into parts A and B for better transparency. Between 5 and 7 min retention time there are some interferences visible, mainly from the origin of the doxepin mass track, but clearly separated chromatographically (part A). Fig. 4 shows the elution sequence of the high level drugs, with concentration of 1000 ng/mL spiked to pool serum and diluted by the factor of 160. Concerning internal

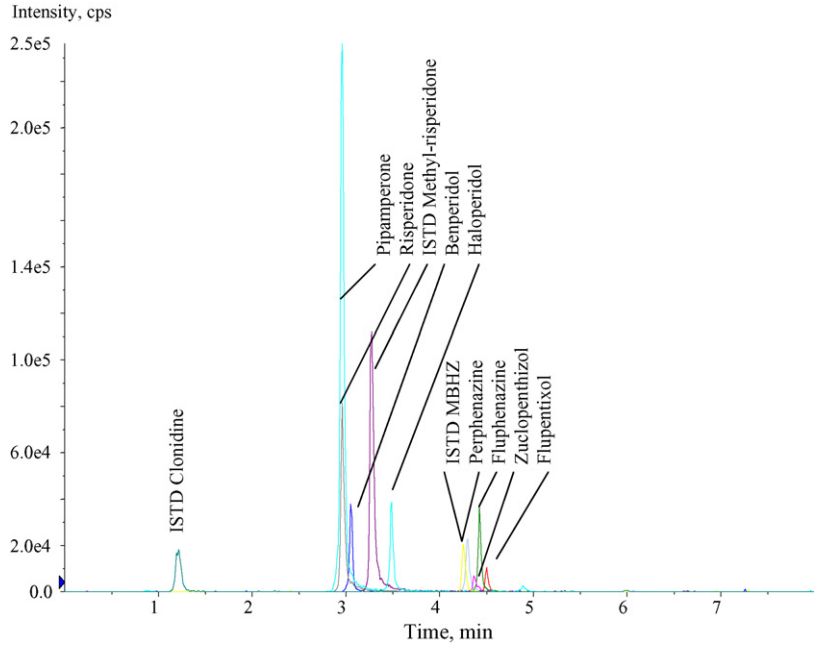

Fig. 2. Total ion current (TIC) chromatograms of low level drugs, 10 ng/mL of each drug spiked to pool serum. Sample preparation and chromatographic conditions as described in Section 2.

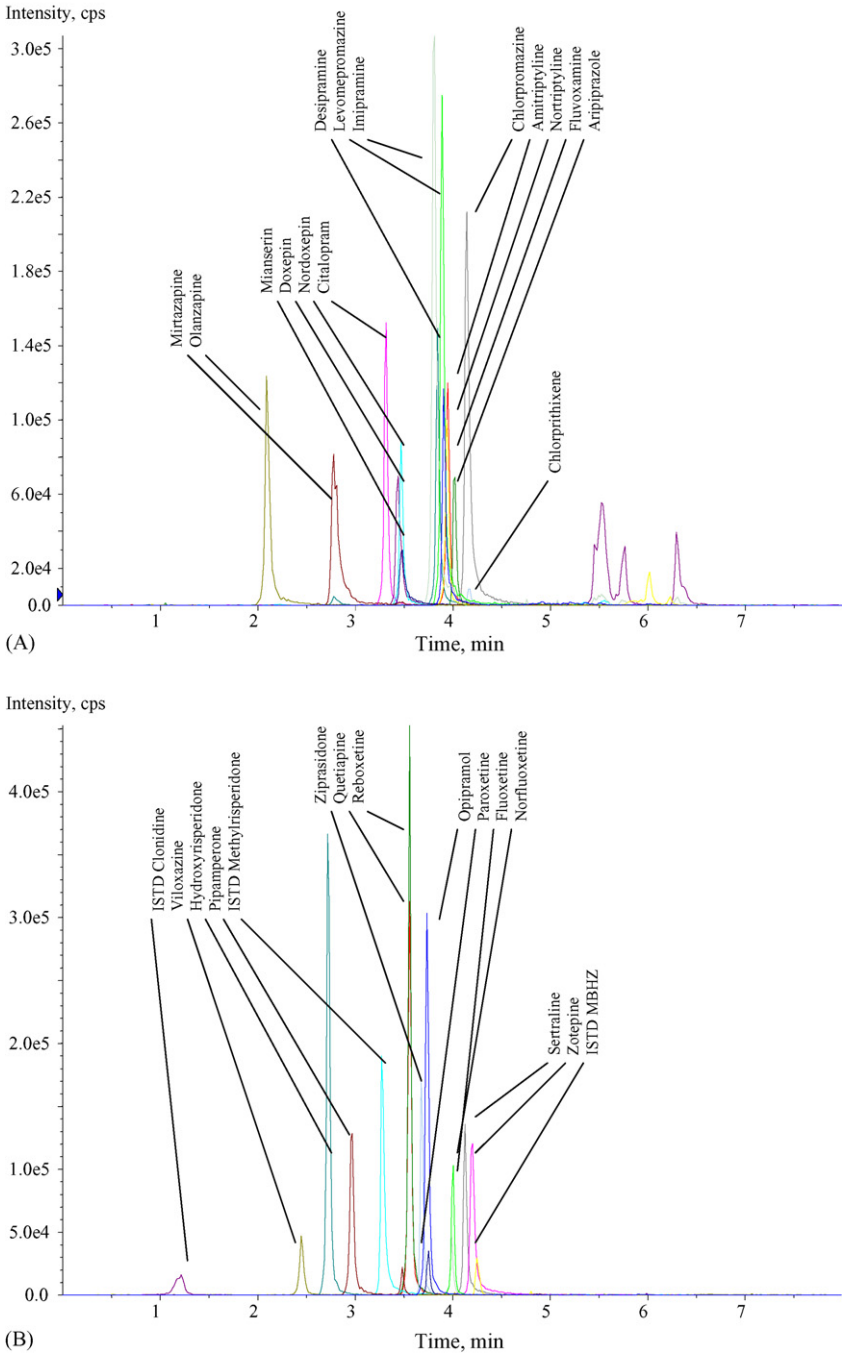

Fig. 3. Total ion current (TIC) chromatograms of medium level drugs, 100 ng/mL of each drug spiked to pool serum. Sample preparation and chromatographic conditions as described in Section 2. (A) part one and (B) part two.

standard correction, it might be argued that clonidine as one of the internal standards could affect results in specimens where it is endogenously present in the course of therapy. Since clonidine, however, is only used for correction of two of the high level drugs (Table 1: amisulpride and sulpiride), it follows from the procedure outlined in Section 2.3 that with maximal therapeutic levels (4 ng/mL), a maximal transfer of 0.025 ng/mL to the previously added 12.1875 ng/mL might occur, and this error (0.2%) is far below the respective coefficients of variation (Table 3).

Table 2 summarizes linear concentration ranges, correlation coefficients, recoveries against standard solutions without biological matrix and limits of quantification (LOQ) as signal/noise ratio of 10. The last column contains the recommended therapeutic ranges of the antidepressants and antipsychotics from ref. [15]. Linear concentration ranges were checked for low level drugs between 1 and 100 ng/mL as described in Section 2.2. Though constricted linearities were found for benperidol, haloperidol, perphenazine, pimozide and risperidone, linear concentration dependencies covered nevertheless the entire thera-

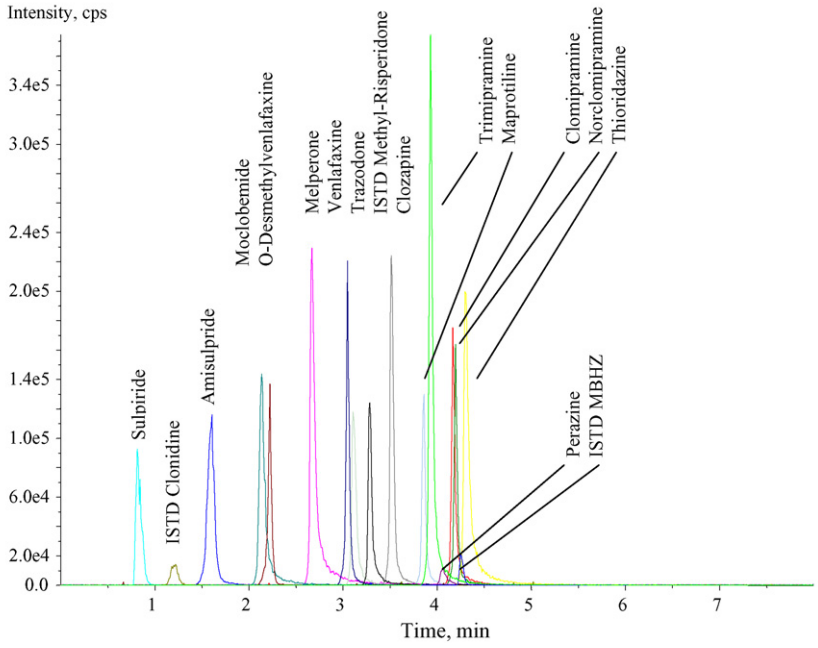

Fig. 4. Total ion current (TIC) chromatograms of high level drugs, 1000 ng/mL of each drug spiked to pool serum. Sample preparation and chromatographic conditions as described in Section 2.

peutic range. For greater concentration ranges, quadratic regression would be an alternative. This is also the case for the medium level drugs nortriptyline, chlorprothixene, desipramine, hydroxyrisperidone, paroxetine, sertraline and zotepine. Correlation coefficients for calibration curves vary from 0.9963 (nordoxepine) to 0.9999 (fluphenazine) with an average of all drugs of 0.9988.

Matrix effects in electrospray ionization could be neglected in all cases but olanzapine. Absolute recovery rates, with the only exception of olanzapine, were found between 92 and 111%, with an average of 101%. The olanzapine peak area is much greater (185%) in the biological matrix than in matrix-free controls. Therefore, calibration with serum matrices is necessary for its quantification. In order to evaluate possible *individual* variations of such a matrix effect, the influence of $n = 10$ different pool serum matrices was compared to parallel serial measurements ($n = 10$) with one constant pool serum matrix in one assay. The resulting intra-assay coefficient of variation was 5.3% with different matrices and 4.4% with constant matrices and thus within the range of imprecision determined at various concentrations (cf. Table 3). It was therefore concluded that individual contributions of serum matrices to measurements of the other drugs could also be neglected. The LOQ data show that concentrations below the therapeutic range can truly be detected in all cases. In Fig. 5, this high sensitivity is demonstrated by illustrating the chromatograms of a low level serum sample, spiked with 0.5 ng/mL and a corresponding serum blank sample. Table 3 lists further quality data of the method for both intra- and inter-assay comparisons, namely imprecision as coefficient of variation (C.V.) and inaccuracy as percent deviation from the amounts added to three concentrations for each drug level. Intra- and inter-assay C.V. with low level drugs averaged 6.9 and 7.1%, while corresponding measures for inaccuracy were 5.4 and 5.4%, respectively. For medium level drugs, averages of intra- and inter-assay C.V. were 6.3 and 8.2% with inaccuracy of 4.9 and 4.0%. High level drugs showed averages of 5.1 and 6.9% for intra- and inter-assay C.V., whereas average data for inaccuracy are 4.0 and 3.2%, respectively. These results indicate that it is possible to generate accurate data with the described multi-level method for antidepressants and antipsychotics.

Sample stability is an important issue whenever samples reach the laboratory after transport from other hospitals or laboratories. On the basis of our experience most antidepressants and antipsychotics are relative stable and there are no transport problems with few exceptions. Perphenazine is very light sensitive and therefore protection from sunlight of serum samples is mandatory. Second, olanzapine is gradually degrading at room temperature, therefore cooled or freezed sample transport is the optimum condition. An assessment of storage and transport stability of some antidepressants and antipsychotics is described by Heller et al. [18].

For routine TDM problems, it is usually not necessary to look at all drugs listed in this method. However, it is possible to select drugs of interest and to generate separate calculation

Fig. 5. Extracted ion current (XIC) chromatograms of low level drugs. Left central part of each panel: pool serum spiked with 0.5 ng/mL drug, right upturned part of each panel: blank pool serum at the same intensity scale. (A) benperidol, retention time (Rt) 3.10 min, (B) flupentixol, Rt 4.60 min, (C) fluphenazine, Rt 4.48 min, (D) haloperidol, Rt 3.60 min, pipamperone (5.0 ng/mL) Rt 3.09 min, (E) perphenazine, Rt 4.35 min, (F) pimozide; Rt 4.14 min, (G) risperidone, Rt 3.01 min, (H) zuclopenthixol, Rt 4.42 min. Sample preparation and chromatographic conditions as described in Section 2.

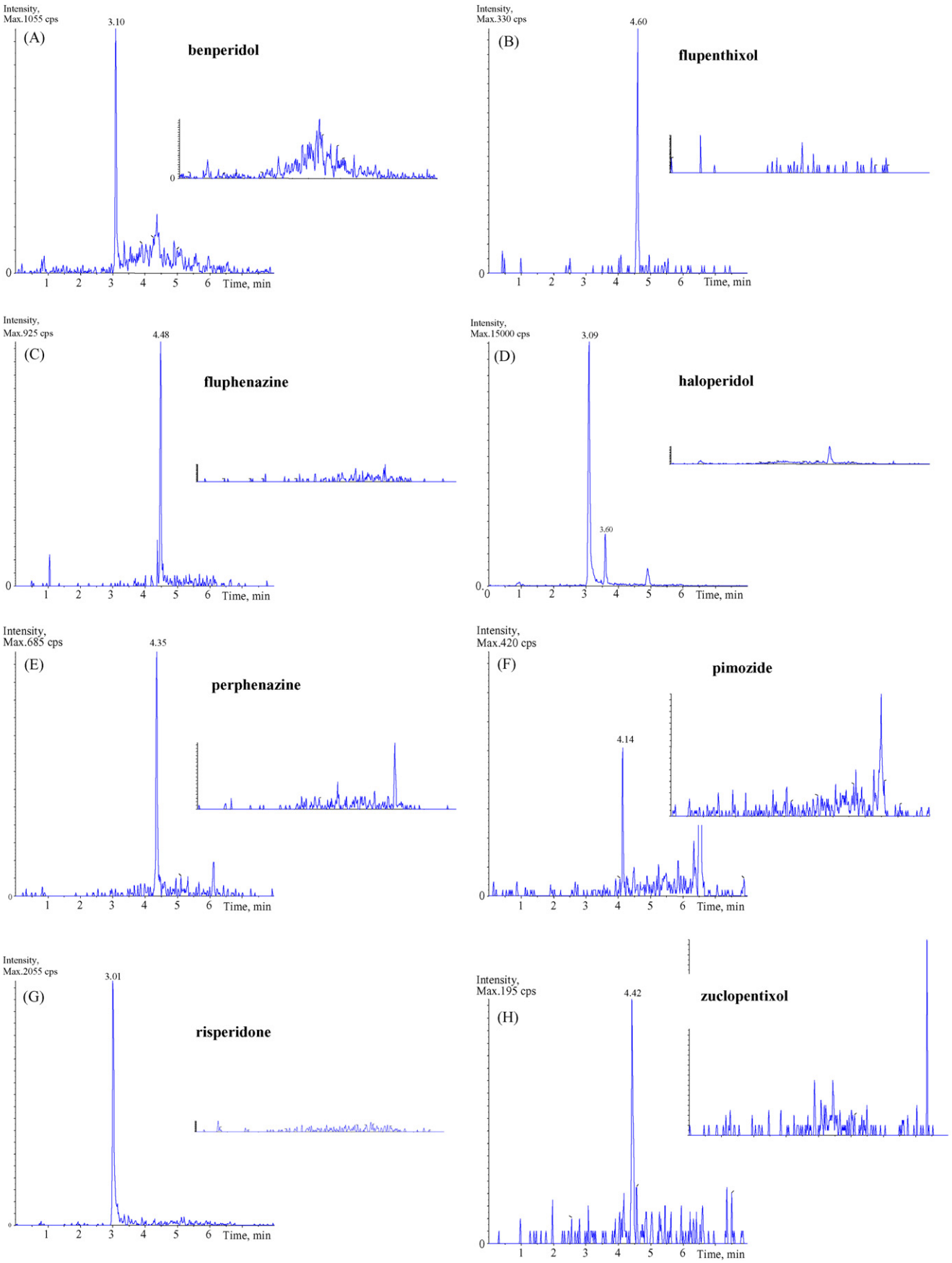
Intensity, Max.1055 cps
(A)
benperidol
3.10
Time, min
Intensity, Max.330 cps
(B)
flupenthixol
4.60
Time, min
Intensity, Max.925 cps
(C)
fluphenazine
4.48
Time, min
Intensity, Max.15000 cps
(D)
haloperidol
3.09
3.60
Time, min
Intensity, Max.685 cps
(E)
perphenazine
4.35
Time, min
Intensity, Max.420 cps
(F)
pimozide
4.14
Time, min
Intensity, Max.2055 cps
(G)
risperidone
3.01
Time, min
Intensity, Max.195 cps
(H)
zuclopentixol
4.42
Time, min

Table 3
Imprecision and inaccuracy

	Concentration added (ng/mL)	Imprecision (C.V.%, $n = 10$)		Inaccuaracy (Dev.%, $n = 10$)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay
Low level drugs					
Benperidol	1	6.9	7.6	6.0	17.0
	5	2.8	5.5	−12.4	12.0
	10	6.1	4.4	−7.8	−4.6
Flupentixol	1	13.4	9.6	4.0	2.0
	5	9.9	5.2	−0.6	2.0
	10	5.2	9.8	8.2	0.8
Fluphenazine	1	5.9	5.7	−5.0	4.0
	5	5.2	5.6	−6.4	4.0
	10	6.4	9.1	2.7	5.3
Haloperidol	1	6.2	6.4	8.0	12.0
	5	3.6	5.3	−6.2	9.0
	10	9.4	5.0	2.5	−1.7
Perphenazine	1	8.9	9.1	2.0	6.0
	5	6.1	5.8	−1.6	7.0
	10	5.1	8.9	−0.5	−0.1
Pimozide	1	12.3	9.8	9.0	6.0
	5	8.6	7.1	−5.8	3.0
	10	9.8	10.6	−4.9	1.8
Risperidone	1	3.7	6.5	11.0	6.0
	5	4.3	5.4	−6.8	6.0
	10	8.8	6.7	−5.1	1.2
Hydroxyrisperidone	10	3.3	8.3	4.0	4.9
	50	3.3	3.9	−7.6	10.0
	100	8.4	8.6	−5.1	−4.2
Zuclopenthixol	1	11.7	8.6	6.0	8.0
	5	5.6	5.0	−1.2	4.0
	10	6.5	9.1	4.5	3.6
Medium level drugs					
Amitriptyline	10	6.0	8.1	8.8	1.0
	50	5.5	7.7	12.8	7.0
	100	6.7	10.9	−0.24	2.4
Nortriptyline	10	9.0	6.0	7.4	−0.7
	50	5.1	7.2	1.6	9.0
	100	5.9	11.6	−4.2	1.7
Aripiprazole	10	10.9	6.0	6.5	−1.4
	50	7.5	7.7	8.4	7.0
	100	6.4	10.8	−5.9	3.4
Chlorpromazine	10	5.2	7.2	−4.3	3.7
	50	6.3	3.0	5.5	6.0
	100	7.0	8.8	−5.3	10.0
Chlorprothixene	10	4.7	8.0	6.7	5.2
	50	8.4	5.0	11.8	7.0
	100	7.1	8.7	0.3	5.7
Citaloprame	10	5.4	8.4	−0.8	−3.2
	50	8.2	9.3	7.6	6.0
	100	6.2	10.3	−2.1	1.9
Desipramine	10	6.8	10.3	5.7	−1.9
	50	3.6	7.1	5.0	9.0
	100	6.1	11.3	1.6	3.1
Doxepin	10	9.3	5.0	−0.2	−3.0
	50	4.5	9.0	3.5	2.0
	100	4.8	9.1	5.6	−0.4

Table 3 (*Continued*)

	Concentration added (ng/mL)	Imprecision (C.V.%, $n = 10$)		Inaccuaracy (Dev.%, $n = 10$)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay
Nordoxepin	10	4.8	6.0	6.2	1.4
	50	3.8	6.5	8.2	7.0
	100	6.2	9.3	2.6	−1.7
Fluoxetine	10	9.8	12.5	4.3	0.8
	50	3.0	7.1	−2.9	6.0
	100	7.4	10.2	−2.6	8.0
Norfluoxetine	10	8.1	10.6	0.2	1.8
	50	6.1	6.6	7.8	6.0
	100	5.3	8.9	−4.5	2.9
Fluvoxamine	10	9.4	7.0	9.5	1.9
	50	4.9	7.5	5.6	8.0
	100	6.3	11.6	1.9	0.8
Hydroxyrisperidone	10	7.6	7.4	−0.01	−1.3
	50	4.2	6.8	4.8	8.0
	100	6.8	8.1	−1.9	0.2
Imipramine	10	5.0	7.5	−0.1	−3.9
	50	4.7	7.5	6.5	6.0
	100	5.8	8.3	−3.6	2.3
Levomepromazine	10	6.4	5.5	−0.1	−0.8
	50	3.9	8.1	6.2	6.0
	100	5.8	10.3	0.6	3.7
Mianserin	10	9.1	8.3	4.7	−3.1
	50	6.6	6.8	3.6	5.0
	100	5.1	6.1	3.9	−0.1
Mirtazapine	10	7.3	9.2	8.2	−1.2
	50	5.5	6.1	6.0	7.0
	100	4.2	9.9	5.0	0.5
Olanzapine	10	6.2	9.2	−7.0	−2.7
	50	4.4	4.3	2.2	−3.0
	100	7.1	7.5	−5.3	5.1
Opipramol	10	7.8	5.0	2.7	0.3
	50	7.4	13.7	9.6	−2.0
	100	5.8	9.9	−2.0	4.2
Paroxetine	10	10.6	11.9	6.1	9.0
	50	9.7	5.7	−3.4	9.0
	100	6.5	8.1	6.2	2.8
Pipamperone	10	7.1	7.6	12.2	−0.8
	50	6.0	7.8	6.9	10.0
	100	5.3	11.5	7.8	2.9
Quetiapine	10	9.1	3.9	−0.02	−1.3
	50	4.1	7.6	8.4	6.0
	100	5.4	8.5	1.4	0.7
Reboxetine	10	8.7	8.8	−1.4	−1.3
	50	2.4	7.9	7.2	5.0
	100	5.5	8.4	6.8	2.2
Sertraline	10	8.1	7.8	6.4	0.7
	50	5.2	5.3	9.7	7.0
	100	7.0	8.6	1.0	10.9
Viloxazine	10	8.6	11.7	1.1	1.1
	50	4.3	8.5	5.1	9.0
	100	6.0	9.9	−2.1	4.1
Ziprasidone	10	3.8	10.6	−5.4	−2.7
	50	5.1	7.3	9.9	5.0
	100	5.0	10.8	−9.7	5.3

Table 3 (*Continued*)

	Concentration added (ng/mL)	Imprecision (C.V.%, $n = 10$)		Inaccuaracy (Dev.%, $n = 10$)	
		Intra-assay	Inter-assay	Intra-assay	Inter-assay
Zotepine	10	6.5	10.4	1.4	0.2
	50	5.5	4.3	7.7	5.0
	100	5.8	5.4	−4.8	7.3
High level drugs					
Amisulpride	100	4.7	5.3	−0.2	0.6
	500	4.8	6.9	−1.8	3.0
	1000	5.0	7.3	13.3	6.2
Clomipramine	100	4.7	8.1	−1.2	−3.3
	500	7.8	6.0	5.7	5.0
	1000	5.2	6.1	−9.2	3.2
Norclomipramine	100	5.6	8.5	4.0	−2.4
	500	4.5	5.3	4.8	5.0
	1000	5.0	8.5	8.9	3.0
Clozapine	100	3.1	9.1	−3.9	−0.8
	500	5.0	5.8	8.2	3.0
	1000	6.3	4.9	2.2	7.0
Maprotiline	100	3.7	6.6	−4.7	−1.3
	500	7.1	7.7	3.2	2.0
	1000	5.4	5.8	−5.7	4.9
Melperone	100	4.8	5.5	−0.01	−1.1
	500	5.0	5.8	6.8	3.0
	1000	3.9	5.5	0.2	3.2
Moclobemide	100	4.5	5.2	0.6	−0.3
	500	3.4	6.5	6.2	3.0
	1000	3.9	6.0	2.7	3.8
Perazine	100	7.7	10.3	0.5	2.2
	500	6.4	7.5	0.4	4.0
	1000	3.6	8.1	−1.1	3.9
Sulpiride	100	6.8	11.9	−3.2	−2.6
	500	5.9	6.4	1.0	5.0
	1000	7.1	11.0	−1.8	6.6
Thioridazine	100	2.1	7.5	3.4	−1.5
	500	7.4	5.3	1.9	3.0
	1000	4.1	8.3	4.4	4.8
Trazodone	100	5.3	7.0	5.8	−2.6
	500	5.6	4.8	9.1	2.0
	1000	7.5	6.0	−2.0	6.1
Trimipramine	100	4.8	4.5	−2.3	0.1
	500	2.1	8.6	7.6	3.0
	1000	6.0	5.8	−1.4	5.1
Venlafaxine	100	3.8	8.3	−3.3	1.2
	500	5.8	5.0	5.0	3.0
	1000	6.1	5.2	−4.5	4.7
O-desmethylvenlafaxine	100	5.4	9.4	4.7	−0.1
	500	1.5	4.8	10.6	4.0
	1000	6.3	6.5	−1.9	5.0

C.V., coefficient of variation; Dev., deviation.

procedures for individual or varying panels of drugs on the basis of this multi-level method. For any laboratory with a wide spectrum of TDM parameters and multiple clients requesting diverse sets of drug combinations, this possibility is a great advantage in contrast to employing several single methods since in the latter case, sequential analysis of individual parameters with short series length of about one to ten samples for one drug result in an over-proportional occupancy of equipment. Using the API 4000 instrument, about 100 samples with different drug profiles can be processed per day with this modified method. The high initial

investment in the tandem mass spectrometer is partly justified by significant reduction of sample preparation efforts and short processing time even for rarely requested parameters. Though this method appears to be possibly less beneficial for laboratories which process a limited spectrum of psychoactive drug combinations, it is easily and succesfully applicable for daily practice in selected laboratories from both the analytical and economical points of view.

4. Conclusion

This paper presents a quantitative screening methodology for a total of 48 antidepressants, antipsychotics and pharmacologically active metabolites in a small sample volume of 0.1 mL of serum which requires only protein precipitation and stepwise dilution for sample preparation. The method allows a general view on the individual intake of psychoactive drugs and its accurate quantification as well. Because of the universally applicable concept in sample preparation and the combination of HPLC and tandem MS separation, it is probably possible to add further drugs into this scheme with no major modifications.

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