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Short communication

Quantification of antidepressants and antipsychotics in human serum by precipitation and ultra high pressure liquid chromatography–tandem mass spectrometry

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ABSTRACT

The present article describes the quantification of mirtazapine, O-desmethylvenlafaxine, quetiapine, venlafaxine, and ziprasidone (group 1), and amitriptyline, citalopram, clomipramine, clozapine, desmethylclomipramine, desipramine, imipramine, and nortriptyline (group 2) in human serum for therapeutic drug monitoring. The method was developed to replace old techniques which applied solid phase extraction and ultra-violet detection. The old methods had reached their limit of capacity regarding the number of samples and co-medicated drugs interfering with the detection. Serum samples were precipitated with zinc sulphate and methanol containing a stable isotope labelled analog for each analyte. Quantitative analysis was performed by ultra high pressure liquid chromatography combined with a tandem mass spectrometer using a Zorbax SB-C8 column (2.0 × 50 mm; 1.8 μm) with a mobile phase consisting of 0.1% formic acid in water and methanol, respectively. The total run time of the chromatography was 4 min. Precision and trueness varied from 2.6% to 14.9% and 87.6% to 103.5%, respectively. At the lower limit of quantification, precision was up to 17.9% and trueness varied from 89.5% to 111.5%. A five point standard curve covering the clinically relevant ranges with a power function fit was applied for calibration. Ion suppression from matrix effects and internal standards were thoroughly investigated and are discussed. Process efficiency rates varied from 42% to 99%. The method has shortened the response time, reduced interference from other drugs, avoided acetonitrile usage, and reduced the amount of serum needed for analysis 50-fold.

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1. Introduction

Therapeutic drug monitoring (TDM) of antipsychotics and antidepressants is a valuable tool for patients in medical treatment for a psychiatric disorder. The indications for using TDM are numerous, including treatment start-up, changes in dose, occurrence of unwanted side effects, lacking therapeutic effect, control for compliance, and pharmacokinetic interactions [1,2]. This large number of indications combined with the marketing of new drugs and increased focus on drug use in psychiatry, augment the use of TDM. Furthermore, the number of laboratories performing TDM has decreased. As a consequence, the total number of samples sent for TDM of antidepressants and antipsychotics at our laboratory has steadily increased through the last decade. However, they are still less frequent analyses due to the large number of different drugs on the market. Therefore, pooling these analyses will be time and cost effective as well as decrease the response time for the clinicians.

Over the last two decades many articles have been published regarding analysis of antidepressants and antipsychotics. Most of them utilize high pressure liquid chromatography with ultra-violet (HPLC) or mass spectrometric (LC–MS–MS) detection, resulting in run times from 3.5 to 20 min [3–11]. The majority applies time consuming and expensive sample preparation like solid phase extraction and liquid–liquid extraction, and not all analytes are quantified with corresponding stable isotope labelled internal standards (SIL-IS). Some methods apply on-line extraction techniques like on-line solid phase extraction and turbulent flow chromatography, both capable of handling low sample volumes, but require expensive and complicated equipment [9–11]. Breaud et al. [3,9] published methods quantifying tricyclic antidepressants using SIL-IS and short columns resulting in a run time of 3.5 min. The methods utilized either precipitation of serum or turbulent flow chromatography, but quantified only three tricyclic antidepressants. Kirchherr and Kuhn-Velten [5] presented a method capable of measuring 48 antidepressants and antipsychotics by precipitation of serum. The method uses structural analogs for quantification, a large column

Abbreviations: TDM, therapeutic drug monitoring; dEMV, delta electron multiplier voltage.

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diameter of 4.6 mm, and a high flow rate. The run time was 8 min. Some of the compounds, including one of the internal standards, eluted very early, increasing the risk of ion suppression.

In the present article, a fast and robust method for quantification of antidepressants and antipsychotics in human serum is presented. The method applies simple precipitation of serum combined with advanced and specific analysis by UHPLC–MS–MS. The method applies SIL-IS for all analytes and has a total run time of 4 min including the injection cycle.

2. Materials and methods

All analytes were grouped into two groups (1 and 2) according to the clinical requirements regarding sensitivity and range of measurement. This grouping was performed to reduce the run time and the number of analytes in standards and quality control samples.

2.1. Chemicals

Reference standards were purchased from the following companies: amitriptyline, clomipramine, clozapine, desipramine, imipramine, and nortriptyline (Sigma–Aldrich Co., St. Louis, USA); O-desmethylvenlafaxine, and venlafaxine (Toronto Research Chemicals Inc., North York, ON, Canada). Other reference standards were kindly provided by the following companies: citalopram (Lundbeck, Copenhagen, Denmark), desmethylclomipramine (Novartis Pharma AG, Basel, Switzerland), mirtazapine (Organon, Oss, The Netherlands), quetiapine (AstraZeneca, Cheshire, United Kingdom), and ziprasidone (Pfizer, Groton, USA). The SIL-IS amitriptyline-d3, nortriptyline-d3, clomipramine-d6, desmethylclomipramine-d3, imipramine-d4, desipramine-d4, citalopram-d6, clozapine-d8, ziprasidone-d8, venlafaxine-d6, O-desmethylvenlafaxine-d6, mirtazapine-d3, and quetiapine-d8 were all purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Bilirubin, intralipid (20%), and zinc sulphate heptahydrate were purchased from Sigma–Aldrich Co. (St. Louis, USA). All other chemicals and solvents were of analytical grade.

2.2. Instrumentation

The UHPLC–MS–MS system consisted of Agilent triple quadrupole 6410 (G6410B) combined with Agilent 1200 LC system (Agilent Technologies, Santa Clara CA, USA). The 1200 LC system can tolerate pressures up to 600 bars and consisted of a degasser (G1379B), a binary pump with a solvent selection valve (G1312B), a thermostated well plate autosampler (G1330B + G1367D), and a column oven with a two column selection valve (G1316B). The LC system was configured for rapid resolution, which includes reduction of dead volume by discarding the solvent mixer and reduction of tube diameter.

2.3. Sample preparation

The sample preparation procedure for both groups was the same. All pipetting and mixing steps were done manually. 60 μl 10% zinc sulphate was added to 60 μl serum in a 96 well plate and mixed by aspirating and dispensing 120 μl three times. Another 60 μl 0.1% formic acid in methanol containing SIL-IS was added and mixed by aspirating and dispensing 180 μl three times. The SIL-IS concentration in 0.1% formic acid in methanol was adjusted to produce the same response of the analyte in the middle of the measurement range. The concentrations were 450 nmol/l amitriptyline-d3, 850 nmol/l nortriptyline-d3, 275 nmol/l clomipramine-d6, 850 nmol/l desmethylclomipramine-d3, 250 nmol/l imipramine-d4, 500 nmol/l desipramine-d4, 200 nmol/l citalopram-d6, 750 nmol/l clozapine-d8, 357.5 nmol/l ziprasidone-d8, 270 nmol/l venlafaxine-d6, 185 nmol/l O-desmethylvenlafaxine-d6, 112.5 nmol/l mirtazapine-d3, and 750 nmol/l quetiapine-d8.

The well plate was sealed by heat and centrifuged for 10 min at 1480 × *g* at 10 °C. The well plate was then placed in the autosampler which was adjusted to inject 5 μl of the supernatant into the UHPLC–MS–MS system and thermostated at 10 °C.

Table 1
Gradient parameters.

Group 1	Group 2
0.00 min/15%B[a]	0.00 min/32%B
1.40 min/90%B	2.35 min/90%B
1.50 min/15%B	2.45 min/32%B
3.00 min/15%B	3.00 min/32%B

[a] Organic solvent.

2.4. Chromatographic and mass spectrometric parameters

All analytes were separated on a Zorbax SB-C8 column (2.0 × 50 mm, 1.8 μm, Agilent Technologies, Santa Clara CA, USA) at a flow rate of 0.6 ml/min and a column temperature of 60 °C. The mobile phase consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in methanol. The gradient profiles were different for the two groups (Table 1). Backpressure was approximately 300 bars. The autosampler washed the needle for 10 s with water:methanol:isopropanol (1:1:1) before injection, resulting in a total run time of 4 min including the injection cycle.

The mass spectrometer operated in positive electrospray mode with the following parameters: nebulizer gas: nitrogen (purity > 99%), gas temperature = 350 °C, gas flow = 10 l/min, nebulizer pressure = 35 psi, capillary voltage = 1500 V, delta EMV = 200, unit resolution, and dwell time = 25 ms for all analytes. The mobile phase in time segment 2 from 0.5 to 2.5 min went into the mass spectrometer, mobile phase in time segment 1 from 0 to 0.5 min and time segment 3 from 2.5 to 3.0 min went to waste. Nitrogen (purity = 99.999%) served as collision gas. Acquisition parameters for each analyte are listed in Table 2.

The UHPLC–MS–MS system was controlled by Agilent Masshunter Workstation Acquisition Software, version B.02.01, and quantification was carried out using Masshunter Workstation Software, Quantitative Analysis, version B.03.02.

Table 2
Acquisition parameters.

Analyte	MS1[a] (*m/z*)	MS2[b] (*m/z*)	Frag[c] (V)	CE[d] (V)	RT[e] (min)
Group 1					
Mirtazapine	269.2	195.0	119	24	1.22
O-desmethylvenlafaxine	264.2	58.0	104	16	1.09
Quetiapine	384.2	253.1	168	20	1.48
Venlafaxine	278.2	58.0	114	16	1.39
Ziprasidone	413.1	194.0	158	28	1.44
Group 2					
Amitriptyline	278.1	233.0	135	15	1.60
Citalopram	325.1	108.9	126	24	1.08
Clomipramine	315.1	86.0	125	15	1.78
Clozapine	327.0	270.0	145	20	0.99
Desmethylclomipramine	301.0	72.0	125	12	1.81
Desipramine	267.1	72.0	120	15	1.53
Imipramine	281.1	86.0	110	15	1.50
Nortriptyline	264.1	233.0	95	10	1.63

[a] Quadrupole 1.
[b] Quadrupole 2.
[c] Fragmentor.
[d] Collision energy.
[e] Retention time.

2.5. Patient samples

The patients were referred to the Clinical Biochemical Laboratory at Aarhus University Hospital, Risskov, Denmark, for TDM. Blood samples were drawn in the morning, approximately 12 h after the last drug intake. Serum was separated from red blood cells by centrifugation. The serum samples were stored at −20 °C until analysis.

2.6. Preparation of stock solutions, standards, quality controls, and validation samples

A ziprasidone stock solution was prepared in dimethylsulphoxide at 2.5 mmol/l. The rest of the stock solutions were prepared in ethanol at 5 mmol/l. The stock solution of ziprasidone was stored at +4 °C, whereas the other stock solutions were stored at −20 °C.

Standards, quality controls, and validation samples were prepared from two different stock solutions and spiked in filtered, pooled, drug-free human serum. Standards 1–4 were prepared by dilution of standard 5, representing the upper limit of quantification (ULOQ) for each analyte. Quality control 1 (low) and 2 (high) and validation samples were prepared independently. Spiked serum pools were equilibrated for 30 min in a water bath at 37 °C in nitrogen filled bottles. Standards, quality controls, and validation samples were stored in aliquots at −80 °C. Organic solvent content in spiked serum was maximum 1%, and amount of stock solutions or dilutions of stock solutions in serum was maximum 5%. All dilutions of stock solutions were performed with 50% ethanol.

2.7. Method validation

2.7.1. Specificity

Specificity was evaluated in three ways. A: post-column infusion of analytes and injection of extracted blank samples from five different donors was performed and evaluated visually. B: 20 samples from patients referred to our laboratory for TDM were spiked at levels corresponding to the lower end of the reference interval for each compound. The latter investigation aimed to evaluate the interference or ion suppression from other drugs, as well as different preanalytical procedures. The variation and bias for this experiment should be below 15%. C: recovery and ion suppression were estimated by pre- and postextraction addition of analytes and internal standards. Blank serum was extracted as samples except that the precipitation solvent 0.1% formic acid in methanol did not contain SIL-IS. The resulting supernatant was spiked with analyte and internal standard in concentrations corresponding to serum levels in the middle of the measurement range. The added volume comprised 2% of the total volume of the supernatant. The spiked supernatant (post-extraction) was then analysed using UHPLC–MS–MS, and areas of the analytes were compared to areas of a spiked serum sample (pre-extraction) and analytes diluted in mobile phase (pure) at corresponding levels. From this experiment extraction recovery, ion suppression and process efficiency could be estimated [12]. The experiment was performed with three preparations and analysed over two days ($n = 11$). Finally, the effects of icteric, haemolytic, and lipemic serum on the quantification were investigated by spiking a serum pool with all analytes at levels corresponding to the LLOQ. Different aliquots of this serum pool were then spiked with bilirubin, haemolysate or intralipid 20% in varying concentrations according to Glick et al. [13].

2.7.2. Precision, trueness, calibration and measurement range

Precision and trueness were estimated at three levels (Table 3) by measuring four repetitions at five days ($n = 20$). Precision values less than 15% and trueness values within the range of 85–115% determined the measurement range except for the lower limit of quantification (LLOQ), which was the level that had a precision less than 20% and trueness between 80 and 120%. Precision and trueness investigations also validated the applied standard curve fit.

2.7.3. Limit of detection and carry-over

Limit of detection (LOD) was determined by the method described by Linnet and Kondratovich [14]. Five different blanks and five different spiked serum samples at sub-LLOQ levels were analysed in four repetitions in five days ($n = 20$). Carry-over was measured by running a blank serum sample after a spiked serum sample (level = ULOQ) in three repetitions. Area ratios had to be less than 1%. The concentrations in the blank serum samples had to be less than 20% of the determined LLOQ.

2.7.4. Stability and method comparison

Stability of spiked serum was evaluated at 3, 6, and 12 months. Spiked serum was compared to a freshly prepared spiked serum by analysis as patient samples over two days. If the difference between old and new spiked serum was less than 15 ± 2 SEM% (SEM: standard error of the mean difference), the stability was acceptable.

The new method was compared to old existing HPLC and LC–MS–MS methods by running approximately 50 patient and external quality control samples.

All calculations were performed in Microsoft Excel 2007 and CBstat version 4.3.2 (www.cbstat.com).

3. Results and discussion

3.1. Precipitation and chromatography

Precipitation was carried out with a 10% zinc sulphate heptahydrate solution (w/v) corresponding to 0.35 M. Precipitation by 10, 1 and 0.1% zinc sulphate was evaluated visually and by UHPLC–MS–MS analysis (results not shown). Generally, 0.1% zinc sulphate resulted in a higher response than 1 and 10%, but made the supernatant unclear and increased the backpressure on the column. One percent zinc sulphate compared to 10% resulted in a better response for clozapine, but lower response for clomipramine and desmethylclomipramine. Therefore, 10% zinc sulphate was chosen as precipitant. There was no difference between 1% and 10% zinc sulphate in ion suppression evaluated by post-column infusion or in the amount of precipitate. Extraction recoveries and the process efficiency ranged from 65% to 104% and 42% to 99%, respectively (Table 3).

The initial mobile phase composition and the gradient were adjusted to elute the analytes of the column between 1 and 2 min (Fig. 1). Fig. 1 also displays a blank chromatogram. The Zorbax SB-C8 column and the mobile phase consisting of water and methanol both with 0.1% formic acid resulted in good peak shape and adequate retention for all analytes including polar metabolites.

3.2. Specificity

The solvent front eluted approximately 0.35 min after the injection, and most of the ion suppressive matrix components eluted between the solvent front and 1 min. The post-column infusion experiment for both chromatographies showed no sudden changes in ion suppression although clozapine, citalopram, mirtazapine and O-desmethylvenlafaxine obviously eluted together with matrix components (Fig. 1). The pre- and postextraction experiment confirmed the ion suppression for clozapine (52%), citalopram (22%) and mirtazapine (25%) (Table 3). Ion suppression of O-desmethylvenlafaxine was estimated to only 7%, which is contrary to the observation in Fig. 1. On the other hand, ziprasidone

Table 3
Validation results of precision, trueness, extraction recovery, ion suppression and process efficiency.

Analyte	Level							Ref. int.[c] (nmol/l)	Recovery (%)	Ion sup.[d] (%)	Proc. eff.[e] (%)
	LLOQ[a]	2	3	4	5	6	ULOQ[b]				
Group 1											
Mirtazapine											
Concentration (nmol/l)	3.8	15.0	37.5	150	300	600	1200	50–350	101	25	76
Precision within run (C.V.[g]%)	7.7	5.5	3.0	4.3	2.7	5.7	4.2				
Precision total (C.V.%)	10.3	6.3	4.2	4.3	3.1	6.3	5.7				
Trueness	111.5	98.4	95.4	90.4	95.7	90.3	96.8				
O-desmethylvenlafaxine											
Concentration (nmol/l)	33.8	84.4	337.5	675	1350	2700	5400	300–2700[f]	103	7	96
Precision within run (C.V.%)	3.8	4.6	2.9	3.6	4.0	3.4	3.8				
Precision total (C.V.%)	8.2	6.5	5.4	4.1	4.0	3.9	5.1				
Trueness	98.3	99.5	94.7	101.0	94.3	99.2	99.4				
Quetiapine											
Concentration (nmol/l)	12.5	50	125	500	1000	2000	4000	50–650	95	7	88
Precision within run (C.V.%)	6.8	4.3	4.0	4.9	4.8	5.0	4.7				
Precision total (C.V.%)	10.0	6.9	6.2	5.3	4.8	5.0	5.0				
Trueness	109.6	96.1	100.0	98.1	103.5	96.8	102.0				
Venlafaxine											
Concentration (nmol/l)	8.4	33.8	84.4	337.5	675	1350	2700	300–2700[f]	104	5	99
Precision within run (C.V.%)	5.1	3.6	2.6	3.4	3.8	3.7	3.5				
Precision total (C.V.%)	6.5	4.4	3.4	3.4	3.8	3.9	5.1				
Trueness	95.6	90.8	89.2	87.8	93.4	91.4	97.6				
Ziprasidone											
Concentration (nmol/l)	4.7	18.8	46.9	187.5	375	750	1500	50–300	70	18	52
Precision within run (C.V.%)	10.8	7.3	4.6	4.3	4.0	4.2	3.3				
Precision total (C.V.%)	16.8	9.1	6.1	6.1	4.9	5.5	4.2				
Trueness	120.9	100.4	92.5	89.3	92.7	95.7	101.2				
Group 2											
Amitriptyline											
Concentration (nmol/l)	5	20	100	400	900	1800	3600	400–900[f]	90	22	69
Precision within run (C.V.%)	13.4	7.9	5.4	5.5	5.0	5.0	3.2				
Precision total (C.V.%)	15.8	8.9	7.9	5.5	7.1	5.2	4.8				
Trueness	109.7	99.3	92.7	95.0	100.1	93.7	100.5				
Citalopram											
Concentration (nmol/l)	20	50	100	400	900	1800	3600	100–400	96	22	74
Precision within run (C.V.%)	4.5	3.6	4.9	5.2	5.5	5.7	2.4				
Precision total (C.V.%)	9.3	8.8	7.6	5.2	6.0	5.7	2.6				
Trueness	109.1	98.5	98.0	97.2	100.6	92.9	98.9				
Clomipramine											
Concentration (nmol/l)	20	50	100	400	900	1800	3600	250–1200[f]	72	5	66
Precision within run (C.V.%)	5.9	3.6	4.1	6.5	4.3	5.0	3.4				
Precision total (C.V.%)	17.9	14.9	12.9	7.9	9.8	7.7	6.5				
Trueness	105.5	94.8	97.2	98.0	101.1	92.9	98.1				
Clozapine											
Concentration (nmol/l)	75	125	500	–	1500	3000	4500	300–1200	95	52	42
Precision within run (C.V.%)	3.6	4.1	6.5	–	4.3	5.0	3.4				
Precision total (C.V.%)	17.1	13.0	7.7	–	6.3	5.0	3.9				
Trueness	89.5	88.7	95.6	–	98.9	101.4	100.2				
Desmethylclomipramine											
Concentration (nmol/l)	100	400	–	–	900	1800	3600	250–1200[f]	65	12	53
Precision within run (C.V.%)	7.4	4.3	–	–	5.8	6.0	3.8				
Precision total (C.V.%)	16.7	8.7	–	–	10.1	6.9	5.3				
Trueness	100.6	96.7	–	–	101.2	92.6	96.9				
Desipramine											
Concentration (nmol/l)	20	50	100	400	900	1800	3600	500–1200[f]	81	12	69
Precision within run (C.V.%)	5.3	5.0	5.7	5.3	5.0	4.6	2.4				
Precision total (C.V.%)	12.9	10.4	11.4	6.0	8.6	6.3	6.0				
Trueness	92.3	87.6	91.3	91.3	98.3	91.7	100.0				
Imipramine											
Concentration (nmol/l)	20	50	100	400	900	1800	3600	500–1200[f]	85	−1	87
Precision within run (C.V.%)	3.8	3.1	5.3	5.3	5.0	4.4	2.7				
Precision total (C.V.%)	16.4	12.8	10.8	6.2	6.8	4.5	3.8				
Trueness	93.8	89.6	91.8	93.8	98.8	92.4	98.4				
Nortriptyline											
Concentration (nmol/l)	20	50	100	400	900	1800	3600	200–600	79	13	66
Precision within run (C.V.%)	9.1	4.5	5.7	4.8	5.2	5.5	3.1				
Precision total (C.V.%)	9.1	6.4	7.9	5.1	7.1	5.8	6.0				
Trueness	97.7	88.6	91.6	91.0	96.6	90.6	97.4				

[a] LLOQ = lower limit of quantification.
[b] ULOQ = upper limit of quantification.
[c] Ref. int. = reference interval.
[d] Ion sup. = ion suppression.
[e] Proc. eff. = process efficiency.
[f] Sum of parent compound and metabolite.
[g] C.V. = coefficient of variation.

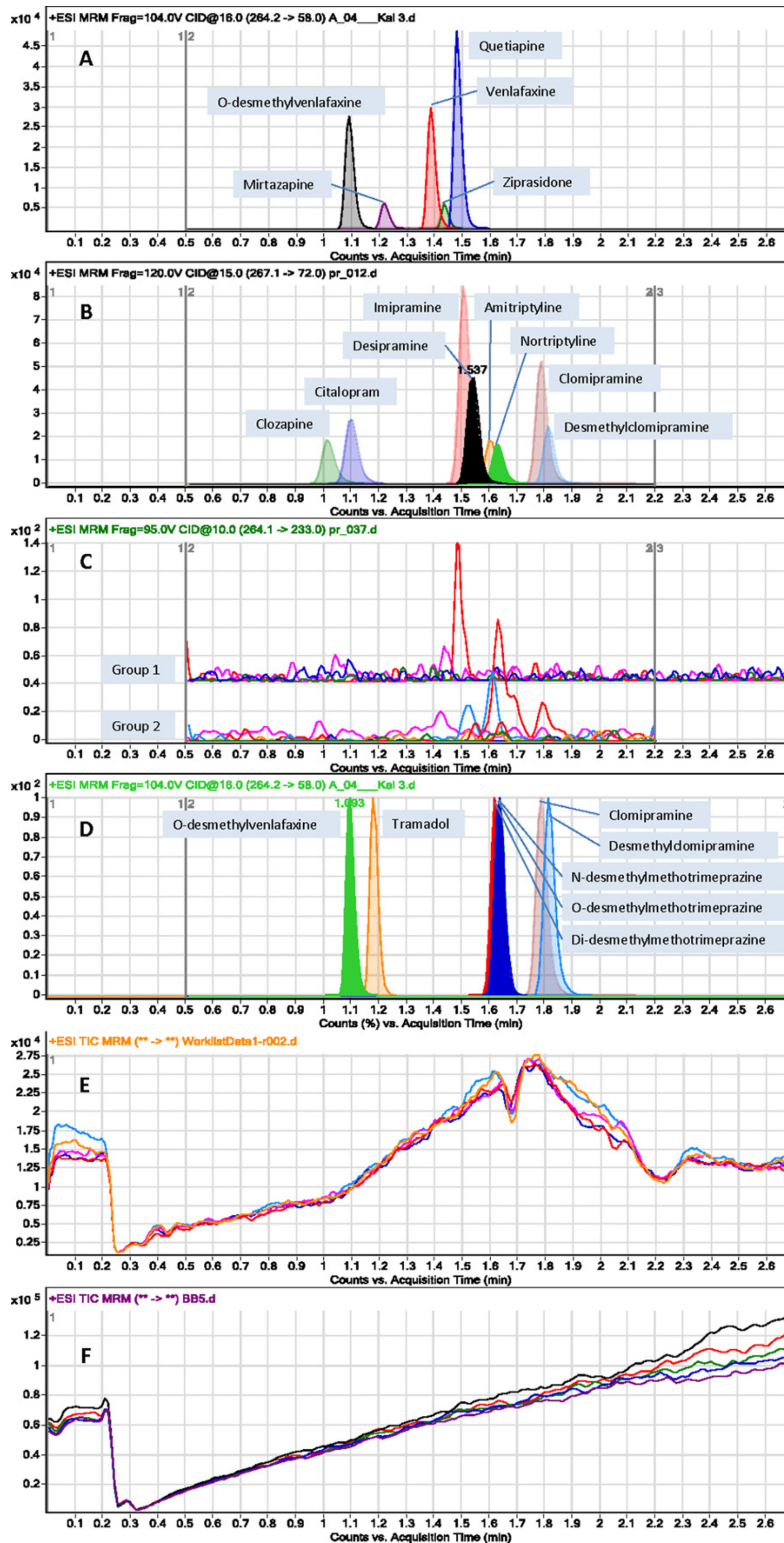

Fig. 1. (A) Extracted ion chromatograms of standard three for group 1. (B) Extracted ion chromatograms of standard three for group 2. (C) Extracted ion chromatograms of blank serum samples for group 1 and 2. (D) Overlayed chromatograms of O-desmethylvenlafaxine and tramadol (group 1) and di-desmethylmethotrimeprazine, N-desmethylmethotrimeprazine, O-desmethylmethotrimeprazine, clomipramine, and desmethylclomipramine (group 2). (E) Ion suppression profile based on post-column infusion for group 1. (F) Ion suppression profile based on post-column infusion for group 2.

and amitriptyline were late-eluting, and the ion suppression profiles did not display any specific ion suppression in the regions (Fig. 1). Still ziprasidone and amitriptyline were suppressed by 18% and 22%, respectively (Table 3). Extracting the MRM transition of ziprasidone and amitriptyline from the post-column infusion experiment did not reveal any explanation. The rest of the ion suppression ranged from −1% to 13%, i.e. no ion enhancement took place. There were no effects of icteric, haemolytic, and lipemic serum on the quantification of the analytes.

3.3. Calibration

Our usual test of linearity of the standard-curve that statistically compares a linear fit with a quadratic fit failed for most analytes which displayed quadratically increasing standard curves. Several functions for curve fit were tested, and a power function ($y = ax^b$) forced through zero, and no weighting was chosen for all analytes. The calibration was validated by precision and trueness investigations. Two explanations for the quadratic increasing standard curve were investigated: the delta electron multiplier voltage (dEMV) and ion suppressive effects between analyte and SIL-IS. Changing the dEMV settings had no effect.

Ion suppressive effects between analytes and their respective SIL-IS were observed. Especially high analyte concentrations suppressed the response of the SIL-IS, leading to quadratically increasing standard curves. Thus several calibration points were needed to describe the non-linear shape. To minimize these suppressive effects, the concentration of each SIL-IS was adjusted to give a response comparable to the response of an analyte concentration in the middle of the measurement range, as suggested by Liang et al. [15] and Sojo et al. [16].

3.4. Mass spectrometry

Ion source parameters, such as gas flow, gas temperature, nebulizer pressure and capillary voltage were optimized to the mobile phase flow of 0.6 ml/min using nortriptyline as a model compound. Fragmentor voltage and collision energy were optimized for each analyte and are listed in Table 2 along with the mass transitions.

The isobaric compounds found in this study were base-line separated and could easily be discriminated by retention time. Tramadol produced a signal in the O-desmethylvenlafaxine multiple reaction monitoring (MRM) transition (Fig. 1), and amitriptyline produced a small signal in the venlafaxine MRM transition. The metabolites of methotrimeprazine N- and O-desmethylmethotrimepramine produced signals in the clomipramine MRM transition and di-desmethylmethotrimepramine produced a signal in the desmethylclomipramine MRM transition as reported by Sauvage et al. [17]. All three metabolites were well separated from clomipramine and desmethylclomipramine (Fig. 1). Tramadol and O-demethyl venlafaxine have similar MRM transitions, which have been reported previously [18]. Generally fragments of m/z 58, 72 and 86 should be avoided if possible [17], but these fragments were chosen in order achieve adequate sensitivity.

3.5. Precision and trueness

Precision and trueness were estimated on the basis of 20 repetitions of each level divided into four series (Table 3). The determined LLOQs and ULOQs are listed in Table 3, thus indirectly an approval of the applied calibration. For group one precision and trueness in the range of measurement were 3.1–6.9% (LLOQ: <10.3%) and 89.2–103.5% (LLOQ: 95.6–111.5%), respectively. For group two precision and trueness in the range of measurement were 2.6–14.9% (LLOQ: <17.9%) and 87.6–101.4% (LLOQ: 89.5–109.7%), respectively. The precision and trueness data are similar to other published methods [3,5,9,10]. Checking robustness by looking at routine quality controls on two levels confirmed the validation data.

The LOD ranged between 1.0 and 5.5 nmol/l, i.e. much lower than the determined LLOQ. In other words, the UHPLC–MS–MS system offers more sensitivity than needed, but the precision and trueness of the method were not acceptable for most analytes at those levels. Carry-over was less than 0.06% of the ULOQ for all analytes and less than 20% of the determined LLOQ. Stability in serum was 12 months for all analytes.

3.6. Method comparison

Method comparisons between the new and old methods were carried out. The old methods comprised HPLC and LC–MS–MS methods with protein precipitation or solid phase extraction. Both external quality controls and patient samples were compared, and all analytes displayed a bias <15% (results not shown), i.e. within the demands of trueness.

In conclusion, the 4 min run time makes it possible to run 360 injections in 24 h. Manual preparation of 96 samples takes approximately 1 h. With this new method, response times and interference from other drugs are reduced, acetonitrile usage is avoided, and the amount of serum used for analysis is decreased by up to a factor of 50. Technician time can be redirected from the manual and laborious handling of sample extraction towards qualified evaluation of analysis quality, pharmacokinetic counselling, and development of new analytical methods.

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References

[1] P. Baumann, C. Hiemke, S. Ulrich, G. Eckermann, I. Gaertner, M. Gerlach, H.J. Kuss, G. Laux, B. Muller-Oerlinghausen, M.L. Rao, P. Riederer, G. Zernig, Pharmacopsychiatry 37 (2004) 243.
[2] A.S. Gross, Br. J. Clin. Pharmacol. 52 (Suppl. 1) (2001) 5S.
[3] A.R. Breaud, R. Harlan, M. Kozak, W. Clarke, Clin. Biochem. 42 (2009) 1300.
[4] E. Choong, S. Rudaz, A. Kottelat, D. Guillarme, J.L. Veuthey, C.B. Eap, J. Pharm. Biomed. Anal. 50 (2009) 1000.
[5] H. Kirchherr, W.N. Kuhn-Velten, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 843 (2006) 100.
[6] H. Juan, Z. Zhiling, L. Huande, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 820 (2005) 33.
[7] Z. Zhou, X. Li, K. Li, Z. Xie, Z. Cheng, W. Peng, F. Wang, R. Zhu, H. Li, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 802 (2004) 257.
[8] U. Gutteck, K.M. Rentsch, Clin. Chem. Lab Med. 41 (2003) 1571.
[9] A.R. Breaud, R. Harlan, J.M. Di Bussolo, G.A. McMillin, W. Clarke, Clin. Chim. Acta 411 (2010) 825.
[10] A. de Castro, M.M. Ramirez Fernandez, M. Laloup, N. Samyn, B.G. De, M. Wood, V. Maes, M. Lopez-Rivadulla, J. Chromatogr. A 1160 (2007) 3.
[11] F.L. Sauvage, J.M. Gaulier, G. Lachatre, P. Marquet, Ther. Drug Monit. 28 (2006) 123.
[12] D.L. Buhrman, P.I. Price, P.J. Rudewicz, J. Am. Soc. Mass Spectrom. 7 (1996) 1099.
[13] M.R. Glick, K.W. Ryder, S.A. Jackson, Clin. Chem. 32 (1986) 470.
[14] K. Linnet, M. Kondratovich, Clin. Chem. 50 (2004) 732.
[15] H.R. Liang, R.L. Foltz, M. Meng, P. Bennett, Rapid Commun. Mass Spectrom. 17 (2003) 2815.
[16] L.E. Sojo, G. Lum, P. Chee, Analyst 128 (2003) 51.
[17] F.L. Sauvage, J.M. Gaulier, G. Lachatre, P. Marquet, Clin. Chem. 54 (2008) 1519.
[18] K.R. Allen, Clin. Toxicol. (Phila) 44 (2006) 147.