Multi-analyte LC-MS/MS quantification of 38 antipsychotics and metabolites in plasma : method validation & application to routine analyses

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Abstract

The past decades have seen a rise in the prescription of antipsychotic drugs in the European population, despite the risk of extra-pyramidal, metabolic and cardiac side effects. A multi-analyte liquid chromatography – triple quadrupole mass spectrometry method was developed for the quantification of 38 antipsychotic drugs in plasma. Samples were extracted by a straightforward liquid-liquid extraction with methyl-tertiary-butyl-ether and the compounds of interest were chromatographically separated within 6 minutes. Calibration curves covered the recommended therapeutic range for all compounds, in addition to sub- and supratherapeutic concentrations for most. The method was successfully validated according to the European Medicines Agency guidelines on bioanalytical method validation. Analysis of medico-legal samples confirmed the relatively common use of the second generation antipsychotics quetiapine and olanzapine, as well as the continued presence of the first generation antipsychotic haloperidol.

Keywords

- Antipsychotics
- Liquid chromatography - tandem mass spectrometry
- Therapeutic drug monitoring
- Forensic toxicology

Abbreviations

Acetonitrile, ACN; autosampler stability, AS; benchtop stability, BT; calibrator level, CAL L; coefficient of variation, CV; collision energy, CE; compound of interest, cpd; European Medicines Agency, EMA; extraction efficiency, EE; first generation antipsychotic, FGA; fragmentor voltage, FV; freeze-thaw

stability, FT; labelled internal standard, ISTD; liquid chromatography, LC; long-term stability, LT; lower limit of quantification, LLOQ; matrix effect, ME; methyl-tertiary-butyl-ether, MTBE; quality control, QC; retention time, RT; second generation antipsychotic, SGA; triple quadrupole mass spectrometry, QQQ; upper limit of quantification, ULOQ.

1 Introduction

Developed in the early 1950s, chlorpromazine showed high potential in the treatment of psychiatric disorders, mainly those related to schizophrenia. This discovery led to the emergence of the first generation antipsychotics (FGAs), all of which are derived from chlorpromazine's phenothiazine core. Second generation antipsychotics (SGAs) were later developed to overcome the extra-pyramidal side-effects associated with the FGAs. However, studies on the occurrence of tardive dyskinesia and anticholinergic medication prescriptions suggest only a minor improvement on the risk of movement disorders for SGAs compared to FGAs [1,2]. SGAs also hold an increased risk for metabolic and cardiac side effects. Nonetheless, placebo-controlled trials highlight the beneficial effect of SGAs on symptoms and quality of life, and the number of patients prescribed antipsychotics is growing worldwide. This rise in numbers is also well-documented for paediatric patients. Their increasing off-label use mainly consists of the treatment of anxiety and insomnia disorders in adults and of ADHD in children [3–5].

The above mentioned undesirable side effects are often aggravated by long-term antipsychotic use or co-prescription with other psychotropic medication. This, together with the increased paediatric use, necessitates strict therapeutic drug monitoring of patients. From a forensic perspective Pelletti et al. showed that daytime traffic accidents are more often caused by psychotropic medication (in combination with alcohol) rather than illicit drugs [6,7]. Of the methods published in literature for clinical or forensic purposes, many only analyse a limited number of antipsychotics [8–12]. More comprehensive methods often have long sample preparation procedures or analysis times, or use less readily available detectors [13–16]. Remane et al. published a noteworthy multi-analyte method spanning multiple drug classes. However, the authors stated that the sensitivity was insufficient to quantify some of the lower therapeutic concentrations and running multiple calibration curves was necessary to account for significant ion enhancing/suppressing effects of co-eluting compounds [17].

This research aimed to address the aforementioned issues by offering a fully validated, quantitative, multi-analyte liquid chromatography (LC) – triple quadrupole mass spectrometry (QQQ) method for use in therapeutic drug monitoring and forensic analyses. The previously published method by Patteet et al. was expanded to include a further 14 analytes (n = 38) [18]. For their applicability in a routine setting, relevant LC and QQQ parameters were optimised to match those of our previously published methods for antidepressants and benzodiazepines [19,20].

2 Materials and methods

2.1 Chemicals and stock solutions

Chemical reference standards were purchased as either powders or methanolic solutions. 7-OH-norquetiapine, 7-OH-norquetiapine-D_8, 7-OH-quetiapine, 7-OH-quetiapine-D_8, amisulpride, amisulpride-D_5, aripiprazole, aripiprazole-D_8, asenapine, asenapine-^{13}C-D_3, bromperidol, clotiapine, clotiapine-D_8, clozapine, clozapine-D_8, dehydro-aripiprazole, dehydro-aripiprazole-D_8, droperidol, flupentixol-D_4, haloperidol, haloperidol-D_4, iloperidone, iloperidone-D_3, levosulpiride, loxapine, loxapine-D_8, lurasidone, lurasidone-D_8, norasenapine, norclozapine, norclozapine-D_8, norolanzapine, norolanzapine-D_8, norquetiapine, norquetiapine-D_8, OH-iloperidone, OH-iloperidone-D_4, olanzapine, olanzapine-D_3, paliperidone, paliperidone-D_4, pimozide, pipamperone, quetiapine, quetiapine-D_8, reduced haloperidol, reduced haloperidol-D_4, risperidone, risperidone-D_4, sertindole, sertindole-D_4, tiapride, zuclopenthixol and zuclopenthixol-D_4 were provided by Toronto Research Chemicals (Toronto, ON, CA). Chlorpromazine, fluspirilene and prothipendyl-D_6 were supplied by Sigma Aldrich International GmbH (St. Gallen, CH), flupentixol by H. Lundbeck A/S (Copenhagen, DK), fluphenazine

by Sanofi (Paris, FR), levomepromazine and prothipendyl by LGC Standards (Teddington, UK) and perphenazine by Merck KGaA (Darmstadt, DE).

Merck KGaA (Darmstadt, DE) also provided formic acid, methyl-tertiary-butyl-ether (MTBE), potassium bicarbonate and potassium hydroxide. Acetonitrile (ACN) was acquired from Fisher Scientific (Loughborough, UK). Ultrapure water was produced in-house using an Elga Purelab water purification system from Veolia Water Technologies (Tienen, BE).

Seven calibrator levels (CAL L1-7, Table S1) were prepared in ACN at ten times the intended plasma concentrations. Calibration curves spanned at least the concentration range expected in blood following commonly administered therapeutic doses of the compounds of interest (cpds; Table S2). The lowest calibrator was chosen based upon recommended minimal concentrations for therapeutic drug monitoring. For the other compounds, calibration ranges were based upon blood concentrations found in literature case reports, or in relation to other analytes. [6,21–23]. Similarly, four quality control solutions (QC LLOQ, low, mid and high) and a mix of all labelled internal standards (ISTDs) were prepared in ACN at ten times the in-sample concentration.

2.2 Sample preparation and instrumental analysis

Blank blood samples were collected from healthy, drug-free volunteers in 9 mL Vacuette® K_2EDTA tubes (Greiner Bio One International GmBH, Kremsmünster, AT). Samples were centrifuged immediately after collection and the plasma was stored at -20 °C. The samples were not pooled in order to account for inter-individual variability during the validation. The donation was approved by the ethical committee of the University Hospital Antwerp (EC/PC/avl/2018.039).

20 µL CAL and ISTD mix (or ACN and ISTD mix for real samples) were spiked to 200 µL blank (or case) plasma. Compounds of interest were subsequently extracted by liquid-liquid extraction using 65 µL carbonate buffer (pH 9.5) and 800 µL MTBE. Following mixing and centrifugation, the upper layer was evaporated to dryness and reconstituted in 25 µL ACN; 1 µL was injected into the chromatographic system.

The cpds and ISTDs were separated on a Zorbax Eclipse Plus C8 column (2.1 x 150 mm, 3.5 µm) using an Agilent 1290 Infinity LC system (Agilent Technologies, Santa Clara, California, US). The mobile phases consisted of A) water + 0.1% formic acid (V/V) and B) ACN:water (9:1) + 0.1% formic acid (V/V) at a flow rate of 0.5 mL/min. The gradient increased from 5% to 95% mobile phase B in 9 min, leading to a total run time of 12 min from injection to injection. A dynamic multiple reaction monitoring method, specific for 38 cpds and 26 ISTDs, was optimised on an Agilent 6460 triple quadrupole mass spectrometer. The detector was operated using Jetstream electrospray ionisation in positive mode. Other MS parameters were: gas (N_2) temperature: 300 °C, sheath gas flow: 11 L/min, nebuliser pressure: 45 psi, capillary voltage: 3500 V, and nozzle voltage: 500 V. Where possible, three transitions were monitored for cpds and two for ISTDs. Retention time windows were set to ± 0.25 min. An overview of the QQQ parameters can be found in Table 1.

2.3 Validation parameters

The method was validated according to the guidelines on Bioanalytical Method Validation as published by the European Medicines Agency (EMA, Table S3) [24]. For those compounds where no deuterated analogue was available as ISTD, the different validation parameters were investigated using both a structurally related and a retention time matched ISTD.

3 Results and discussion

3.1 Method validation

3.1.1 Selectivity, matrix effects and extraction efficiency

The uniqueness of the transitions was verified against the other cpds and ISTDs, as well as against twelve blank plasma samples and mixtures of commonly co-prescribed medications: 38 antidepressants or their metabolites (+ 32 labelled analogues) and 54 benzodiazepines or their metabolites (+ 20 labelled analogues). No interferences were observed at the specific retention times of the cpds. The liquid-liquid extraction with MTBE was previously reported to be time efficient, easy to use and highly efficient in extracting basic compounds from plasma [20]. For the antipsychotics included in this method, average extraction efficiencies were 76% ± 9% (Figure 1). Only levosulpiride (49% ± 9%), olanzapine (50% ± 12%) and norolanzapine (41% ± 12%) experienced lower but reproducible recoveries, none of which affected the LLOQ. Furthermore, the sample preparation and chromatographic settings were highly efficient at removing matrix interferences, as can be derived from the overall absence of ion suppression or enhancement (average matrix factors 0.98 ± 0.07 and 0.97 ± 0.04 for QC low and QC high respectively, Figure 1). No significant differences were observed between absolute and ISTD corrected matrix factors, nor was a coefficient of variation (CV) greater than ± 15% observed (n = 6). The aberrant values for asenapine (0.83 ± 0.20 for QC low) and norasenapine (0.99 ± 0.26 for QC low and 0.56 ± 0.22 for QC high) are likely linked to a stability issue rather than being related to the sample preparation (see section 3.1.3 below).

3.1.2 Calibration range, accuracy and precision

The most simple yet adequate relationship between the CAL samples for each of the cpds was investigated based on twelve calibration curves, each spiked in plasma from a different source. Calibration curves were linear for all but 10 cpds, with a weighing factor of $1/x^2$ for most (Table S1). Where multiple ISTDs were investigated, the method seemed to favour a structurally related over a retention time matched one, although no significant differences were noted. Accuracies and precisions, both within and between batches, did not exceed ± 15% (± 20% at LLOQ) over the entire calibration range (Table S4), as stipulated in the EMA guidelines. Based upon six replicates, the average within-run accuracy and precision was 111% ± 4% at LLOQ, 100% ± 3% for QC low, 103% ± 3% for QC mid and 102% ± 3% for QC high. Between batches, these values were 111% ± 9%, 100% ± 7%, 103% ± 6% and 101% ± 6%, respectively. CAL L1 had to be excluded for asenapine and its demethylated metabolite due to a previously reported sensitivity issue [18]. Carry-over was not observed for any of the cpds. With the exception of olanzapine, where the average back calculated concentration of 128% ± 5% exceeded the ± 15% requirements for accuracy and precision, a dilution factor of 10 could be reliably applied to samples with a concentration higher than that of CAL L7 (average accuracy and precision 98% ± 4%). To further ensure the accuracy of the method, its performance was successfully evaluated against externally acquired quality control samples (Figure S2).

3.1.3 Stability

Stability was assessed throughout the different steps of the sample preparation and analysis, as well as for different storage conditions. Based upon four replicates at two concentration levels (QC low and QC high), variations within ± 15% of the nominal concentration were deemed acceptable. The stability of spiked samples was investigated both at ambient temperature and at -20 °C (Figure S1). In unextracted samples the cpds were stable for at least 3 hours at room temperature (average bench-top stability 99% ± 6% for QC low, 99% ± 5% for QC high). In their frozen state, unextracted samples could be kept for a minimum of 3 months (average long-term stability 99% ± 12% for QC low, 97% ± 7% for QC high), with the exception of iloperidone (77% ± 29% for QC low at 3 months), norolanzapine

(54% ± 61% for QC low and 83% ± 9% for QC high at 3 months) and perphenazine (84% ± 6% for QC low and 80% ± 9% for QC high at 3 months). The concentrations of the latter three compounds are guaranteed for up to 1 month. Samples could also be confidently thawed and re-frozen three times (average freeze-thaw stability 106% ± 10% for QC low and 96% ± 4% for QC high), with the exception of two times for lurasidone (average relative concentration 123% ± 63% for QC low after the third thawing cycle). Once extracted and reconstituted in ACN, all compounds were stable for 72 hours on the autosampler at ambient temperature (average autosampler stability 98% for both QC low and QC high), except clotiapine which dropped to 77% of the nominal concentration after 72 hours.

Asenapine showed considerable instability under most of the conditions tested for, particularly at lower concentrations. Acceptable benchtop stabilities were found for QC low and QC high. During long-term storage at -20 °C, the calculated concentrations for QC low and QC high respectively averaged 124% ± 39% & 99% ± 8% after 1 week, 112% ± 15% & 112% ± 9% after 1 month and 203% ± 47% & 125% ± 29% after 3 months. Consecutive freeze-thaw cycles further increased its instability, resulting in relative respective average QC low and QC high concentrations of 127% ± 14% & 100% ± 7% after one cycle, 111% ± 6% & 90% ± 12% after two cycles and 421% ± 17% & 37% ± 14% after 3 cycles. Lastly, after 24 h, 48 h and 72 h on the autosampler, QC low and QC high concentrations respectively averaged 48% & 93%, 48% & 89% and 79% & 80% compared to their nominal concentrations. Norasenapine only deviated from the acceptability criteria for its long-term and freeze-thaw stability. Long-term storage of over 1 month is not advised as the average relative concentrations increased to 153% ± 53% for QC low and to 119% ± 40% for QC high. For samples spiked at QC low, average relative concentrations of 88% ± 41%, 121% ± 35% and 322% ± 43% were found after one, two and three consecutive thawing cycles, respectively. For QC high, these results were 83% ± 9%, 85% ± 11% and 8% ± 18%, respectively. Besides these both compounds' significant deviations of the calculated concentrations from those expected, the high CVs indicate an additional issue besides in-sample degradation. Indeed, preliminary data by Feng et al. suggest that neutral and basic conditions (the pH was raised to 9.5 in our sample preparation) may poorly affect the stability of asenapine. Their hypothesis is supported by Ansermot et al., who report excellent stability of asenapine under all conditions after reconstitution in an acidified mobile phase (pH 3.0) [8,25].

3.1.4 Rationale for an updated method

The previously published method by Patteet et al. served as starting point for the current method [18]. Developed for therapeutic drug monitoring, this quantitative LC-QQQ method was applicable for 24 commonly used (mainly second generation) antipsychotics or their metabolites. The sample preparation consisted of a liquid-liquid extraction using 200 µL serum and 1 mL MTBE. The final extract (in ACN) was injected onto an Agilent SB C18 column (2.1 × 50 mm, 1.7 µm). 10 mM aqueous ammonium acetate and acetonitrile were used as mobile phase A and B, respectively. The current method expanded the number of cpds to 38, including more FGAs, as well as less commonly used SGAs, several of which could be detected in our routine forensic analyses (see section 3.2 below). The LC and QQQ settings were also updated to match those of the quantitative methods for antidepressants and benzodiazepines [19,20]. This will greatly enhance throughput for routine analysis as no switching of LC columns or mobile phases is required. Due to the aforementioned absence of interference by cpds and/or ISTDs from the other methods, the three methods could easily be combined. The latter holds the potential to act as a multi-analyte (n = 130) screening method for different drug classes, or even as a quantitative method, although LLOQs and accuracy/precision might need to be re-evaluated.

3.2 Application to routine analyses

137 medico-legal cases that screened positive for the presence of psychoactive substances through GC-MS and/or LC-DAD were analysed with the current method. A third of them (n = 46) were positive for one or more antipsychotic drug, leading to a total of 176 cpds identified (Table 2). The calibration curves generally covered the concentration range of the samples. The majority of cases were positive for quetiapine (and its metabolites), the most commonly prescribed antipsychotic drug throughout Europe. Besides its approved use, quetiapine is also administered off-label in the treatment of anxiety and insomnia in adults [3,26–28]. The next most detected compound was olanzapine, which could be quantified in 7 cases, together with its demethylated metabolite. With only 1 and 2 samples positive for risperidone/paliperidone and aripiprazole (a so-called third generation AP), respectively, these drugs seemed less prevalent than expected from the literature. However, these compounds are mainly prescribed for paediatric use, an age group usually underrepresented in medico-legal cases [3,4,27]. Haloperidol (6 positive samples) was the most detected FGA. Recent safety warnings on its involvement in QT_c prolongation are reported to have caused a steady decrease in its use [27]. Other FGAs present were flupentixol (n = 1), zuclopenthixol (n = 2) and levomepromazine (n = 1), all of which are currently approved for use in Belgium. A quarter of the detected cpds were not included in the original method by Patteet et al. (Table 2) [18].

Confidence in the measured results was continuously evaluated by participating in proficiency testing schemes by the Gesellschaft für Toxikologische und Forensische Chemie [Society of Toxicological and Forensic Chemistry] (Arvecon GmbH, Walldorf, DE). 157 cpds were successfully identified and, apart from one clozapine result, quantified with acceptable z-scores (Figure S3).

4 Conclusion

The described method offers a fully validated, time efficient, quantitative, multi-analyte (n = 38) method for therapeutic drug monitoring and forensic analysis of first, second and third generation antipsychotics in plasma. Asenapine and its metabolite may exhibit reduced stability and suspected positive samples should be analysed upon the earliest convenience. The sample preparation and chromatographic settings were previously successfully applied in the quantification of other psychoactive drug classes, allowing for easy transitioning between or merging of methods. The method was further applied to over 100 medico-legal cases and its performance was thoroughly evaluated through proficiency testing schemes.

5 Conflict of Interest

The authors declare that they have no conflicts of interest.

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Figures

Figure 1. Matrix effects (ME) and extraction efficiencies (EE). *The mean value is plotted with its standard deviation (error bars), based on six replicates per concentration level. Values lower than 100% indicate loss of cpds (EE) or ion suppression (ME), higher values indicate ion enhancement (ME).*

Tables

Table 1. Dynamic multiple reaction monitoring settings. *The ion ratios (between brackets) are relative to that of the quantifier ion (underlined). Collision energy, CE; fragmentor voltage, FV; retention time, RT.*

Compound	Precursor ion (*m/z*)	FV (V)	Product ions (*m/z*)	CE (V)	RT (min)
7-OH-norquetiapine	312.1	172	226.0 (100%)	26	2.00
			164.0 (82.5%)	62	
			208.0 (62%)	38	
7-OH-norquetiapine-D_8	320.2	172	226.0 (100%)	26	1.97
			164.0 (78.5%)	62	
7-OH-quetiapine	400.2	172	269.0 (100%)	18	2.30
			237.1 (16%)	42	
			295.0 (14%)	22	
7-OH-quetiapine-D_8	408.2	196	274.1 (100%)	22	2.26
			302.1 (30.5%)	26	
amisulpride	370.2	188	242.0 (100%)	26	2.52
			196.0 (46%)	42	
			112.1 (34%)	22	
amisulpride-D_5	375.2	188	242.0 (100%)	26	2.50
			196.0 (47%)	42	
aripiprazole	448.2	228	285.1 (100%)	22	4.60
			98.1 (41.5%)	38	
			176.1 (37.5%)	30	
aripiprazole-D_8	456.2	220	293.1 (100%)	26	4.59
			176.0 (46.5%)	30	
asenapine	286.1	172	229.0 (100%)	18	4.28
			166.0 (99.5%)	34	
			215.0 (43.5%)	30	
asenapine-^{13}C-D_3	290.1	172	229.0 (100%)	22	4.28
			166.0 (105.5%)	34	
bromperidol	420.1	172	165.0 (100%)	22	4.42
			123.0 (46%)	46	
			402.0 (7%)	14	
chlorpromazine	319.0	95	86.1 (100%)	15	4.79
			58.1 (57%)	40	
			214.0 (3.5%)	40	
clotiapine	344.0	65	287.0 (100%)	16	4.52
			255.0 (49%)	30	
			209.0 (6.5%)	30	
clotiapine-D_8	352.0	60	292.0 (100%)	17	4.50
			260.0 (50.5%)	32	
clozapine	327.1	172	270.0 (100%)	18	3.49
			192.0 (52%)	46	
			164.0 (14.5%)	90	
clozapine-D_8	335.2	172	275.1 (100%)	22	3.45
			192.0 (59%)	50	
dehydro-aripiprazole	446.1	176	285.1 (100%)	18	4.42
			98.1 (24%)	42	
			84.1 (3.5%)	62	
dehydro-aripiprazole-D_8	454.2	214	293.1 (100%)	22	4.41
			102.1 (18.5%)	46	
droperidol	380.0	125	165.0 (100%)	23	3.73

			194.0 (74.5%)	10	
			123.1 (59%)	53	
flupentixol	435.2	175	305.1 (100%)	25	4.99
			265.1 (68.5%)	37	
			390.1 (59.5%)	20	
flupentixol-D_4	439.2	170	305.1 (100%)	27	4.98
			265.1 (76%)	40	
fluphenazine	438.0	165	171.1 (100%)	22	4.84
			143.1 (57.5%)	28	
			70.1 (31%)	50	
fluspirilene	476.1	165	98.0 (100%)	33	5.34
			371.1 (30%)	15	
			55.1 (15%)	65	
haloperidol	376.2	172	165.0 (100%)	22	4.33
			123.0 (81%)	42	
			95.1 (20%)	82	
haloperidol-D_4	380.2	172	165.0 (100%)	22	4.31
			123.0 (75.5%)	42	
iloperidone	427.2	196	261.1 (100%)	26	4.28
			233.1 (61%)	30	
			190.0 (57.5%)	42	
iloperidone-D_3	430.2	196	261.1 (100%)	26	4.27
			190.0 (55%)	42	
levomepromazine	329.1	65	100.1 (100%)	15	4.62
			58.1 (63%)	40	
			242.0 (7.5%)	18	
levosulpiride	342.2	188	112.1 (100%)	22	1.73
			214.0 (29.5%)	30	
			110.1 (1%)	42	
loxapine	328.0	60	271.0 (100%)	18	4.24
			193.0 (26%)	48	
			164.0 (12%)	78	
loxapine-D_8	336.0	55	276.0 (100%)	20	4.22
			193.0 (23%)	50	
lurasidone	493.3	260	166.1 (100%)	42	5.12
			177.0 (33%)	46	
			120.1 (30%)	66	
lurasidone-D_8	501.3	260	166.1 (100%)	46	5.12
			120.1 (43.5%)	66	
melitracen	292.2	75	247.1 (100%)	13	4.93
			217.1 (108%)	35	
			232.1 (98%)	22	
melitracen-D_6	298.2	73	253.1 (100%)	15	4.92
			217.1 (95%)	35	
norasenapine	272.1	90	165.0 (100%)	57	4.17
			229.0 (128%)	13	
			166.0 (74.5%)	30	
norclozapine	313.1	172	192.0 (100%)	42	3.21
			270.0 (71%)	22	
			227.0 (17%)	26	
norclozapine-D_8	321.2	172	192.0 (100%)	46	3.17
			275.1 (33%)	22	
norolanzapine	299.1	176	198.0 (100%)	38	1.88
			256.0 (98%)	22	
			213.0 (66%)	26	
norolanzapine-D_8	307.2	176	198.0 (100%)	38	1.91
			213.0 (62%)	26	
norquetiapine	296.1	110	210.0 (100%)	30	3.65
			139.0 (60%)	66	
			183.0 (47%)	42	
norquetiapine-D_8	304.2	115	210.0 (100%)	30	3.60
			139.0 (34.5%)	70	
OH-iloperidone	429.2	196	261.1 (100%)	18	4.04

			190.0 (20.5%)	42	
			233.1 (16.5%)	30	
OH-iloperidone-D_4	433.3	196	261.1 (100%)	18	4.01
			190.0 (20%)	42	
olanzapine	313.2	176	256.0 (100%)	18	1.93
			198.0 (22%)	42	
			169.0 (12.5%)	42	
olanzapine-D_3	316.2	176	256.0 (100%)	18	1.95
			198.0 (22%)	42	
paliperidone	427.2	176	207.1 (100%)	26	3.29
			110.0 (19%)	46	
			82.1 (6%)	58	
paliperidone-D_4	431.2	176	211.1 (100%)	26	3.29
			114.1 (17.5%)	46	
perphenazine	404.1	140	171.1 (100%)	20	4.49
			143.1 (64%)	27	
			70.1 (34%)	45	
pimozide	462.1	70	109.1 (100%)	55	5.14
			328.1 (75.5%)	27	
			147.1 (71%)	38	
pipamperone	376.2	166	165.0 (100%)	26	2.34
			123.0 (62.5%)	50	
			291.1 (48%)	14	
prothipendyl	286.1	65	241.0 (100%)	10	3.99
			213.0 (48%)	26	
			181.0 (40.5%)	43	
prothipendyl-D_6	292.1	60	241.0 (100%)	10	3.98
			213.0 (45.5%)	27	
quetiapine	384.2	172	253.0 (100%)	18	3.84
			221.1 (46%)	38	
			279.1 (16.5%)	22	
quetiapine-D_8	392.2	172	226.1 (100%)	38	3.81
			257.7 (103.5%)	2	
reduced haloperidol	378.2	166	149.0 (100%)	26	4.08
			109.0 (41%)	58	
			342.1 (13.5%)	18	
reduced haloperidol-D_4	382.2	166	149.0 (100%)	26	4.07
			109.0 (45%)	54	
risperidone	411.2	188	191.1 (100%)	26	3.30
			110.0 (6%)	54	
			82.1 (4.5%)	66	
risperidone-D_4	415.3	188	195.1 (100%)	26	3.30
			114.1 (4.5%)	54	
sertindole	441.2	214	113.1 (100%)	30	5.08
			71.2 (10.5%)	54	
sertindole-D_4	445.2	214	117.1 (100%)	34	5.08
			73.2 (13%)	58	
tiapride	329.0	55	256.0 (100%)	15	1.98
			213.0 (27.5%)	32	
			212.0 (14%)	28	
zuclopenthixol	401.2	176	100.1 (100%)	26	4.68
			230.9 (100%)	38	
			221.0 (85.5%)	58	
zuclopenthixol-D_4	405.2	176	221.0 (100%)	58	4.68
			231.0 (141%)	34	

Table 2. Antipsychotics identified in medico-legal casework (n = 137). *The concentration is expressed as the average with the range between brackets. Calibration, CAL; compound of interest, cpd; lower limit of quantification, LLOQ; upper limit of quantification, ULOQ.*

Compound	n° samples	CAL range (ng/mL)	Concentration (ng/mL)	n° samples < LLOQ[a] or > ULOQ
7-OH-norquetiapine	21	1.0 - 512.0	46.8 (1.0 - 333.3)	5
7-OH-quetiapine	18	1.0 - 512.0	78.1 (1.5 - 355.8)	4
amisulpride	4	10.0 - 5120.0	33.1 (22.4 - 47.2)	1
aripiprazole	2	10.0 - 5120.0	195.1 (144.1 - 246.0)	
clotiapine[b]	4	1.0 - 512.0	12.7 (6.8 - 17.9)	1
dehydro-aripiprazole	2	4.0 - 2048.0	17.2 (13.7 - 20.7)	
flupentixol[b]	1	0.5 - 256.0	4.8	
haloperidol	5	0.5 - 256.0	3.9 (2.5 - 4.7)	1
levomepromazine[b]	1	2.0 - 1024.0	4.9	
levosulpiride	3	10.0 - 5120.0	23.6 (15.9 - 36.6)	
norolanzapine	7	1.0 - 512.0	75.4 (2.9 - 180.0)	
norquetiapine[b]	28	3.0 - 1536.0	216.6 (3.0 - 1277.5)	4
olanzapine	7	1.0 - 512.0	115.4 (1.5 - 215.8)	
paliperidone	1	1.0 - 512.0	18.1	
pipamperone	5	4.0 - 2048.0	99.8 (4.0 - 312.1)	1
prothipendyl[b]	5	4.0 - 2048.0	1479.5 (30.7 - 3701.8)	2
quetiapine	25	10.0 - 5120.0	905.6 (25.9 - 4368.1)	11
reduced haloperidol	4	0.5 - 256.0	59.1 (8.0 - 162.2)	
zuclopenthixol	2	1.0 - 512.0	95.8	1

[a] Cpds detected at a concentration < LLOQ were included when (i) they displayed near-Gaussian peak shapes, (ii) the signal of the quantifier ion (relative to the ISTD) was greater than three times that of blanks samples analysed within that batch and (iii) qualifier ion ratios were within ± 20% of the theoretical ones (Table 1).

[b] Cpds not included in the method by Patteet et al. [18].