Clinica Chimica Acta 485 (2018) 243–257

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Validation of a simple, fast liquid chromatography-tandem mass spectrometry method for the simultaneous quantification of 40 antidepressant drugs or their metabolites in plasma

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ARTICLE INFO

Keywords:
Antidepressant
Liquid chromatography-tandem mass spectrometry
Triple quadrupole
Therapeutic drug monitoring

ABSTRACT

Introduction: Antidepressant (AD) use has increased significantly over the last decades. Therapeutic drug monitoring is recommended for compliance, toxicity and treatment efficiency. ADs also show a high prevalence in forensic cases. Few methods have been developed that combine a fast, easy sample clean-up with a quantification based on liquid chromatography-triple quadrupole mass spectrometry (LC-QQQ).

Methodology: A liquid-liquid extraction (LLE) was performed using 200 µL of plasma. The evaporated and reconstituted upper fraction was injected on a LC-QQQ system monitoring 3 transitions per compound. The method was fully validated according to international guidelines.

Results & discussion: The chromatographic run time was under 12 min. The LLE was successful in removing interferences with minimal sensitivity loss. Calibration curves ranged from sub-therapeutic to toxic concentrations. Quality control samples showed high accuracy (81%–119%) and precision (≤14%) within and between batches. Stability was tested at ambient temperature and −20 °C. The method was successfully applied to external quality control and case samples.

Conclusion: The presented method successfully quantifies 40 compounds of interest. Because of a simple sample clean-up, a relatively short chromatographic run and a wide calibration range this method can be implemented in therapeutic drug monitoring, forensic research and related fields.

1. Introduction

According to the latest information from the World Health Organisation, depression can be classified as a non-uniform disease with a large variation between patients in for example the number of depressive episodes, their duration and their severity. The lifetime prevalence of a major depressive disorder is around 15%–20%, with a 1 year prevalence of 5%–10%. Over 300 million people worldwide are assumed to be affected, though under- and misdiagnoses are likely to have skewed this number [1–4]. Treatment usually consists of either psychotherapy or the use of antidepressants (ADs), with a global increase in the prescription of the latter [4–8]. A large national survey in the US revealed that ADs were the 3rd most prescribed drug class with their usage having increased by 400% over the last 10 to 20 years [9]. Similarly, a UK study from 2011 found a 35% increase in AD prescriptions in people aged 65 and over; 55% of the prescribed ADs belonged to the class of the selective serotonin reuptake inhibitors (SSRIs) [10]. There has also been a rise in the off-label use of SSRIs for the treatment of panic disorders, obsessive compulsive disorder and post-traumatic stress disorder, and of tricyclic ADs for that of insomnia, neuropathic pain, migraine and fibromyalgia [7, 11, 12].

Numerous articles stress the need for therapeutic drug monitoring of ADs to assess patient compliance, improve therapeutic efficacy and diminish unwanted side effects [13–16]. It is recommended to monitor both parent and metabolite compounds as both contribute to the therapeutic and toxic effects. Their ratio could also give an indication of

Abbreviations: ACN, acetonitrile; AD, antidepressant; AP, antipsychotic; CAL, calibration; R^2, coefficient of determination; CV, coefficient of variation; CE, collision energy; cpd, compound of interest; CI, confidence interval; dMRM, dynamic multiple reaction mode; ESI, electrospray ionisation; EE, extraction efficiency; EMA, European Medicines Agency; FV, fragmentor voltage; ISTD, labelled internal standard; LC-QQQ, liquid chromatography-triple quadrupole mass spectrometry; LLE, liquid-liquid extraction; LLOQ, lower limit of quantification; mCPP, *m*-chlorophenylpiperazine; MS, mass spectrometry; MF, matrix factor; MTBE, methyl-tertiary-butyl-ether; MP, mobile phase; QC, quality control; RT, retention time; SSRI, selective serotonin reuptake inhibitor; STDV, standard deviation; ULOQ, upper limit of quantification

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https://doi.org/10.1016/j.cca.2018.06.047
Received 30 April 2018; Received in revised form 19 June 2018; Accepted 29 June 2018

Table 1
Plasma concentrations for the calibration and quality control samples. L1 served as the LLOQ, calibration solutions were prepared in accordance with the EMA guidelines: LLOQ, low (≤3× LLOQ), medium (30–50% of the CAL range), high (≥75% of CAL L10).

Cpd	Calibration levels (ng/mL)										Quality control levels (ng/mL)			
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	LLOQ	QC_{low}	QC_{med}	QC_{high}
Agomelatine	2	4	10	20	40	100	200	400	1000	2000	2	6	60	1500
Amitriptyline	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Atomoxetine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Bupropion	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Citalopram	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Clomipramine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Desipramine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Dosulepin	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Doxepin	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Duloxetine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Fluoxetine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Flupentixol	0.5	1	2.5	5	10	25	50	100	250	500	0.5	1.5	15	375
Fluvoxamine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Imipramine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Maprotiline	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
mCPP	2	4	10	20	40	100	200	400	1000	2000	2	6	60	1500
Melitracen	0.5	1	2.5	5	10	25	50	100	250	500	0.5	1.5	15	375
Mianserin	2	4	10	20	40	100	200	400	1000	2000	2	6	60	1500
Mirtazapine	2	4	10	20	40	100	200	400	1000	2000	2	6	60	1500
Moclobemide	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Norcitalopram	2	4	10	20	40	100	200	400	1000	2000	2	6	60	1500
Norclomipramine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Nordosulepin	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Nordoxepin	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Norfluoxetine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Normaprotiline	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Normianserin	0.5	1	2.5	5	10	25	50	100	250	500	0.5	1.5	15	375
Normirtazapine	0.5	1	2.5	5	10	25	50	100	250	500	0.5	1.5	15	375
Nortrimipramine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Nortriptyline	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
O-desmethylvenlafaxine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
OH-bupropion	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Opipramol	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500
Paroxetine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Reboxetine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Sertraline	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Tianeptine	2	4	10	20	40	100	200	400	1000	2000	2	6	60	1500
Trazodone	25	50	125	250	500	1250	2500	5000	12,500	25,000	25	75	750	18,750
Trimipramine	5	10	25	50	100	250	500	1000	2500	5000	5	15	150	3750
Venlafaxine	10	20	50	100	200	500	1000	2000	5000	10,000	10	30	300	7500

the time of ingestion and whether therapeutic or excessive ingestion has occurred [17]. Tricyclic antidepressants have a narrow therapeutic window with a large intra- and inter-individual variation in metabolism and clearance [18–20]. SSRIs have a much lower risk of toxicity but the relationship between their plasma concentration and therapeutic effect is less clearly understood [16, 17]. Additionally, patients are often treated with a combination of psychiatric drugs, thus increasing the risk of drug-drug interactions [21–23]. Furthermore, ADs are often detected in the blood of those involved in injurious crashes, second to alcohol and benzodiazepines. Blood AD levels seem unrelated to the incidence of a crash, however initiation and changes in treatment regimen have been found to show a positive correlation [24, 25]. Whether or not the ADs or the underlying disorder are the cause of the crashes remains undecided [26, 27].

Despite the widespread consensus for the need to monitor ADs, a limited number of liquid chromatography methods has been published. Many articles describe the detection of only a small number of compounds using liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QQQ) [28–32]. Of those methods that are able to quantify > 10 ADs simultaneously, most do not make use of any or many labelled internal standards for quantification purposes [33–39]. More wholesome methods often require long chromatographic run times and/or multiple injections [15, 35, 40]. Lastly, several noteworthy multi-analyte methods have been published. They however describe the use of alternative matrices, specialised sample clean-up procedures or more costly instruments, none of which are readily available to a routine laboratory [41–47].

We therefore aimed to develop a fast, simple bioanalytical assay based on liquid chromatography-tandem mass spectrometry for the quantitative detection of ADs in plasma, for use in therapeutic drug monitoring and routine forensic analysis without the need for any time-consuming sample preparation or highly specialised instrumentation. The described method allows for the simultaneous quantification of 40 compounds of interest (cpds) and is, to our knowledge, the most comprehensive published method for the quantitative analysis of ADs to date. The antipsychotic (AP) flupentixol has also been added given that the AD melitracen is only prescribed in combination with this AP in Belgium.

2. Material & methods

2.1. Chemicals & reagents

Amitriptyline, bupropion, bupropion-D_9, citalopram, clomipramine, dosulepin, fluoxetine, imipramine, mirtazapine, nortriptyline, paroxetine, *O*-desmethylvenlafaxine-D_6, OH-bupropion, OH-bupropion-D_6, *m*-chlorophenylpiperazine-D_8 (mCPP-D_8), sertraline-D_3 and trazodone standards were purchased from Cerilliant (Round Rock, Texas, US) as