Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 576–582

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Liquid chromatography/tandem mass spectrometry method for determination of olanzapine and N-desmethylolanzapine in human serum and cerebrospinal fluid

Martin Josefsson[a,b,*], Markus Roman[a], Elisabeth Skogh[c], Marja-Liisa Dahl[d,e]

[a] Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, SE-58133 Linköping, Sweden
[b] Department of Physics, Chemistry and Biology, Linköping University, SE-58183 Linköping, Sweden
[c] Department of Clinical and Experimental Medicine, Psychiatry Section, Faculty of Health Science, Linköping University, SE-58183 Linköping, Sweden
[d] Department of Medical Sciences, Clinical Pharmacology, University Hospital, SE-75185 Uppsala, Sweden
[e] Department of Clinical Pharmacology, Karolinska University Hospital, SE-17176 Stockholm, Sweden

ARTICLE INFO

Article history:
Received 15 February 2010
Received in revised form 26 March 2010
Accepted 29 March 2010
Available online 10 April 2010

Keywords:
Olanzapine
N-desmetylolanzapine
Serum
Cerebrospinal fluid
Tandem mass spectrometry
LC–MS/MS

ABSTRACT

A validated, accurate and sensitive LC–MS/MS method for determination of olanzapine and its metabolite N-desmethylolanzapine has been developed. The analytes were quantified by tandem mass spectrometry operating in positive electrospray ionization mode with multiple reaction monitoring. Olanzapine and desmethylolanzapine were extracted from serum or cerebral spinal fluid samples, 200 μl, with tert-butyl methyl ether using olanzapine-D3 as internal standard. Calibrations for olanzapine and desmethylolanzapine were linear within the selected range of 0.2–30 ng/ml (6–96 nM) in cerebral spinal fluid and for olanzapine in plasma, in the range of 5–100 ng/ml (16–320 nM). The method was successfully used for the analysis of samples from patients treated with olanzapine in the dose range of 2.5–25 mg/day.

1. Introduction

Olanzapine (OLZ) is an atypical antipsychotic agent with thienobenzodiazepinyl structure (Fig. 1). It has efficacy against both positive and negative symptoms of schizophrenia [1,2] and has recently been suggested to be also useful during acute manic and the maintenance phase of bipolar disorder [3]. The side-effect profile is characterized by a low degree of extra-pyramidal side-effects [1,2] and a low risk of tardive dyskinesia [4] but an increased risk of metabolic abnormalities [5].

Following oral administration, OLZ is predominantly metabolized to 4′-N-desmethylolanzapine (DMO) via the cytochrome P-450 (CYP) 1A2 isoenzyme and to OLZ-N-oxide via a flavin-containing mono-oxygenase (FMO3) [6]. The two most important phase-II reactions include glucuronidation to OLZ-10-N-glucuronide and to OLZ-4′-N-glucuronide. 10-N-glucuronide is the most abundant circulating metabolite at steady state after repeated oral dosing but DMO is the most commonly investigated OLZ metabolite.

OLZ shows a large inter-individual variability in serum concentrations adjusted for dose (C/D) [7]. Analysis of OLZ concentrations in serum (therapeutic drug monitoring, TDM) is therefore a potentially useful tool for dose optimization, especially in patients developing adverse drug effects, with suboptimal response or potential drug interaction problems. However, the pharmacological effects of OLZ could be expected to be more closely related to its concentrations at the site of action, i.e. in the central nervous system (CNS), than in serum. In general, there are only few studies on the relationship between serum and cerebrospinal fluid (CSF) concentrations of antipsychotics in man [8]. Such data would be important in light of the recent findings on the potential importance of active transport proteins such as P-glycoprotein, for the penetration of drugs into CNS [9]. Several methods have been published for the analysis of OLZ in plasma or serum (human or animal), using high-performance liquid chromatography (HPLC) with ultraviolet (UV) [10], electrochemical (EC) [7,11–13] and, more recently, sensitive and specific tandem mass spectrometric detection (MS/MS) [14–17]. Moreover, for TDM use, a single state LC–MS method has recently been used [18]. In some of the methods reviewed several other antipsychotic drugs have been analysed [10,14,17,18] while methods for OLZ and its metabolites are few [7,13,15]. Methods for the analysis of drugs in CSF are in general rare, and, to the knowledge of the authors, no method has yet been published for OLZ in CSF. In a previous paper a LC–MS/MS

* Corresponding author at: Department of Forensic Genetics and Forensic Toxicology, National Board of Forensic Medicine, Artillerigatan 12, SE-58133 Linköping, Sweden. Tel.: +46 13 252323; fax: +46 13 104875.
E-mail address: martin.josefsson@rmv.se (M. Josefsson).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.03.040

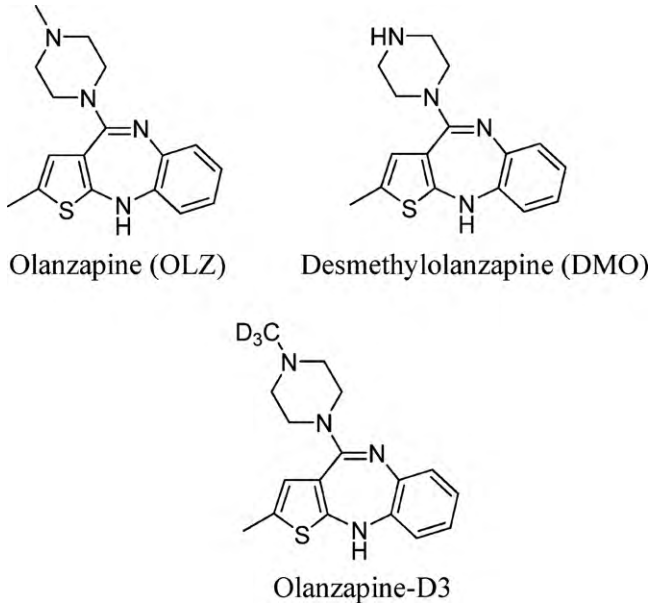

Fig. 1. Structures. Olanzapine (OLZ), N-desmethylolanzapine (DMO) and the deuterated internal standard olanzapine-D3 (OLZ-D3)

method was described for 20 different antipsychotics and a great number of their metabolites in human blood, urine and hair [19]. In this multi-target method OLZ was less retained than the other compounds and showed unfavourable peak shape. Recently, an LC–MS/MS method for five antipsychotic drugs, including OLZ, in rat plasma [17] was adapted to rat brain tissue [20]. In an application for human autopsy cases OLZ was determined by LC–MS/MS in whole blood [21]. Interferences from complex sample matrices may cause either enhancement or suppression in the ionization of the target analyte and may affect precision, sensitivity and accuracy of the method [22]. Thus, even if LC–MS/MS is considered a selective and sensitive tool for quantitative determinations of drugs, sample clean up most often is required for the analysis of biofluids. Different strategies have been used for sample preparation of OLZ including simple precipitation [21], liquid–liquid extraction (LLE) [7,10,12,14,16,17,20], and solid phase extraction (SPE) [11,13,15,18,19] but LLE is by far the most frequently used. Significant matrix effects were shown with electrospray LC–MS/MS when mixed-mode SPE was used for sample preparation of plasma with various anticoagulant agents [15]. Moreover, in some studies problems with stability of OLZ in plasma at room temperature have been described [14,17]. In other studies, problems with adsorption and instrument carry over have been observed [21]. For studies of drug transport over the blood–brain barrier sensitive analytical methods are required and co-determination of the parent drug and important metabolites is desirable. In a clinical study investigating patients with schizophrenia or schizoaffective disorder treated with olanzapine as the only antipsychotic drug, we aimed to analyse the concentrations of olanzapine and its desmethyl metabolite in serum and CSF. The concentrations of the drug were expected to be significantly lower in CSF than in serum and thus not quantifiable by the routine HPLC methods. Therefore, a novel LC–MS/MS method was developed for this purpose, as described in this paper.

2. Experimental

2.1. Chemicals, reagents and reference compounds

Acetonitrile and methanol, gradient grade, tert-butyl methyl ether and formic acid (98%, 25.5 M) were purchased from Merck (Darmstadt, Germany). Tris(hydroxymethyl)aminomethane (TRIS) and sodium hydroxide, both analytical-reagent grade, were purchased from Merck as well. Ammonium formate, analytical-reagent grade, was purchased from Fluka (Buchs, Schwitzerland). Deionized water purified with a Milli-Q Gradient grade water purifying system (Millipore Corporation, Bedford, MA), was used.

The reference compounds olanzapine (OLZ) (M.W. 312.4 g/mol) and N-desmethylolanzapine (DMO) (M.W. 298.4 g/mol) were purchased from Lilly Research Lab. (Indianapolis, IN) and olanzapine-D3 (OLZ-D3) from Molcan Corporation (Toronto, ON, Canada). Structures are shown in Fig. 1.

2.2. Solutions

Ammonium formate stock solution (1 M) was prepared by dilution of 12.6 g ammonium formate salt in 200 ml Milli-Q water. Ammonium formate mobile phase buffer (10 mM, 0.05% formic acid) was prepared by dilution of 5 ml ammonium formate stock solution and 250 μl formic acid (98%) to a final volume of 500 ml. TRIS buffer (pH 11 ± 0.2, 1 M) was prepared by dilution of 121.1 g tris(hydroxymetyl)aminomethane in 900 ml Milli-Q water, and after pH adjustment with HCl or NaOH, water was added to a final volume of 1000 ml.

Stock solutions of the reference compounds were prepared at 1.0 mg/ml in methanol.

Working solutions were prepared as mixtures of OLZ and DMO at 0.01, 0.1 and 1.0 μg/ml in methanol. The internal standard was prepared at 1.0 μg/ml in methanol.

For the infusion studies on the LC–MS/MS instrument, working solutions at 0.2 μg/ml were freshly prepared for each analyte individually in methanol and ammonium formate (pH 3, 20 mM) (1:1, v/v).

2.3. Samples

Plasma was used for preparation of standards and quality controls since human blank serum was not available in higher quantities. Drug-free plasma, collected from healthy volunteers, was purchased from the local University Hospital (Linköping, Sweden). CSF standards were prepared in plasma diluted 1:10 (v/v) with water. OLZ free reference samples of CSF were collected from patients ($n = 6$) during brain surgery and drug-free plasma from healthy volunteers from the laboratory staff ($n = 6$). Serum and CSF samples were collected from patients with schizophrenia or schizoaffective disorder, treated with olanzapine (Zyprexa®) as the only antipsychotic drug, at steady state conditions. The daily doses ranged from 2.5 to 25 mg. The selected 12 patients were 6 men and 6 women, aged 26–50 years (median 37 years) and a body mass index (BMI) of 19–29 kg/m^2 (median 24 kg/m^2). The study design and patient characteristics are described in detail elsewhere [22].

The Ethics Committee of the Medical Faculty of Linköping University, Sweden, the Swedish Medical Products Agency and the Swedish Data Inspection Board approved the collection of patient samples. All patients gave written informed consent before inclusion in the study.

2.4. Instrumentation

An electrospray liquid chromatography–tandem mass spectrometry system (ESI-LC–MS/MS) for gradient chromatography was used. The instrumentation consisted of an Acquity, Ultra Performance Liquid Chromatographic system (UPLC), equipped with a solvent manager, a sample manager and a column manager for handling of four columns (Waters, Milford, MA). Mass detection was performed on an API 4000 triple quadrupole instrument equipped with an electrospray interface (TURBO V™ source, TurbolonSpray®

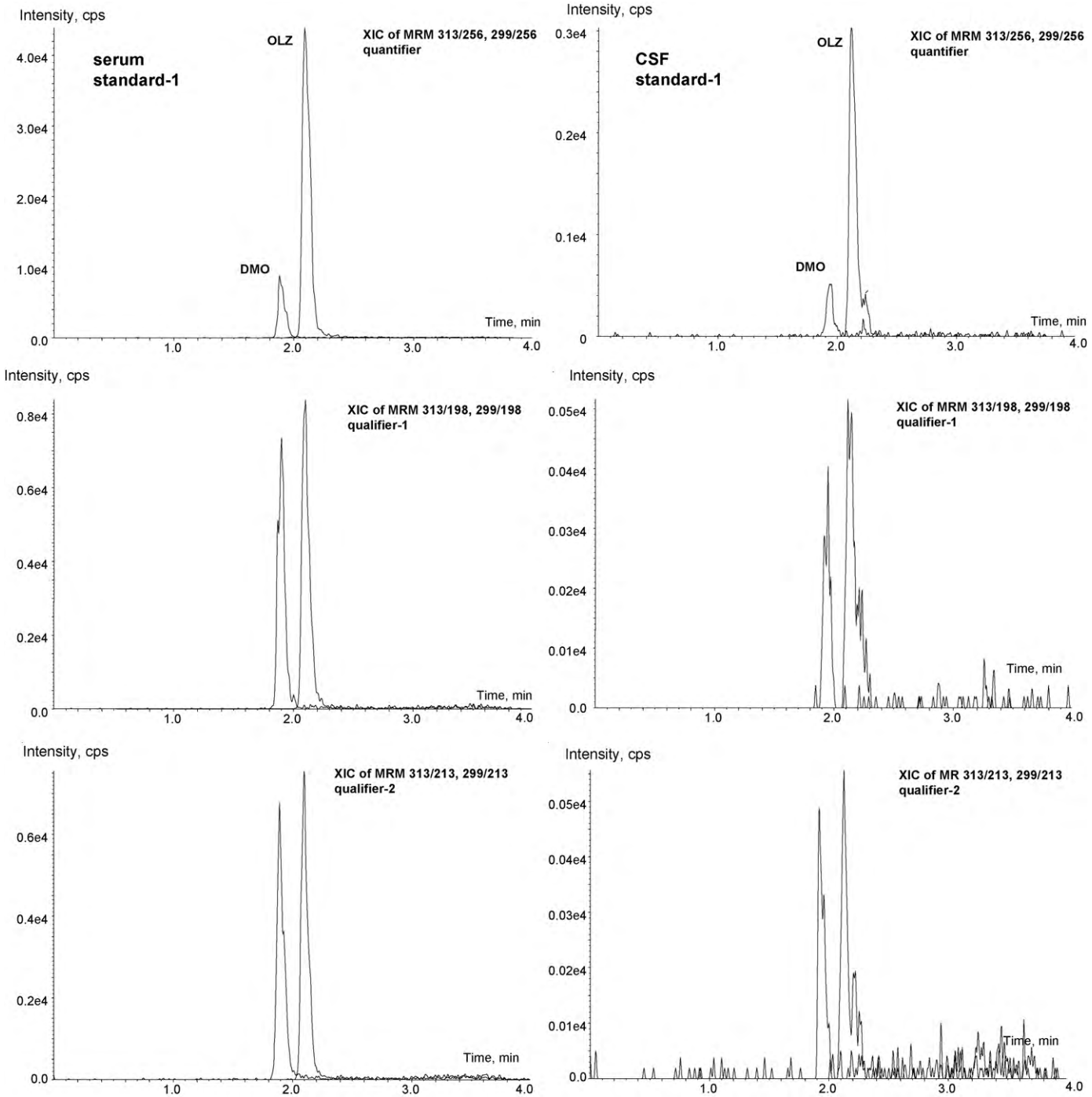

Fig. 2. LC–MS/MS chromatograms. Ion chromatograms for olanzapine (OLZ) and N-desmethylolanzapine (DMO) at the lowest calibration level, 5 ng/ml (16 nM) for plasma/serum and 0.2 ng/ml (6 nM) for cerebrospinal fluid (CSF), respectively.

probe) operating in positive ion mode (Applied Biosystem/MSD Sciex, Stockholm, Sweden). The interface probe was set at 500 °C and the ion-spray needle was operated at +5000 V. Nitrogen was used as nebulizer gas (172 kPa), auxiliary gas (345 kPa), curtain gas (207 kPa), and as Collision Activated Dissociation gas, CAD (set on 5). Instrument control, integration, and calculation were performed using Analyst™ 1.4.2 software. Infusion experiments for multiple reaction monitoring (MRM) optimizations and ion-suppression studies were performed with a Harvard Apparatus II Plus syringe pump (Hollston, MA) at a flow rate of 10 μl/min. High-performance liquid chromatography (HPLC) was carried out on a Synergi Hydro-RP 50 mm × 2 mm i.d., 2.5 μm (Phenomenex, Torrance, CA) equipped with an Opti-Solv 2 μm column inlet filter (Optimize, Portland, OR). Sample volumes of 2 μl were injected, followed by a 0.5-ml strong needle wash consisting of 2% formic acid in methanol/acetonitrile/2-propanol/water (25/25/25/25, v/v/v/v) and a 1.0-ml week needle wash consisting of formic acid (0.1 M) in methanol (50/50, v/v). The mobile phases consisted of ammonium formate buffer (10 mM) with 0.05% formic acid for solvent A and methanol with 0.05% formic acid for solvent B. A linear gradient chromatography from 0% to 60% B-phase over 2.5 min was run. Total runtime including wash and reconditioning was 4 min. A flow rate of 0.3 ml/min at 40 °C was used. Reference chromatograms are shown in Fig. 2 and the retention times in Table 1. An MRM

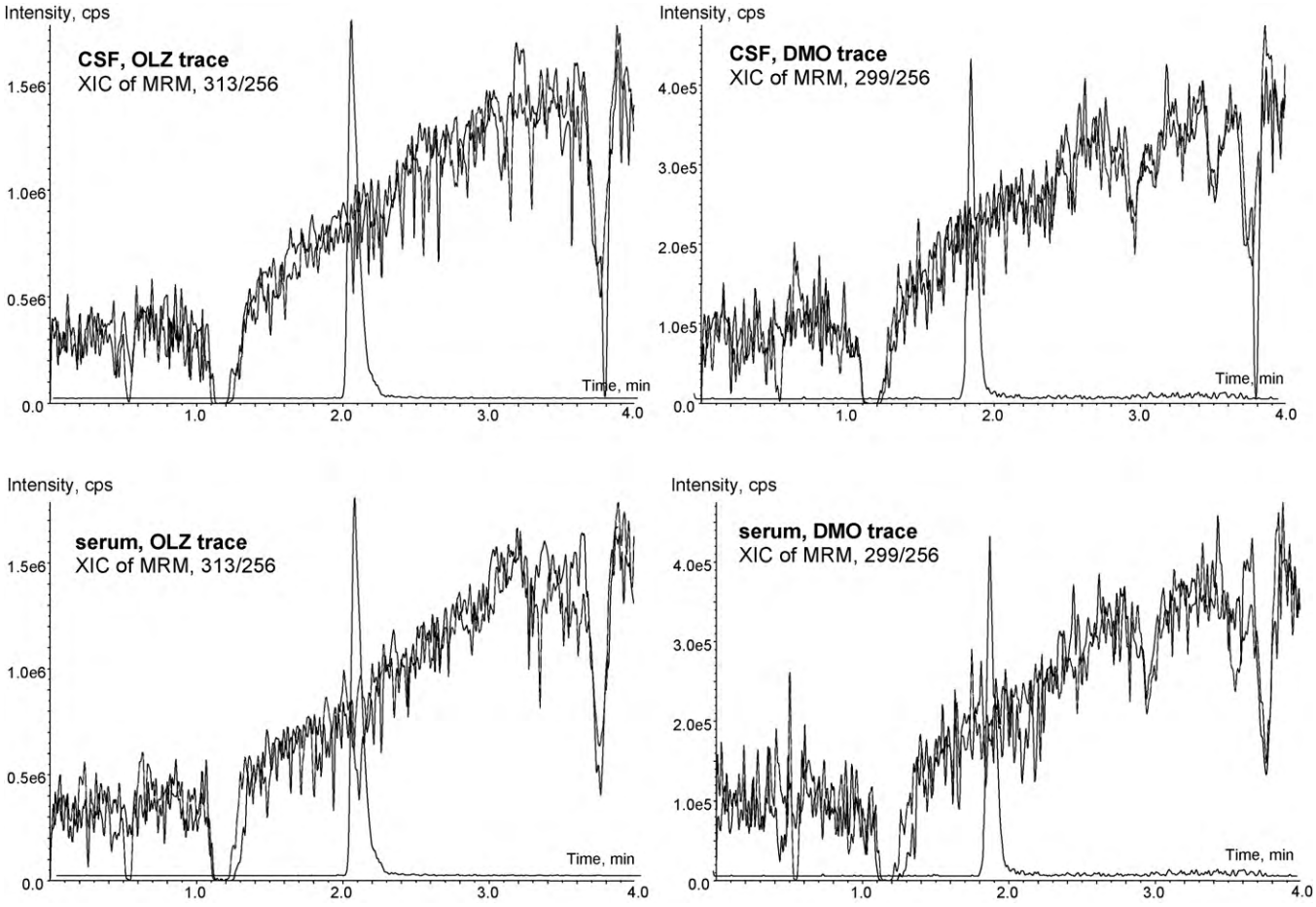

Fig. 3. Ion-suppression profiles. Ion chromatograms for olanzapine (OLZ) and N-desmethylolanzapine (DMO) from injections of reference cerebrospinal fluid (CSF) (top) and serum samples (bottom) compared with blank injections.

method was prepared including the three most intense transitions for OLZ, DMO and the internal standard OLZ-D3, Table 1. The most intense transition was used for quantification while the remaining two transitions could be used as qualifiers, Fig. 2. Matrix effects on the ESI signal during chromatography were investigated with an instrumental set-up with post-column infusion of the test analyte. The extracted ion chromatograms (XICs) from each transition were investigated individually, Fig. 3.

2.5. Preparation of samples and quality controls

To 200 μl sample, serum/plasma or CSF, 25 μl of IS (OLZ-D3, 1 μg/ml) and 100 μl TRIS buffer (1 M, pH 11) were added. The samples were vortex-mixed for 10 s and 2 ml of tert-butyl methyl ether was added as extraction solvent. After 10 min of horizontal shaking, the mixture was centrifuged at 4169 × *g* for 10 min. The organic phase was then transferred to clean 10-ml conical glass tubes and evaporated to dryness under nitrogen at 40 °C in a TurboVap LV (Zymark, Hopkinton, MA) evaporator. Residues were reconstituted in 50 μl 20% methanol in water. A 2-μl aliquot was injected into the LC–MS/MS. With each batch of samples, a set of controls was prepared and analysed. To 1 ml plasma reference compounds were added at a low concentration, LQC (7 ng/ml,), at medium level, MQC (35 ng/ml) and at high level, HQC (70 ng/ml). To 1 ml CSF reference (10% plasma in water) the reference compounds were added at a low concentration, LQC (0.4 ng/ml), at medium level, MQC (4 ng/ml) and at high level, HQC (15 ng/ml). Controls were then treated as authentic samples.

Table 1
MRM transitions and retention times (Rt). Optimized collision energy (CE) and collision exit potential (CXP) at a declustering potential (DP) of 100 V, an entrance potential (EP) at 14 V and a dwell time for each transition of 20 ms.

Analyte	Transition Q1/Q3		CE (V)	CXP (V)	Rt (min)
	Molecular ion	Fragment ion			
OLZ quantifier	313	256	32	15	2.1
OLZ qualifier-1	313	198	56	11	2.1
OLZ qualifier-2	313	213	42	11	2.1
DMO quantifier	299	256	34	15	1.9
DMO qualifier-1	299	198	51	11	1.9
DMO qualifier-2	299	213	38	11	1.9
OLZ-D3	316	256	33	15	2.1
OLZ-D3	316	198	55	11	2.1
OLZ-D3	316	213	41	11	2.1

For estimations of extraction recovery, spiked human plasma and CSF reference (10% plasma in water) was used. Reference compounds were added in five replicates at a low and a high level for plasma (7 and 70 ng/ml) and CSF reference (1.5 and 15 ng/ml), respectively. For ion-suppression studies, test samples (serum and CSF) from six different individuals were analysed. Test samples were then treated as authentic samples before injection.

2.6. Calibration

For quantitation, calibration samples were prepared in human drug-free plasma or CSF reference (10% plasma in water) by addition of the reference compound mixture (OLZ and DMO) to final concentrations of 5, 10, 20, 40, 60, 80 and 100 ng/ml in plasma,

Table 2
Validation data. Precision and accuracy for olanzapine (OLZ) and N-desmethylolanzapine (DMO) determined for quality control (QC) samples.

Analyte	Added concentration, ng/ml (nM)	Within-day ($n = 5$)			Between-day ($n = 8$)		
		Found concentration (ng/ml)	CV (%)	Accuracy (%)	Found concentration (ng/ml)	CV (%)	Accuracy (%)
Plasma[a]							
OLZ	7 (22)	7.02 ± 0.05	0.7	100	6.06 ± 1.3	22	87
	35 (112)	36.5 ± 0.9	2.5	104	33.0 ± 4.6	14	94
	70 (224)	70.2 ± 1.8	2.5	100	66.2 ± 4.6	14	94
DMO	7 (23)	6.33 ± 0.48	7.6	91	8.24 ± 1.9	23	117
	35 (117)	34.2 ± 3.5	10	98	37.7 ± 7.0	18	107
	70 (235)	79.5 ± 6.3	7.9	114	78.5 ± 11.5	14	112
CSF							
OLZ	0.4 (1.3)	0.40 ± 0.02	5.9	100			
	4 (13)	3.47 ± 0.18	4.8	97			
	15 (48)	14.7 ± 1.3	7.1	106			
DMO	0. 4 (1.3)	0.36 ± 0.07	19	90			
	4 (13)	3.47 ± 0.27	7.7	87			
	15 (50)	14.7 ± 1.7	12	98			

[a] QC prepared in plasma, used for analysis of serum samples.

and 0.2, 0.5, 2, 5, 10, 20, 30 ng/ml in CSF reference, representing calibration ranges of 16–320 nM in plasma and 6–96 nM in CSF, respectively. Standard samples for calibration were then treated as authentic samples and control samples.

3. Results and discussion

3.1. Method outline

A sensitive HPLC assay for OLZ and DMO with electrochemical detection was used in a previous work [7]. However, 4% of the samples in that TDM study were excluded due to technical problems such as interferences and for the analysis of the drug in CSF, a lower limit of detection below 5 nM (1.5 ng/ml) was needed.

Therefore, a selective chromatography was developed for OLZ and DMO with sufficient separation from interfering matrix components. According to our previous experience, the sulphur containing OLZ is more difficult to retain than other related antipsychotics such as clozapine, and peak shapes are often bad [19]. By changing chromatography from a Zorbax Stable Bond Cyano column (50 mm × 2.1 mm i.d., 3.5 μm) previously used to a Synergi Hydro-RP column (50 mm × 2 mm i.d., 2.5 μm), retention and peak performance were improved. In both cases acidic mobile phases with methanol were found to be the best choice. Sub-2 μm particles for high resolution LC were not available for the Synergi Hydro-RP material but peak performance was found better than on other Acquity sub-2 μm materials tested. By using tandem MS a specific detection was achieved. Three transitions based on fragments of the parent ion were included in the assay but only the most abundant transition was used for quantifications. The remaining transitions could be used as confirmation ions (qualifiers). In this controlled study no qualifier ratio was used for identification.

Initially, during method development when no deuterated OLZ standard was commercially available, Mianserine-D3 was used as internal standard and poor precision was seen, probably due to uncontrolled adsorption of OLZ and DMO on surfaces during sample preparation and analysis. Moreover, severe carry-over was observed in the autosampler, indicating that OLZ and DMO are adhesive compounds. Similar results have recently been reported by others [21]. By changing the injection procedure, e.g. reducing needle overfill volume and increasing the needle wash volumes (strong and weak wash) carry over was markedly reduced but not completely avoided. Thus, blank samples were run between every unknown sample in the sequences. Carry-over of less than 0.2% for OLZ and 0.3% for DMO was seen in the blank samples after the HQC samples. Moreover, by changing internal standard to OLZ-D3 the precision was improved, more so for OLZ than for DMO.

3.2. Validation

3.2.1. Linearity and sensitivity

Calibration showed good linear response in the range of 0.2–30 ng/ml (6–96 nM) in CSF for both OLZ ($r > 0.999$) and DMO ($r > 0.998$). In plasma linear response was achieved for OLZ ($r > 0.998$) in the range of 5–100 ng/ml (16–320 nM) while a quadratic curve fit was used for DMO ($r > 0.993$). In all cases standards at seven levels were used. Calibration was stable over several days and the same calibration was used for between-day precision studies. LOQ (signal-to-noise peak to peak ≥10) was estimated from replicates of the lowest standard samples used for calibration and was found to be 0.3 ng/ml for OLA and 0.9 ng/ml for DMO in plasma and 0.1 ng/ml for OLA and 0.3 ng/ml DMO in CSF.

3.2.2. Precision and accuracy

Precision and accuracy measurements for OLZ and DMO were acquired from the analysis of QC samples at three levels in plasma (7, 35 and 70 ng/ml) and CSF (0.4, 4 and 15 ng/ml), Table 2. For OLZ the within-day precision (CV) was equal or better than 7% in plasma and CSF while the precision for DMO was within 7–19%. A higher, but still acceptable, CV (19%) was achieved for DMO in the LQC samples (0.4 ng/ml) in CFS since this concentration was close to the LOQ for the method. Moreover, the better precision shown for OLZ was due to the use of the corresponding deuterated internal standard (i.e. OLZ-D3), not available for DMO. In this study, historical calibrations were used. Between-day precision data (with CV 14–23%) was based on concentrations calculated from the calibration curves generated day one. These CVs are somewhat higher than what usually found in comparable methods for less adhesive compounds. Most likely, better precision would have been achieved if daily calibrations had been used.

The accuracy was 100–104% for OLZ and 91–114% for DMO in plasma, and 97–106% for OLZ and 87–98% for DMO in CSF, in within-day measurements. Lower accuracies were found for DMO at low concentrations which could be explained by losses from adsorption on surfaces that were not compensated for by a corresponding deuterated internal standard.

3.2.3. Recovery, matrix effect and stability

Relative recoveries were estimated from mean values of five replicates at high and low QC level. Recoveries of 107% (7 ng/ml)

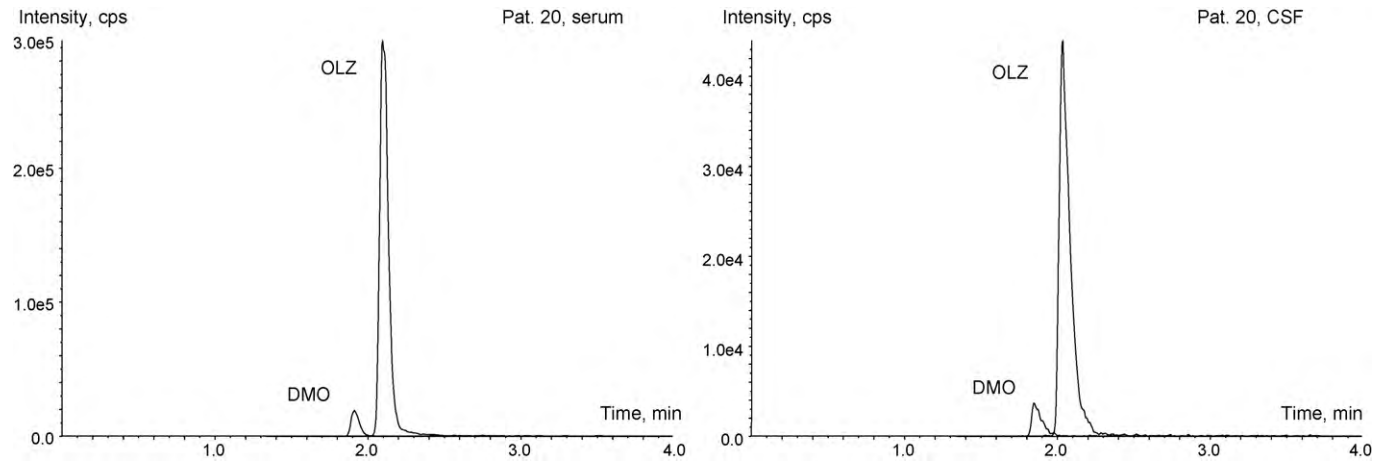

Fig. 4. Authentic sample. Ion chromatograms from an authentic sample, with 70 nM (22 ng/ml) olanzapine (OLZ) and 23 nM (7.0 ng/ml) N-desmethylolanzapine (DMO) in serum, and 12 nM (3.6 ng/ml) OLZ and 7 nM (1.8 ng/ml) DMO in cerebrospinal fluid (CSF), respectively.

and 85% (70 ng/ml) were found in plasma and 75% (1.5 ng/ml) and 81% (15 ng/ml) in CSF, respectively.

No matrix effects were observed when drug-free reference samples, plasma or CFS, were injected during constant infusion of OLZ and DMO. Ion-suppression profiles are shown in Fig. 3.

Previous studies have shown that OLZ is stable in human plasma samples, without any added preservatives, for at least 12 months at −20 °C while a decrease with approximately 30% in total was seen for DMO (unpublished work). For OLZ and DMO losses of <10% and 23%, respectively, were seen after one freeze/thaw procedure. However, after two additional freeze/thaw cycles no further losses were seen. In the present study samples were stored at −80 °C before analysis and no severe degradation has been observed in samples that have been re-analysed. Sample extracts, however, were not stable for re-analysis after one freeze/thaw procedure or storage 24 h in refrigerator +4 °C. Losses were especially pronounced for DMO.

3.3. Application for authentic samples

The method was successfully applied for the analysis of serum and CSF from patients treated with OLZ doses ranging from 2.5 to 25 mg/day. Chromatograms from the analysis of serum and CSF samples from a representative patient at a dose of 10 mg are shown in Fig. 4. No interferences in the extracted ion-chromatogram or severe ion suppression on the internal standard signal were seen. With few exceptions, all measurements were within the calibration range of the method for both OLZ and DMO in serum and CSF, Table 3 and Fig. 5. OLZ and DMO concentrations found in serum were in accordance with previously described concentrations in TDM data [7]. As expected, lower concentrations were found in CSF compared with serum. Especially the concentrations of DMO in CSF were low and as described in 3.2 the precision of the measurements was lower for DMO than for OLZ. OLZ serum concentration correlated to the daily dose with tenfold higher concentrations found in

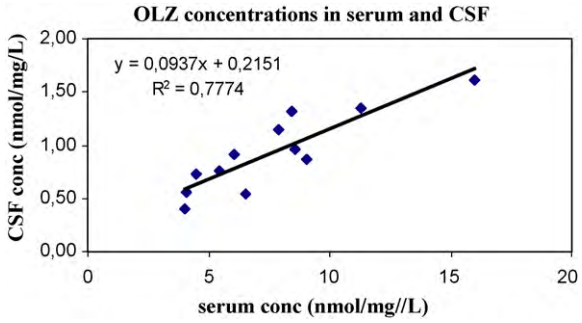

Fig. 5. Patient data. Correlation of olanzapine (OLZ) concentrations found in serum and cerebrospinal fluid (CSF) in 12 patients.

Table 3
Patient data. Concentrations of olanzapine (OLZ) and N-desmethylolanzapine (DMO) found in serum and cerebrospinal fluid (CSF) in 12 patients treated with olanzapine.

Patient	Dose (mg)	Serum			CSF		
		OLZ (nM)	DMO (nM)	DMO/OLZ ratio	OLZ (nM)	DMO (nM)	DMO/OLZ ratio
Pat 26	2.5	11	9	(0.78)	1.8	≪LOQ	–
Pat 01	2.5	28	10	0.37	3.4	2.4	0.71
Pat 27	5	30	10	0.32	4.6	0.5	0.10
Pat 05	5	80	13	0.17	8.1	0.5	0.06
Pat 08	10	54	22	0.41	7.6	2.4	0.31
Pat 13	10	65	32	0.50	5.5	6.2	1.12
Pat 30	15	119	29	0.24	17	1.7	0.10
Pat 23	15	126	26	0.21	20	5.4	0.27
Pat 03	20	81	20	0.25	11	3.3	0.30
Pat 17	20	171	51	0.30	19	10	0.53
Pat 04	25	101	40	0.39	10	2.4	0.24
Pat 06	25	226	58	0.26	22	4.0	0.18
Mean		91	27	0.31	11	3.2	0.36
Min	2.5	11	9	0.17	1.8	0.5	0.06
Max	25	226	58	0.50	22	10	1.12

Outlying result between brackets not included in statistics.

patients treated with 25 mg compared with patients treated with 2.5 mg. Correlation between serum and CSF OLZ concentrations was seen, Fig. 5. High correlation between serum and CSF OLZ is desirable when discussing the concentration–effect relationship and the concept of therapeutic drug monitoring of OLZ. The DMO/OLZ ratio in serum was not dose dependent and ranged moderately between individuals (from 0.17 to 0.50). The concentrations of DMO in CSF varied more than those of OLZ. The CSF DMO concentrations, however, were low and close to the lower limit of quantification in many patients. The limited analytical precision with respect to CSF DMO concentrations cannot be excluded as a cause of these findings.

The method has recently been successfully applied in a larger clinical study examining the relationship between OLZ and metabolite concentrations in serum and CSF in patients with schizophrenia or schizoaffective disorder [23]. In this study the influence of gender, age, smoking and pharmacogenetics were investigated.

4. Conclusion

A sensitive and specific LC–MS–MS method for OLZ and its metabolite DMO was developed for human serum and CSF. By using a deuterated internal standard, acceptable precision was achieved. The method could successfully be used for the analysis of samples from patients with daily doses varying between 2.5 and 25 mg. To our knowledge, this is the first published method applied for analysis of the very low concentrations of OLZ and DMO found in human CSF. The method has been applied in a larger clinical study examining the relationship between OLZ and metabolite concentrations in serum and CSF and the factors influencing it. CSF concentration data are also of interest from a clinical point of view when dose–effect relationships are addressed.

Acknowledgment

The study was supported by grants from the Swedish Research Council (2008-2578 and 2009-4740).

References

[1] C. Beasley, T. Sanger, W. Satterlee, G. Tollefson, P. Tran, S. Hamilton, Olanzapine versus placebo: results of a double-blind, fixed-dose olanzapine trial, Psychopharmacology 124 (1996) 159–167.
[2] F. Bymaster, K. Perry, D. Nelson, D. Wong, K. Rasmussen, N. Moore, D. Calligaro, Olanzapine: a basic science update, Br. J. Psychiatry 174 (1999) 36–40.
[3] K. Fountoulakis, E. Vieta, Treatment of bipolar disorder: a systematic review of available data and clinical perspectives, Int. J. Neuropsychopharmacol. 11 (2008) 999–1029.
[4] C. Beasley, M.A. Dellva, R. Tamura, H. Morgenstern, W. Glazer, K. Ferguson, G. Tollefson, Randomised double-blind comparison of the incidence of tardive dyskinesia in patients with schizophrenia during long term treatment with olanzapine or haloperidol, Br. J. Psychiatry 174 (1999) 23–30.
[5] K.I. Melkersson, M.-L. Dahl, Adverse metabolic effects associated with atypical antipsychotics. Literature review and clinical implications, Drugs 64 (2004) 701–723.
[6] J.T. Callaghan, R. Bergstrom, L. Ptak, C. Beasley, Olanzapine pharmacokinetic and pharmacodynamic profile, Clin. Pharmacokinet. 37 (1999) 178–193.
[7] E. Skogh, M. Reis, M.-L. Dahl, J. Lundmark, F. Bengtsson, Therapeutic drug monitoring data on olanzapine ant its N-demethyl metabolite in the naturalistic clinical setting, Ther. Drug Monit. 24 (2002) 518–526.
[8] C. Nordin, B. Almé, U. Bondesson, CSF and serum concentrations of clozapin and its demetyl metabolite: a pilot study, Psychopharmacology 122 (1995) 104–107.
[9] J.S. Wang, R. Taylor, Y. Ruan, J.L. Donovan, J.S. Markowitz, C. Lindsay De Vane, Olanzapine penetration into brain is greater in transgenic Abcb1a P-glycoprotein-deficient mice than FVB1 (wild-type) animals, Neuropsychopharmacology 29 (2004) 551–557.
[10] G. Zhang, A.V. Terry Jr., M.G. Bartlett, Simultaneous determination of five antipsychotic drugs in rat plasma by high performance liquid chromatography with ultraviolet detection, J. Chromatogr. B 856 (2007) 20–28.
[11] J.T. Catlow, R.D. Barton, M. Clemens, T.A. Gillespie, M. Goodwin, S.P. Swanson, Analysis of olanzapine in human plasma utilizing reversed-phase high-performance liquid chromatography with electrochemical detection, J. Chromatogr. B 668 (1995) 85–90.
[12] M. Aravagiri, D. Ames, W.C. Wirshing, S.R. Marder, Plasma level monitoring of olanzapine in patients with schizophrenia: determination by high-performance liquid chromatography with electrochemical detection, Ther. Drug Monit. 19 (1997) 307–313.
[13] M.A. Raggi, G. Casamenti, R. Mandrioli, V. Volterra, A sensitive high-performance liquid chromatographic method using electrochemical detection for the analysis of olanzapine and desmethylolanzapine in plasma of schizophrenic patients using a new solid-phase extraction procedure, J. Chromatogr. B 750 (2001) 137–146.
[14] Z. Zhou, X. Li, K. Li, Z. Xie, Z. Cheng, W. Peng, F. Wang, R. Zhu, H. Li, Simultaneous determination of clozapine, olanzapin, risperidon and quetiapine in plasma by high-performance liquid chromatography–electrospray ionization mass spectrometry, J. Chromatogr. B 802 (2004) 257–262.
[15] C. Chin, Z.P. Zhanz, H.T. Karnes, A study on matrix effect on an LC/MS/MS assay for olanzapine and desmethyl olanzapine, J. Pharm. Biomed. Anal. 35 (2004) 1149–1167.
[16] R.V.S. Nirogi, V.N. Kandikere, M. Shukla, K. Mudigonda, S. Maurya, R. Boosi, A. Yerramilli, Development and validation of a sensitive liquid chromatography/electrospray tandem mass spectrometry assay for the quantification of olanzapine in human plasma, J. Pharm. Biomed. Anal. 41 (2006) 935–942.
[17] G. Zhang, A.V. Terry Jr., M.G. Bartlett, Liquid chromatography/tandem mass spectrometry method for the simultaneous determination of olanzapine, risperidone, 9-hydroxyrisperidone, clozapine, haloperidol, and ziprasidone in rat plasma, Rapid Commun. Mass Spectrom. 21 (2007) 920–928.
[18] E. Choonga, S. Rudazb, et al., Therapeutic drug monitoring of seven psychotropic drugs and four metabolites in human plasma by HPLC–MS, J. Pharm. Biomed. Anal. 50 (2009) 1000–1008.
[19] M. Josefsson, R. Kronstrand, J. Andersson, M. Roman, Evaluation of electrospray ionisation liquid chromatography–tandem mass spectrometry for rational determination of a number of neuroleptics and their metabolites in human body fluids and tissues, J. Chromatogr. B 789 (2003) 151–157.
[20] G. Zhang, A.V. Terry Jr., M.G. Bartlett, Sensitive liquid chromatography/tandem mass spectrometry method for the simultaneous determination of olanzapine, risperidone, 9-hydroxyrisperidone, clozapine, haloperidol, and ziprasidone in rat brain tissue, J. Chromatogr. B 858 (2007) 276–281.
[21] M.K. Klose Nielsen, S. Stybe Johansen, Determination of olanzapine in whole blood using simple protein precipitation and liquid chromatography–tandem mass spectrometry, J. Anal. Toxicol. 33 (2009) 212–217.
[22] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[23] E. Skogh, I. Sjödin, M. Josefsson, M.-L. Dahl, Olanzapine and its N-desmethyl metabolite in serum and cerebrospinal fluid in patients with schizophrenia or schizoaffective disorder—influence of gender, age, smoking and pharmacogenetics, J. Clin. Psychopharmacol., submitted for publication.