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A UPLC–MSMS method for the analysis of olanzapine in serum—with particular emphasis on drug stability testing

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ABSTRACT

A method including a rapid and automated extraction of olanzapine from serum followed by ultra performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) has been developed and validated. Serum aliquots (100 μL) and internal standard (olanzapine-d_3, 25 μL) were pipetted onto an Ostro™ 96-well filtration plate and protein precipitated with acidic acetonitrile (300 μL) before removal of endogenous phospholipids by filtration followed by analysis. Chromatography was achieved using an HSST 3 (2.1 × 100 mm, 1.8 μm) column and gradient elution with acidic water in combination with methanol at a flow rate of 0.5 mL/min. The runtime was 1.5 min. The mass spectrometer was monitored in positive mode and multiple reaction monitoring (MRM). The *m/z* 313.1 > 256.1 and 313.1 > 198.0 transitions were monitored for olanzapine (*m/z* 316.1 > 256.1 for olanzapine-d_3). The quadratic calibration curves ranged from 5 to 500 nM ($R^2 \geq 0.999$). Limit of quantification was 0.5 nM (CV 9.6%, accuracy 110%). Within-assay and between-assay inaccuracies were 2.6–11.9% (CV ≤ 4.8%). Recovery was 84–95% (CV ≤ 1.4%) and matrix effects ranged from 100 to 103% (CV ≤ 2.6%). Extensive stability testing showed that at ambient temperature, olanzapine in patient serum samples were stable for at least seven hours on the laboratory bench and for at least 48 h in darkness. When exposed to 3000 lux, however, significant degradation had occurred after 48 h. Notably, olanzapine in spiked serum was unstable already after four hours when exposed to 3000 lux. At 4–8 °C and exposure to 550 lux, both patient serum and spiked serum were stable for more than 48 h but less than a week, whereas in darkness, the samples were stable for at least 14 days. The cumulative light exposure causing significant degradation of olanzapine in patient serum was 50,000–100,000 lux-h. In some individual samples, however, the effect of light exposure was more pronounced. Therefore, it seems pertinent to recommend protecting all samples from light, although we found no indication that a few hours of exposure to standard indoor illumination will affect the olanzapine concentration to any significant degree.

1. Introduction

The antipsychotic drug olanzapine (Fig. 1a) is used in treatment of schizophrenia and bipolar disorder [1–4]. It is mainly metabolized in the liver by the cytochrome 450 (CYP) enzyme CYP1A2, but also by glucuronosyltransferases and flavine monooxygenases [5]. The serum concentrations achieved by a certain dose are influenced by factors such as sex, age, smoking, genetics [6–9] and concomitant use of CYP1A2 inducers or inhibitors [10–12].

Therapeutic drug monitoring (TDM), i.e. the quantification of the serum or plasma concentration of a drug, is an important tool to ensure that a patient's drug dose is optimized. It can also be used to monitor drug adherence and reveal drug interactions. TDM is widely used in patients treated with olanzapine e.g. in the UK, in Germany and in Scandinavia [13–16]. As the analysis is often requested among psychiatrists in these countries, a simple, rapid and robust analytical method, enabling a high throughput, is a prerequisite to meet the needs from the clinicians.

Previously published liquid chromatography—tandem mass spectrometry (LC–MSMS) methods for olanzapine utilize sample volumes ranging from 0.1 to 1.5 mL with run times up to 15 min and include either simultaneous quantification of several antipsychotics in serum [17,18], detection of olanzapine or olanzapine and fluoxetine in plasma [19–21], determination of olanzapine in whole blood [22], serum or cerebrospinal fluid [23] or more automated methods for olanzapine TDM in serum or plasma [24,25]. A

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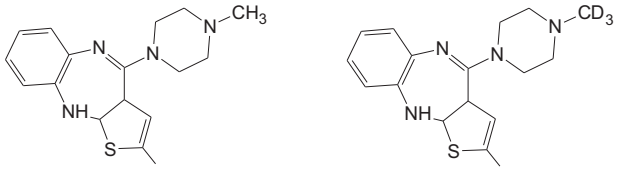

a) Olanzapine b) Olanzapine-d_3

Fig. 1. Molecular structures of olanzapine and olanzapine-d_3.

majority of these LC–MSMS methods have time-consuming extraction steps like solid phase [19–21,24,25] or liquid–liquid extraction [17,23,26]. There are also methods with protein precipitation followed by evaporation and reconstitution of the samples [18,22] requiring additional time, thus making these methods less suitable for high throughput analysis.

A potentially complicating factor in the process of TDM of olanzapine is that its stability depends on sample matrix and physical storage conditions such as light exposure and temperature [17,27–29]. The results are however inconsistent and puzzling, and often the exact testing conditions, e.g. related to the degree of light exposure, whether the samples are spiked or are real patient samples, and the types of tubes used, are not specified. Thus, there is a need for conclusive data regarding the various factors possibly affecting olanzapine stability. As an integrated part of the development of a new analytical method for olanzapine, factors concerning stability and storage conditions of patient samples, calibrators and quality controls (QCs) should be included in the validation procedure.

The aim of this study was to develop an automated and robust routine method for the analysis of olanzapine in serum, using a low sample volume and a simple and rapid sample preparation followed by a high throughput ultrahigh pressure liquid chromatography—tandem mass spectrometry (UPLC–MSMS) analysis. A secondary aim was to thoroughly evaluate the factors possibly affecting olanzapine stability in vitro.

2. Materials and method

2.1. Chemicals and reagents

Olanzapine were purchased from Lilly Research Laboratories (Indianapolis, IN, USA) and Chiron (Trondheim, Norway). Olanzapine-d_3 was purchased from Toronto Research Chemicals Inc. (Toronto, Canada) whereas formic acid (100%) was obtained from VWR International (Trondheim, Norway). Other chemicals were of LC–MS, high-performance liquid chromatography (HPLC) or analytical grade from various commercial sources. Ultrapure water was obtained using a Barnstead Smart2Pure system (Thermo scientific, Germany) with a resistivity of 18.2 MΩ × cm at 25 °C and TOC values of 5–10 ppm. External quality control samples were obtained from LGC Standards Proficiency Testing (Bury, UK). Human blank serum and full blood were obtained from healthy blood donors not using any medication (St. Olav University Hospital, Trondheim, Norway).

All olanzapine concentrations are given in nM. The conversion factor from nmol/l to ng/ml is 0.312 for olanzapine and 0.315 for olanzapine-d_3. As the method was developed for routine TDM purposes, the inactive metabolite *N*-desmethylolanzapine was not included in the assay.

2.2. Stock solutions

Stock solutions of olanzapine from two different suppliers were prepared to a concentration of 1 mM in methanol, and the two stock solutions were diluted with methanol to yield working solutions for calibrators and quality control (QC) samples. The working solutions were used to spike calibrators and QCs in blank serum. The internal standard olanzapine-d_3 (Fig. 1b) was diluted in water with methanol (20% v/v) to a concentration of 800 nM and stored at 4–8 °C. All other solutions, calibrators and QCs were stored at −20 °C.

2.3. Sample preparation

Automatic sample preparation was performed using a Tecan Freedom Evo pipetting robot (Tecan Nordic, Mölndal, Sweden). Aliquots of serum samples (100 μL) and internal standards (25 μL) were pipetted onto an Ostro™ 96-well filtration plate (25 mg; Waters corp., Milford, MA, USA). Freeze cold acetonitrile with formic acid (1% v/v, 300 μL) was mixed with the samples for protein precipitation. The samples were then filtrated using a positive pressure unit (Positive Pressure-96, Waters) capturing the phospholipids and precipitated proteins in the filtration plate. The eluates were collected in 2 mL sample collection wells (96-well Square collection plate, Waters). After sealing the collection plate (Cap-mat square plugs, silicone/PTFE treated, pre-slit, Thermo Scientific VWR, Oslo, Norway), the samples were ready for UPLC–MSMS analysis.

2.4. Instrumentation

All experiments were carried out on a UPLC (Waters Acquity System, Waters) coupled to a tandem-quadrupole mass spectrometer (Xevo TQS, Waters).

2.4.1. UPLC conditions

Chromatography was performed using a Waters HSST3 column (2.1 × 100 mm, 1.8 μm) with pre-column and oven temperature 50 °C. The gradient elution was performed with a binary solvent system consisting of 0.1% formic acid in water (mobile phase A) and methanol (mobile phase B) at a flow rate of 0.5 mL/min. The gradient run was from 20% to 95% of mobile phase B within 1:25 min, and 45 s with 10% B was found sufficient to equilibrate the column before next injection. The injection volume was 0.5 μL and the autosampler temperature was set to 4 °C.

2.4.2. Mass spectrometry

The MS/MS-detection was performed with electrospray ionization (ESI) in the positive mode and multiple reaction monitoring (MRM). The capillary voltage was set to 3.0 kV, the source block temperature was 120 °C and the nitrogen desolvation gas was heated to 650 °C with a flow rate of 1000 L/h. The *m/z* 313.1 > 256.1 and 313.1 > 198.0 transitions (cone voltages 40 V, collision energy 20 eV) were monitored for olanzapine and *m/z* 316.1 > 256.1 transition (cone voltage 24 V, collision energy 22 eV) was monitored for olanzapine-d_3.

System operation and data acquisition were controlled using Mass Lynx 4.1 software (Waters). All data were processed with the Target Lynx quantification program (Waters). Olanzapine was identified by comparing the retention time of the corresponding calibrator and QC samples.

2.5. Method validation

Validation of the method included selectivity, specificity, calibration model, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, extraction recovery, matrix effects, sample dilution and stability.