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A high-performance liquid chromatography assay with ultraviolet detection for olanzapine in human plasma and urine

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Abstract

Olanzapine is a commonly used atypical antipsychotic medication for which therapeutic drug monitoring has been proposed as clinically useful. A sensitive method was developed for the determination of olanzapine concentrations in plasma and urine by high-performance liquid chromatography with low-wavelength ultraviolet absorption detection (214 nm). A single-step liquid–liquid extraction procedure using heptane-iso-amyl alcohol (97.5:2.5 v/v) was employed to recover olanzapine and the internal standard (a 2-ethylated olanzapine derivative) from the biological matrices which were adjusted to pH 10 with 1 *M* carbonate buffer. Detector response was linear from 1–5000 ng (r^2>0.98). The limit of detection of the assay (signal:noise=3:1) and the lower limit of quantitation were 0.75 ng and 1 ng/ml of olanzapine, respectively. Interday variation for olanzapine 50 ng/ml in plasma and urine was 5.2% and 7.1% (n=5), respectively, and 9.5 and 12.3% at 1 ng/ml (n=5). Intraday variation for olanzapine 50 ng/ml in plasma and urine was 8.1% and 9.6% (n=15), respectively, and 14.2 and 17.1% at 1 ng/ml (n=15). The recoveries of olanzapine (50 ng/ml) and the internal standard were 83±6 and 92±6% in plasma, respectively, and 79±7 and 89±7% in urine, respectively. Accuracy was 96% and 93% at 50 and 1 ng/ml, respectively. The applicability of the assay was demonstrated by determining plasma concentrations of olanzapine in a healthy male volunteer for 48 h following a single oral dose of 5 mg olanzapine. This method is suitable for studying olanzapine disposition in single or multiple-dose pharmacokinetic studies.

Keywords: Olanzapine

1. Introduction

Olanzapine is one of the most commonly used atypical antipsychotic medications in the United States for the treatment of schizophrenia and other psychotic disorders [1]. Plasma concentrations of antipsychotics have been shown to correlate with clinical response for some of the atypical agents [2], but there appears to be considerable interindividual variability in their pharmacokinetics [3]. Thus, therapeutic drug monitoring of patients taking antipsychotics such as olanzapine may in many cases provide a basis for individualized dosing, rather than

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empirically titrating dose against clinical response or side effects.

High-performance liquid chromatography (HPLC) currently represents a convenient and practical method for the analysis of olanzapine from biological samples. Initial reports of HPLC assays for olanzapine employed electrochemical or amperometric detection [4–6] to achieve the necessary sensitivity in the low nanogram range seen following oral dosing [3,7,8]. While electrochemical detection methods are useful and are certainly sensitive and specific for olanzapine, they tend to require higher maintenance and are less reproducible than methods using ultraviolet absorption or fluorescence detectors due to solute adsorption and degradation of the electrode surface over time [9]. Other assays reported for olanzapine have employed liquid chromatography–mass spectroscopy [10,11]. To date, only one method has been reported that employs ultraviolet absorption detection for olanzapine which was a normal-phase assay employing 270 nm detection [12]. This report describes an HPLC assay for olanzapine in human plasma that employs the convenience of reversed-phase operation with the sensitivity of low-UV wavelength detection without the capital outlay required for mass spectroscopy detection. The utility of the assay is demonstrated in a pharmacokinetic study in a healthy volunteer following oral dosing with a single oral low dose of olanzapine.

2. Materials and methods

2.1. Materials

Reference standards for olanzapine (LY170053) and a 2-ethylated analog (LY170222) as an internal standard, both 99.5% pure, were gifts from Eli Lilly and Company, Corporate Reference Standards (Indianapolis, IN). Their structures are shown in Fig. 1. HPLC grade acetonitrile and methanol (Burdick and Jackson Division, Muskegon, MI) and distilled, deionized water was used in mobile phases. All other reagents were of analytical grade or better. Drug-free human plasma and urine was collected from healthy volunteers by the authors, and frozen within 1 h of collection until required.

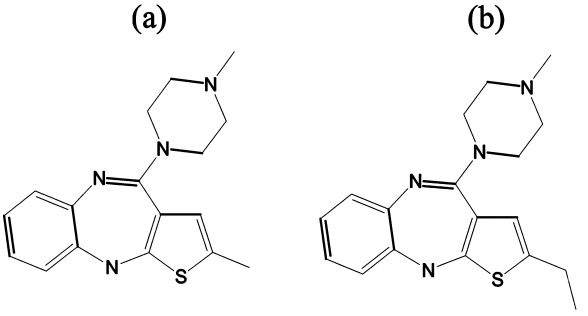

Fig. 1. Structures of (a) olanzapine (LY170053) and (b) the internal standard (LY170222).

2.2. Chromatography

The assay was performed on a Waters 2690 separations module (combined pump and autosampler, Waters, Milford, MA, USA). The mobile phase flow-rate was 1 ml/min and consisted of 10% methanol, 25% acetonitrile and 65% 50 m*M* phosphate buffer pH 6.0. The mobile phase was degassed under vacuum and filtered (0.45 μm) prior to use. The analytical column was a 25 cm×4.6 mm I.D. Supelcosil LC-CN (Supleco, Bellefonte, PA, USA). The assay was performed at ambient temperature. Detection of olanzapine was by ultraviolet detection at 214 nm (Waters 2487). Chromatographic data were acquired by a computerized integration system (Millenium^{32} 3.05, Waters). Olanzapine and the internal standard were identified from their peak retention times through comparisons with standard solutions.

2.3. Sample preparation

Olanzapine was extracted from biological samples by liquid–liquid extraction based on the method of Olesen and Linnet [12]. Briefly, in a 10 ml borosilicate test tube, 50 ng of internal standard (20 μl of a 2.5 μg/ml aqueous solution) was added to the biological sample (1 ml of human plasma or human urine). The mixture was then adjusted to pH 10 with 1 ml of 1 *M* carbonate buffer. Five milliters of heptane-iso-amyl alcohol (97.5:2.5 v/v) was then added, the tube capped, and the mixture was gently agitated on a shaker for 20 min at room temperature.

The mixture was then centrifuged at 2000 *g* for 5 min and the organic layer, which contained the analyte of interest, was aspirated into a clean tube and the solvent evaporated under a stream of nitrogen. The residue was reconstituted in 150 μl of mobile phase and 100 μl injected onto the HPLC.

2.4. Quantification and validation

Recovery values for the extraction procedure were calculated by comparing chromatographic responses obtained from spiked extracted samples (50 ng/ml, $n=5$ in both plasma and urine) and drug-free plasma and urine samples spiked with the same concentration of olanzapine immediately after extraction.

To obtain a standard curve for the assay, spiked samples were prepared by evaporating appropriate volumes of ethanolic solutions of olanzapine (1 mg/ml or 1 μg/ml) under a stream of nitrogen. Three independently prepared ethanolic stock solutions were used to obtain triplicate data. Six final concentrations ranging from 1, 2.5, 10, 100, 1000 and 5000 ng/ml in 1 ml aliquots of either drug-free human plasma or drug-free human urine were prepared. Fifty nanograms (20 μl of a 2.5 μg/ml aqueous solution) of the internal standard was added to all samples. The integrated chromatogram peak areas of olanzapine and the internal standard were used to construct a standard curve from the peak area ratio versus nominal olanzapine concentration using linear regression analysis with $1/x$ weighting. The estimated concentrations of olanzapine in unknown samples used the slope of the standard curve generated in the same analytical run. The lower limit of quantitation was defined as the lowest concentration of standard that was above the limit of detection. The specificity of the assay was determined by analyzing extracted drug-free plasma from six different individuals and examining the resultant chromatograms for interfering peaks.

Drug-free spiked plasma samples at 1 and 50 ng/ml of olanzapine, which are concentrations in the range of clinical interest, were used as quality control standards. Intraday variation of the assay was assessed by injecting five samples at each concentration on the same day. Interday variation was assessed by injecting a further five samples of each concentration on two subsequent days.

2.5. Single oral dose pharmacokinetic study

The pharmacokinetics of olanzapine was examined in a 24-year-old, 72 kg male volunteer judged healthy following a physical exam and routine blood biochemistry. The protocol was approved by the Medical University of South Carolina Institutional Review Board and the volunteer gave written informed consent to participate in the study. A single 5 mg dose of olanzapine (Zyprexa®, Eli Lilly) was administered orally following an overnight fast. Venous blood samples were collected frequently for 48 h following olanzapine administration. Urine was collected cumulatively for 24 h following administration of olanzapine. Plasma (3 ml) and an aliquot of urine (20 ml) were stored immediately following collection at −80°C until analyzed. Blood was collected via a catheter in a forearm vein or by venepuncture into 7 ml vacuum tubes containing heparin (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) and the plasma was collected following centrifugation. Plasma samples were split, with 1 ml being analyzed by the method reported here and the remainder analyzed by a contract research laboratory (National Medical Services, Willow Grove, PA) by an unpublished validated HPLC–MS method. The HPLC–MS results were compared to the results of the assay reported here as a secondary confirmation of its accuracy. Urine was assayed for olanzapine by HPLC–UV alone.

2.6. Data analysis

Separate linear regression analysis was performed for standard curves and for comparison between the plasma olanzapine concentration values obtained by method reported here and those found by the HPLC–MS assay. The Student's *t*-test was employed to test if the *y*-intercept of the line of best fit was different from zero. The level of significance was set at $P=0.05$. Data are reported as mean±SD.

3. Results and discussion

3.1. Chromatography

Representative chromatograms from blank plasma, spiked plasma and a plasma sample obtained follow-

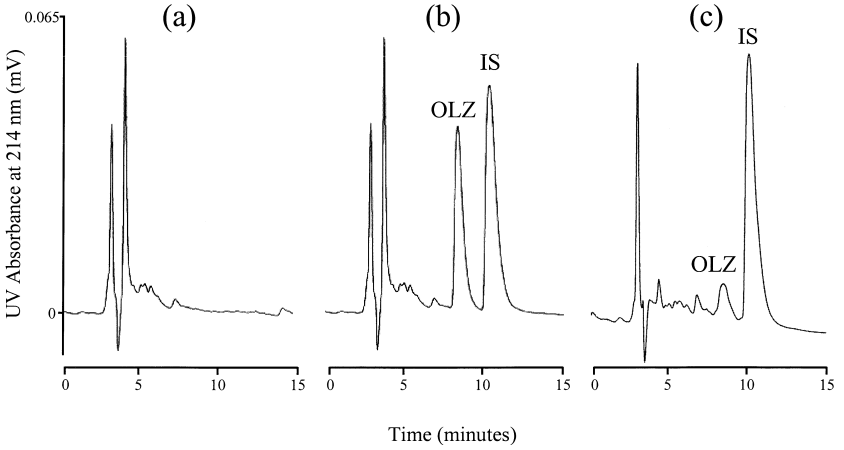

Fig. 2. HPLC chromatograms of (a) a blank extracted plasma sample (b) a drug-free extracted plasma sample spiked with 50 ng olanzapine and 50 ng of the internal standard (IS) and (c) an extracted plasma sample collected 5 h following a 5 mg dose of olanzapine to a healthy volunteer, calculated to contain 6.8 ng/ml olanzapine.

ing oral administration of olanzapine to a healthy volunteer are shown in Fig. 2. The blank was free of interference at the retention time of the analytes of interest. It was found that 214 nm provided the optimum limit of detection (signal:noise) under the conditions employed.

3.2. Quantification and validation

The recoveries of olanzapine (50 ng/ml) and the internal standard were 83±6 and 92±6% in plasma, respectively, and 79±7 and 89±7% in urine, respectively (all $n=5$ at 50 ng/ml). The limit of detection of the assay (signal:noise=3:1) was 0.75 ng of olanzapine. The lower limit of quantification (the lowest concentration of spiked standard employed) from 1 ml of biological fluid was 1 ng/ml. Interday variation for olanzapine 50 ng/ml in plasma and urine was 5.2% and 7.1% ($n=5$), respectively, and 9.5 and 12.3% at 1 ng/ml ($n=5$). Intraday variation for olanzapine 50 ng/ml in plasma and urine was 8.1% and 9.6% ($n=15$), respectively, and 14.2 and 17.1% at 1 ng/ml ($n=15$). The calibration curves for olanzapine in both plasma and urine matrices were linear over the range of 1 to 5000 ng/ml ($r^2>0.98$, $P<0.001$) and the *y*-intercepts of their lines of best fit was not significantly different from zero ($P>0.05$, Student's *t*-test). No substantial peaks were observed in extracted drug-free plasma from six different individuals. Standard curves were included in each day's run. Accuracy at 50 ng/ml was 96% (mean of found/added, $n=5$) and 93% at 1 ng/ml ($n=5$).

3.3. Single oral dose pharmacokinetic study

The assay method was able to detect the relatively low (less than 10 ng/ml) plasma concentrations of olanzapine for at least 48 h following a low (5 mg) oral dose. The plasma concentration vs. time profile is shown in Fig. 3a. The correlation between the olanzapine concentrations estimated from the same plasma samples independently-analyzed by HPLC–UV and HPLC–MS methods was satisfactory ($y=0.94x+0.3409$, $r^2=0.79$, $P<0.001$) and the relationship is shown in Fig. 3b. The amount of olanzapine excreted unchanged in the urine was 63 μg over 24 h and was determined by HPLC–UV analysis only.

4. Summary

The assay reported here represents a convenient and sensitive alternative to electrochemical methods of detection for olanzapine. This assay compliments a previously published normal-phase HPLC method using UV absorbance detection and demonstrates an improvement in sensitivity by employing a lower UV wavelength and more conventional reversed-phase

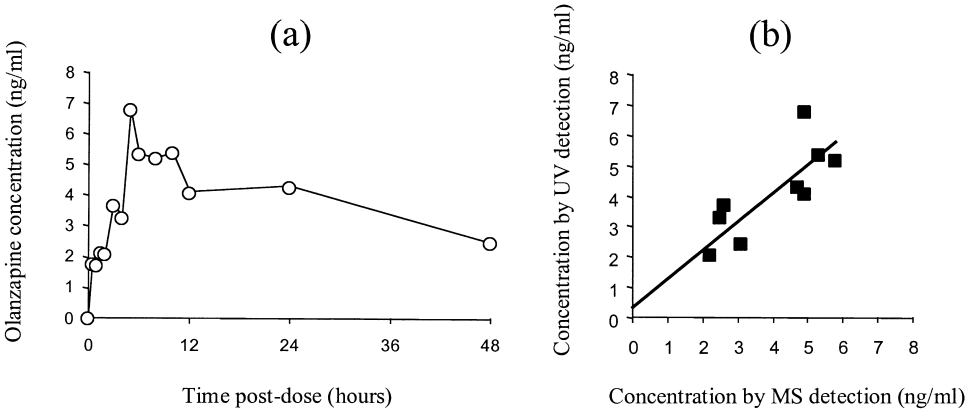

Fig. 3. (a) Plasma concentration vs. time profile for olanzapine following a 5 mg dose to a healthy volunteer and (b) comparison between the ultraviolet detection method presented in this report and an independent validated liquid chromatography/mass spectroscopy assay.

methodology. In addition, the assay reported here will be useful for labs that do not have LC–MS equipment. While this assay will need to be validated for potential interference by any known co-administered medication on a case-by-case basis, it should be useful in pharmacokinetic studies examining olanzapine-induced side effects, therapeutic failure, patient compliance, or to examine for formulation bioequivalence or potential drug–drug interactions.

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