A Liquid Chromatographic-Electrospray-Tandem Mass Spectrometric Method for Quantitation of Quetiapine in Human Plasma and Liver Microsomes: Application to a Study of In Vitro Metabolism

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Abstract

Quetiapine is an atypical antipsychotic agent for the treatment of schizophrenia. After an oral dose it is absorbed rapidly and extensively metabolized in the liver, resulting in low plasma concentrations of the parent drug. A sensitive analytical method is needed. A liquid chromatographic-electrospray-tandem mass spectrometric (LC-ESI-MS-MS) method combined with a simple liquid-liquid extraction has been developed for the measurement of quetiapine in human plasma and in human liver microsomes (HLM). Clozapine is used as internal standard. Plasma samples or microsomes quenched with methanol (100 µL) were made basic and extracted with 3 mL n-butyl chloride. The reconstituted extracts were analyzed by LC-ESI-MS-MS. Selective reaction monitoring of MH+ at m/z 384 and 327 resulted in strong fragment ions at m/z 253 and 192 for quetiapine and clozapine, respectively. Recovery of quetiapine and clozapine ranged from 62 to 73%. Intrarun accuracy and precision determined at 1.0 (lower limit of quantitation), 2.5, 200, and 400 ng/mL did not exceed 7% deviation from target and the %CV did not exceed 5.5%. The % target %CV for interrun accuracy and precision were at least 95% ± 7.4% at concentrations of 2.5, 200, and 400 ng/mL. Plasma samples (2.5 and 400 ng/mL) stored at room temperature for 24 h or after 3 cycles of freeze/thaw were all stable (maximum % deviation ≤ 11.0%). Processed extracts (2.5 and 400 ng/mL) stored for 7 days at -20°C or 6 days on the autosampler were all stable (maximum % deviation ≤ 11.5%). The method has been used to study quetiapine utilization during incubation with HLM or with cDNA-expressed human cytochrom P450s (CYP). Quetiapine is extensively metabolized by CYP 3A4 and CYP 2D6 and to a lesser extent by CYP 3A7, CYP 3AS, and CYP 2C19.

Introduction

Quetiapine (Seroquel), a dibenzothiazepine derivative, is an atypical antipsychotic with demonstrated efficacy for treatment of acute schizophrenia (1). Quetiapine's relatively benign tolerability profile distinguishes it from other commonly used atypical agents, particularly with respect to changes in body-weight, extrapyramidal symptoms, and levels of plasma prolactin. For these reasons, it is considered a suitable first-line option for the treatment of schizophrenia (1). The pharmacokinetics of a single 10-mg oral dose of quetiapine in healthy volunteers showed a Cmax of 28 ng/mL at 1.5 h and an apparent terminal elimination half-life of 3.45 h (2).

Quetiapine is extensively metabolized in the liver. A preliminary abstract (3) on in vitro experiments indicated that CYP3A4 and CYP2D6 were the main CYP enzymes involved in the metabolic degradation. Metabolic interactions with inducers or inhibitors of CYP 3A4 and CYP 2D6 can have significant effects on the pharmacokinetics of quetiapine (4-6). The combined data suggest that an analytical method with high sensitivity (low ng/mL) and specificity and fast sample throughput time for quantitation of quetiapine would be desirable for additional studies on the metabolism of quetiapine.

Quetiapine is considered to have little propensity for toxic reactions (1). However, somnolence is a common side effect; this leads to warnings concerning driving and use of heavy equipment until tolerance for the drug has developed. Inhibition of quetiapine metabolism may enhance this effect or re-instate it in individuals believed to have become tolerant. Quetiapine has been reported to cause false-positive results with immunoassays for tricyclic antidepressants (7). Clarification of such cases presents forensic toxicologists with yet another reason for quantitative determination of quetiapine.

At present four liquid chromatographic (LC) methods (8-11), one gas chromatography–mass spectrometry (GC–MS) (8) and two LC–MS methods (8,12) for determination of quetiapine and/or metabolites in human plasma have been described. The LC methods (8,10,11) provide linear responses from 50 to 5000, 2.5 to 500, and 4 to 500 ng/mL, respectively. All LC methods, even those for analysis of the parent drug only, require long
analytical times (> 14 min). The GC–MS method provides a linear response from 2.12 to 106 ng/mL and requires a 14-min cycle. The most recent published selective ion monitoring (SIM) LC–ESI-MS method (12) provides a linear response from 10 to 2000 ng/mL with a sample-throughput time of > 15 min to allow simultaneous determination of quetiapine and three metabolites in human plasma.

We report a new, validated LC–ESI-MS–MS method for quantitation of quetiapine in human plasma that includes a simple liquid–liquid extraction. The method is more sensitive than any published procedures, has a faster cycle time, and is suitable for the quantitation of quetiapine in human liver microsomes (HLM). We have used it to study quetiapine utilization in HLM and cDNA-expressed human cytochrome P450s (CYPs), with results that demonstrate the primary involvement of CYP 3A4 and CYP 2D6 in the metabolism of quetiapine in vitro.

Experimental

Materials

Quetiapine fumarate (ICI 204,636, Seroquel) was provided by AstraZeneca (Macclesfield, U.K.). Clozapine for use as internal standard and ketoconazole were purchased from ICN Biochemicals (Aurora, OH). HPLC-grade methanol and n-butyl chloride were purchased from Burdick & Jackson (Muskegon, MI) and formic acid from J.T. Baker (Phillipsburg, NJ). NaOH pellets were purchased from Mallinckrodt Specialty Chemicals Inc. (St Louis, MO). Diluted bases and acids were prepared with Milli Q water. The LC column was a 50 × 2 mm YMC Materials and the LC–ESI-MS–MS instrument performance was evaluated by injecting a 10-pL solution consisting of 0.33 ng/pL each of quetiapine and clozapine.

Calibrator and control preparation

Calibrator samples were prepared in duplicate just prior to extraction by spiking 0.1 mL human plasma with quetiapine to give concentrations of 1, 2.5, 5, 10, 30, 90, 150, 250, and 500 ng/mL. Quality-control samples (QCs) were prepared in advance at concentrations of 2.5, 200, and 400 ng/mL; 100-μL aliquots were placed in 16 × 100-mm silanized borosilicate glass tubes and stored at −20°C until removed for analysis.

Extraction

QCs and appropriate study samples were removed from the freezer and allowed to thaw at room temperature. As soon as the QCs and study samples were thawed, these samples along with freshly prepared calibrators were spiked with internal standard solution to give 25 ng/mL. Samples were made basic by adding 25 μL 1 N NaOH, vortex mixed, and extracted with 3 mL n-butyl chloride. The n-butyl chloride layers were transferred into 3 × 100-mm glass tubes and evaporated to dryness under a stream of air at 40°C. The residues were reconstituted in 200 μL of 0.1% formic acid in water and methanol (90:10) and transferred to labeled autosampler vials.

LC–ESI-MS–MS

Analyses were performed using a Finnigan TSQ Quantum (Thermo Electron Corporation, San Jose, CA). The gradient LC program was as follows: flow rate was constant at 0.2 mL/min; solvent A was 0.1% formic acid in water and solvent B was HPLC-grade methanol; the initial mobile-phase composition was 40% B and held at this composition for 2 min; % B was then linearly increased to 90% in 50 s and held at this composition for 3 min; then the % B was returned to 40% in 10 s and remained at this composition for 2 min before the next cycle of analysis. This process was repeated every 8 min until the last sample of the batch was analyzed, at which time, a final solvent sample was injected. The LC program proceeded forward to include at least 87 min of column washing with 100% B, and returned to 40% B for 10 min before the LC flow was stopped.

A standard auto-tune of the Finnigan TSQ Quantum was performed before each new batch run, using an infusion containing quetiapine and clozapine at 0.5 ng/μL. First the TSQ Quantum was auto-tuned to maximize the MH+ ions of quetiapine and clozapine at m/z 384 and 327, respectively. Then the auto-tune proceeded to maximize the selective reaction monitoring (SRM) ions of m/z 384 to 253 for quetiapine and of m/z 327 to 192 for clozapine. Finally, before each batch run the instrument performance was evaluated by injecting a 10-μL solution consisting of 0.33 ng/μL each of quetiapine and clozapine.

Quantitation

The LCquan software of the Quantum TSQ provided a standard method for quantitative calculations. The peak areas for all SRMs were automatically integrated, and the area ratios (quetiapine/clozapine) of the calibrators were used to generate quadratic response curves with 1/Y^2 weighting. The response curves were used to calculate the concentrations of calibrators, QCs and study samples.

In vitro metabolic experiment

Studies with HLM and cDNA-expressed CYPs were carried out following incubation protocols previously described (13,14). A final volume of 500 μL contained 0.25 mg HLM or 25 pmole CYP, 0.1M phosphate buffer (pH 7.4), 1.0mM EDTA, 5.0mM MgCl2, and a NADPH-generating system which was composed of 10mM D-glucose-6-phosphate, 1.2mM β-NADP, and 1.2 units of glucose-6-phosphate dehydrogenase. Quetiapine (250 ng/mL unless noted otherwise) was added to the mixture and incubated for the times indicated. Reactions were terminated by addition of 200 μL methanol. These samples were stored at −75°C until removed for analysis.
sommes were used at the mean protein concentration averaged over all of the CYPs. Inhibition experiments were carried out using three different sources of HLM. Quetiapine utilization was determined in the presence of 10μM quinidine, a specific inhibitor for CYP 2D6; 2μM ketoconazole, a specific inhibitor for CYP 3A4; or 15μM TAO, a mechanism-based inhibitor of CYP 3A4. Quinidine or ketoconazole was added to the incubation mixture at the same time as quetiapine. TAO was incubated with the HLM and NADPH-generating system for 15 min before mass spectrum of clozapine (MH+ at 327 as the precursor ion) showed. Six product ions with % relative ion intensities > 5 were detected: m/z 158 (6.5%), 210 (11%), 221 (56%), 247 (13%), 253 (100%), and 279 (23%). The most intense ion at m/z 253 was selected for use in the SRM scan mode. The product-ion mass spectrum of clozapine (MH+ at 327 as the precursor ion) showed m/z 192 (100%) to be the major ion. Three additional ions were detected with % relative ion intensity > 5: m/z 138 (18%); 164 (62%), and 226 (6%). The m/z 192 was used as the SRM ion.

Chromatography
SMR chromatograms of blank plasma (Figure 1A) fortified with internal standard (Figure 1C) showed a small peak at the retention time for quetiapine. A sharp peak was seen when quetiapine was added at the lower limit of quantitation (LLOQ) of 1 ng/mL (Figure 1B). The ratio of the peak areas between the spiked LLOQ and the blank plasma in this case is greater than 18-fold, suggesting that background interference has minimum impact on the quantitation of quetiapine.

Calibration and linearity
Calibrators ranging from 1 to 500 ng/mL were run in duplicate for each batch analysis. A separate quadratic-response curve was generated for each run during validation. The response curve was used to recalculate the calibrator concentrations; the mean recalculated calibrator concentrations did not deviate more than 6.7% from the target concentrations and the %CVs did not exceed 5.6%. The parameters of the quadratic curves were fairly consistent, that is, r² = 0.997 when all three separate curves generated during validation were compared.

Recovery
Recovery of quetiapine was calculated by comparing the peak area of the analyte from extracted plasma standards to peak areas obtained from unextracted standard at the same concentrations. Quetiapine recovery was tested at concentrations of 2.5, 200, and 400 ng/mL; clozapine recovery was tested at 250 ng/mL, and five extracted and unextracted samples at each concentration were compared. The mean values of the five peak areas at each concentration were used to calculate percent recovery. For quetiapine, the percent recoveries were as follows: 72.9% at 2.5 ng/mL, 62.0% at 200 ng/mL, and 70.1% at 400 ng/mL. The recovery for clozapine was 71.3%.

Specificity
Plasma from six separate sources was used for validation. For each individual source of plasma, three aliquots were spiked with only the internal standard; one aliquot was spiked with the internal standard and quetiapine at the LLOQ (1 ng/mL); and one aliquot remained blank. Chromatograms of all blank plasma samples involved in the specificity study showed small detectable peaks at the retention time of quetiapine (Figure 1A). Detectable peaks at the retention time of clozapine were also present in the chromatograms of blank plasma not fortified with this compound (not shown); these detectable peaks also appear in chromatograms when solvents used to reconstitute sample extracts were injected. We concluded that these peaks resulted from minor background interference persisting within the LC system that became detectable because of the high sensitivity of the TSQ quantum. These small background peaks expressed as percent of the extracted LLOQ ranged from 1.23 to 2.16% with the highest individual peak at 3.27%. The trace amounts of background interference, while detectable, had minimal impact on the quantitation of quetiapine in human plasma.
Interference study

As patients taking quetiapine may also take many different drugs, we tested potential interferences of these drugs on the analytical method, using four sets of samples each containing three QCs at concentrations of 2.5 ng/mL. Twenty commonly prescribed, over-the-counter, and/or illicit drugs were divided into four groups. All drugs in each group were collectively spiked to the QCs of the set, at concentrations that exceeded twice the maximum plasma concentration determined from standard references or experience in our lab. (Table I). The presence of these compounds had no significant effect on quantitation of quetiapine. Measured concentration did not deviate more than 10.4% from targets, and % CVs did not exceed 13.6% (Table I).

Accuracy and precision

Intrarun precision and accuracy expressed as % target ± %CV were determined from analyses of QCs at 1.0, 2.5, 200, and 400 ng/mL. The quantitative results from six different sources of human plasma spiked with LLOQ quantities of quetiapine used in the specificity study gave a % target ± %CV of 98.0 ± 5.1. At other concentrations, the % target ± %CV of five replicates at concentrations of 2.5, 200 and 400 ng/mL were 95.2 ± 4.6, 93.0 ± 5.4, and 94.8 ± 5.5, respectively.

Intererrun precision and accuracy were determined from three separate runs of QCs each with five replicates. The % target ± %CV averaged over all the 15 replicates at each fortified concentration were as follows: 96.0 ± 4.2 at 2.5 ng/mL, 95.0 ± 7.4 at 200 ng/mL and 95.3 ± 5.2 at 400 ng/mL.

Stability

Davis et al. (10) reported long-term stability of quetiapine in fortified human plasma stored at -20°C for 15 months. The stability of quetiapine in stock solutions prepared in methanol/water (50:50) was determined by comparing peak areas of a freshly made stock to a 42-day-old stock stored at -20°C and to a portion of the new stock stored at room temperature for 19 h. Five replicates at each condition were analyzed. Peak-area ratios for the 42-day old stock and 19-h room temperature-stored stock were 98.9 and 97.8% of the fresh stock, respectively.

In vitro metabolism of quetiapine

The only published information available on the in vitro metabolism of quetiapine by human CYPs is limited to a preliminary communication that suggests the primary involvement of CYP 3A4 and CYP 2D6 (3). We therefore used our new analytical method to perform an initial study on the in vitro metabolism of quetiapine. As we did not have reference material available for quetiapine metabolites, the study was limited to the utilization of quetiapine in either HLM or cDNA-expressed human CYPs. When quetiapine was incubated with HLM and an NADPH-generating system we observed a concentration- and time-dependent loss of quetiapine. For starting concentrations of 120 and 250 ng/mL, this loss was linear over the 30-min time course investigated; at concentration of 400 ng/mL, a break from linearity was noted after 10 min of incubation (Figure 2A). The 250 ng/mL concentration was used for subsequent experiments in which quetiapine was incubated with cDNA-expressed human CYPs.

Table I. Interference Study*

<table>
<thead>
<tr>
<th>Interferant</th>
<th>Concentration (ng/mL)</th>
<th>Interferant</th>
<th>Concentration (ng/mL)</th>
<th>2.5 ng/mL QC (% Target ± %CV)</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>Set 1 (n = 3)</td>
<td></td>
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<tr>
<td>Buprenorphine</td>
<td>200</td>
<td>Norbuprenorphine</td>
<td>200</td>
<td>110.4 ± 13.0</td>
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<td>Methadone</td>
<td>500</td>
<td>EDDP</td>
<td>500</td>
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<tr>
<td>Morphine</td>
<td>1000</td>
<td>Codeine</td>
<td>1000</td>
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<tr>
<td>Cocaine</td>
<td>1500</td>
<td>Benzoylegonine</td>
<td>1500</td>
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<tr>
<td>Egonine methyl ester</td>
<td>1500</td>
<td></td>
<td></td>
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<td>Set 2 (n = 3)</td>
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<td>Salicylic acid</td>
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<td>Acetaminophen</td>
<td>30,000</td>
<td>104.4 ± 7.3</td>
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<tr>
<td>Ibuprofen</td>
<td>100,000</td>
<td>Ketoconazole</td>
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<tr>
<td>Set 3 (n = 3)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Triazolam</td>
<td>40</td>
<td>Alprazolam</td>
<td>100</td>
<td>91.2 ± 13.2</td>
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<tr>
<td>Oxazepam</td>
<td>300</td>
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<td></td>
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<tr>
<td>Diphenhydramine</td>
<td>200</td>
<td>Psuedoephedrine</td>
<td>1500</td>
<td></td>
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<tr>
<td>Set 4 (n = 3)</td>
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</tr>
<tr>
<td>Fluoxetine</td>
<td>10,000</td>
<td>Paroxetine</td>
<td>10,000</td>
<td>91.6 ± 10.9</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>10,000</td>
<td></td>
<td></td>
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</table>

* Samples were prepared at the same concentration as the low quality control. Each set was fortified with the drugs listed above to achieve the listed concentrations. No significant difference was found in comparison of sets using one-way analysis of variance (p < 0.05). EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
and troleandromycin, significantly reduced quetiapine utilization by 90 and 67%, respectively. The CYP 2D6 inhibitor, quinidine, reduced quetiapine utilization by 60% (Figure 2C).

Conclusions

Quetiapine can be quantified from 0.1-ml aliquots of human plasma using liquid–liquid extraction and LC–ESI-MS-MS with acceptable precision and accuracy down to a LLOQ of 1.0 ng/mL. The study also demonstrates that quetiapine is stable in plasma samples or in processed extracts under the following experimental conditions: plasma samples, storage at room temperature for 24 h or 3 freeze/thaw cycles separated by 12-h intervals; for processed samples, storage at –20°C or on the autosampler for 6–7 days. The method has been successfully applied to a study of quetiapine metabolism using HLM or cDNA-expressed CYPs. The results demonstrated that CYPs 3A4 and 2D6 are the primary CYPs involved in hepatic metabolism of quetiapine. Co-administration of quetiapine with CYP 3A4 inducers or with 2D6 and 3A4 inhibitors, or use of quetiapine in poor or ultrarapid 2D6 metabolizers, should be approached with suitable caution.

Table II. Stability of Quetiapine in Human Plasma and in Processed Samples

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>2.5 ng/mL</th>
<th>400 ng/mL</th>
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</thead>
<tbody>
<tr>
<td>Fortified plasma at</td>
<td>% target ± %CV</td>
<td>% target ± %CV</td>
</tr>
<tr>
<td>RM Temp for:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-h</td>
<td>94.4% ± 3.8%</td>
<td>89.3% ± 3.6%</td>
</tr>
<tr>
<td>24-h</td>
<td>90.4% ± 3.5%</td>
<td>90.0% ± 1.1%</td>
</tr>
<tr>
<td>3 freeze/thaw Cycles</td>
<td>96.8% ± 1.7%</td>
<td>89.0% ± 2.2%</td>
</tr>
<tr>
<td>Processed Samples Stored:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days at –20°C</td>
<td>97.6% ± 2.9%</td>
<td>90.5% ± 6.6%</td>
</tr>
<tr>
<td>6 days on Autosampler</td>
<td>97.6% ± 3.3%</td>
<td>88.5% ± 7.1%</td>
</tr>
</tbody>
</table>

Figure 2. In vitro metabolism of quetiapine. Quetiapine utilization during incubation with HLM and an NADPH generating system at the following concentrations of quetiapine: O, 400 ng/mL; A, 250 ng/mL; and Q, 120 ng/mL. Data are the mean of duplicate incubations from a single source of microsomes (A). Quetiapine utilization during incubation with 25 pmol of cDNA-expressed CYPs and an NADPH generating system. Data are the mean of duplicate incubations per CYPs; quetiapine starting concentration was 250 ng/mL. Control microsomes were added at the mean protein concentration of the other P450s (B). The effect of the P450 2D6 (10μM quinidine) and P450 3A4 (12μM ketoconazole and 15μM TAO inhibitors on quetiapine utilization in HLM. Inhibitors were added to HLM and an NADPH-generating system; quetiapine was added at this time, except for the TAO experiment where it was added after a 15-min preincubation. Data are the mean ± SD for three different sources of microsomes each incubated in duplicate (C).

Acknowledgments

The authors are thankful to Ruth Foltz for critical reading of the manuscript. This work was supported by NIDA contract N01DA-3-8829 and NIDA grant R01-DA-10100.

References

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