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Analysis and pharmacokinetics of quetiapine and two metabolites in human plasma using reversed-phase HPLC with ultraviolet and electrochemical detection

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Abstract

A sensitive and specific HPLC assay for the measurement of the antipsychotic compound quetiapine in human plasma has been developed and validated. The assay employs a three-step liquid–liquid extraction of quetiapine and its 7-hydroxylated and 7-hydroxylated, *N*-dealkylated metabolites from human plasma, and utilizes ultraviolet (UV) detection of quetiapine and electrochemical detection of the metabolites. The method provides a linear response from a quantitation limit of 2.50 to 500 ng ml^{-1} for each analyte using 0.4 ml plasma. The assay is applicable from 500 to 5000 ng ml^{-1} by sample dilution with de-ionized water. The inter-assay precision of quetiapine in plasma calibration standards across 4 validation days averaged 11.9% relative standard deviation (RSD) over the range 2.50 to 500 ng ml^{-1}, with intra-assay precision averaging 16.0% RSD and mean accuracy of 98.6% of theory. Similarly, the inter-assay precision of the 7-hydroxylated metabolite in plasma calibration standards across 4 validation days averaged 13.7% RSD over the range 2.50 to 500 ng ml^{-1}, with intra-assay precision averaging 17.6% RSD and mean accuracy of 109% of theory. The 7-hydroxylated, *N*-dealkylated metabolite demonstrated inter-assay precision of 16.2% RSD, intra-assay precision of 19.9% RSD, and mean accuracy of 104% of theory over the range 2.50 to 500 ng ml^{-1}. The present assay method was used to support a study comparing the pharmacokinetic profile of quetiapine with the time course of dopamine D_2 and serotinin 5-HT_2 receptor occupancy in the brain using positron emission tomography (PET). We describe in this paper the bioanalytical method and the plasma concentrations of quetiapine and its metabolites resulting from this study.

Keywords: Quetiapine; Metabolites; Plasma; HPLC; Antipsychotics; Positron emission tomography

1. Introduction

Quetiapine fumarate ('Seroquel'; bis[2-(2-[4-(dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl] ethoxy) ethanol] fumarate) is a dibenzothiazepine derivative developed by Zeneca Pharmaceuticals

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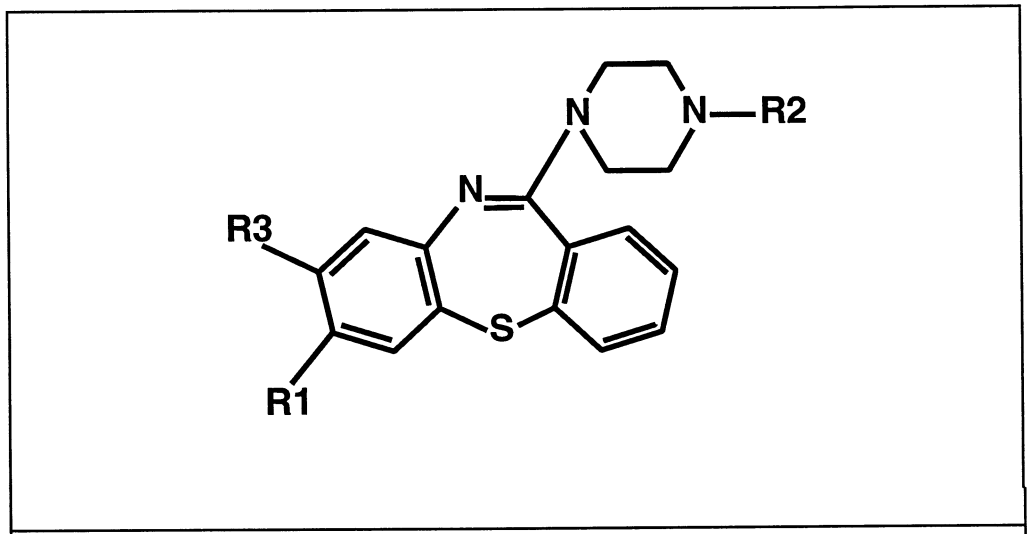
and approved for the management of manifestation of psychotic disorders. The preclinical profile of quetiapine predicts that it would have the pharmacologic properties of atypical antipsychotics [1]. This is supported by the results of clinical studies which indicate that, in the therapeutic dose range of 150 to 750 mg day^{-1}, quetiapine is effective in improving the positive and negative symptoms of schizophrenia, and that quetiapine treatment is not associated with sustained elevation of plasma prolactin concentrations and induction of extrapyramidal symptoms (EPS) across the clinical dose range [2]. To investigate the pharmacokinetics of quetiapine in humans, a sensitive and specific assay for quetiapine and two of its active metabolites was required. A previously developed HPLC method [3] performed well for quetiapine, but lacked sensitivity to support low-dose studies and was non-reproducible for the 7-hydroxylated metabolite. A GC/MSD method was developed [3] which afforded sensitivity, but lacked ruggedness. This HPLC procedure has been successfully developed and validated, with a quantitation limit of 2.50 ng ml^{-1} for each analyte in human plasma. The assay employs a three-step liquid–liquid extraction of quetiapine and its 7-hydroxylated (ICI 214,227) and 7-hydroxylated, *N*-dealkylated (M 236,303) metabolites from human plasma, and utilizes ultraviolet (UV) detection of quetiapine and electrochemical detection of the metabolites (Fig. 1). An example from our laboratory of the application of this methodology to the determination of the pharmacokinetics of quetiapine and its



COMPOUND	R1	R2	R3
Quetiapine	H	$(CH_2)_2OCH_2CH_2OH$	H
ICI 214,227	OH	$(CH_2)_2OCH_2CH_2OH$	H
M 236,303	OH	H	H
M 214,652	H	$(CH_2)_2OCH_2CH_2OH$	Cl

Fig. 1. Chemical structures of quetiapine, ICI 214,227 (7-hydroxylated metabolite of quetiapine), M 236,303 (7-hydroxylated, *N*-dealkylated metabolite of quetiapine) and M 214,652 (internal standard).

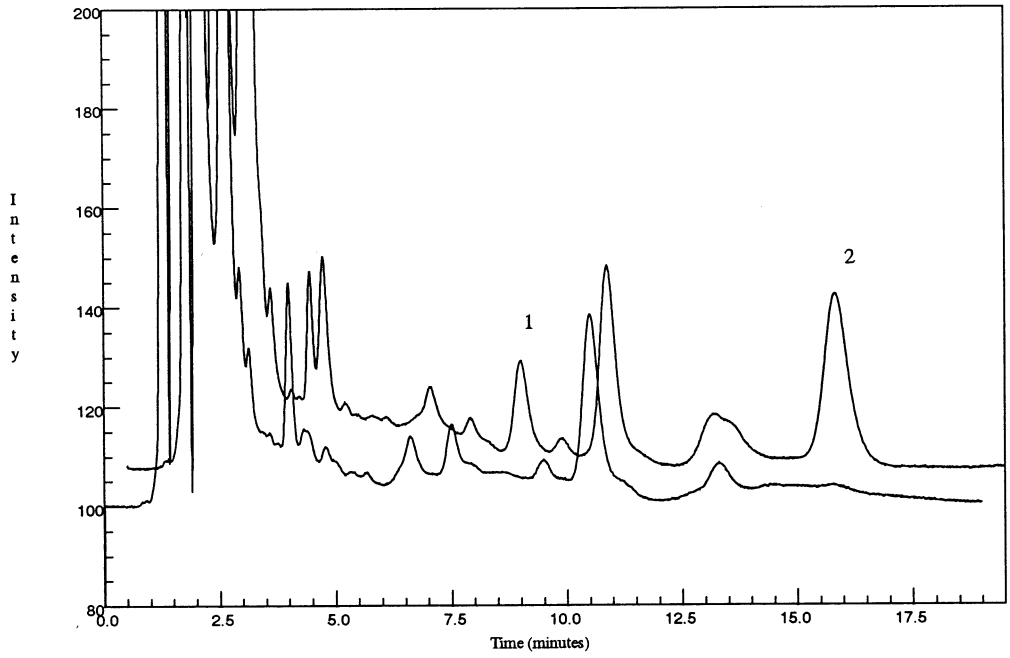


Fig. 2. Chromatogram of extracted, control human plasma with 20 ng ml^{-1} (top) and without (bottom) quetiapine (1) and M 214,652 (2) (internal standard).

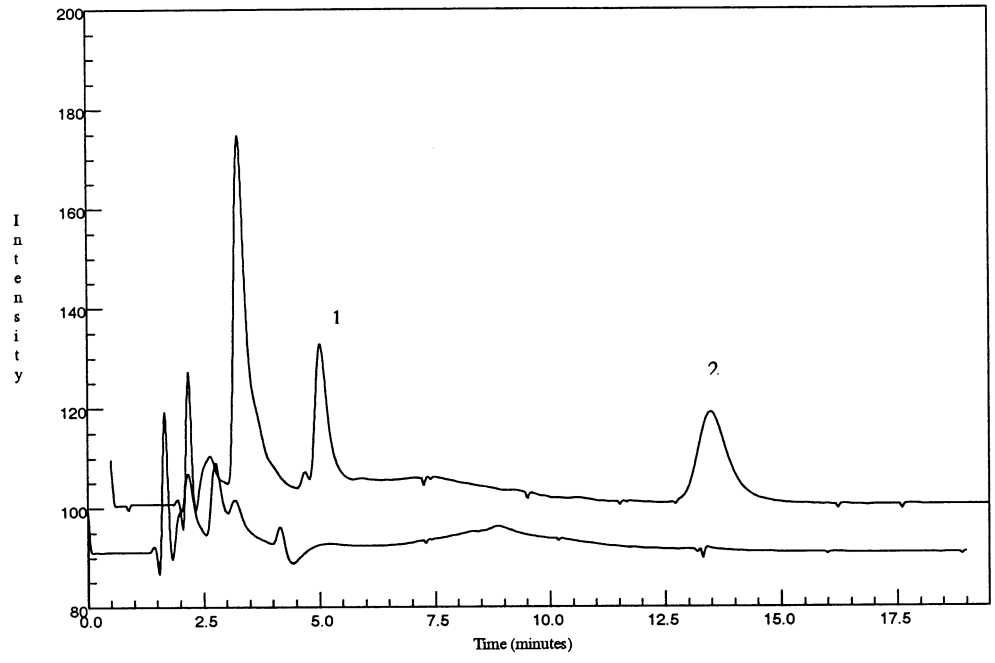


Fig. 3. Chromatogram of extracted, control human plasma with 20 ng ml^{-1} (top) and without (bottom) ICI 214,227 (1) (7-hydroxylated metabolite of quetiapine) and M 236,303 (7-hydroxylated, *N*-dealkylated metabolite of quetiapine).

metabolites in human plasma with the time course of dopamine D_2 and serotinin 5-HT_2 receptor occupancy in the brain using positron emission tomography (PET) is presented. The PET method and results will be presented in a separate paper.

Table 1
Validation of quetiapine analysis in human plasma calibration standards

Concentration (ng ml^{-1})	Pooled mean (ng ml^{-1})	Accuracy (%)	Intra-assay precision (% RSD)	Inter-assay precision (% RSD)	Recovery (%)
2.50	2.08	83.2	25.7	22.0	39.1
5.00	4.14	82.8	20.8	13.0	35.4
10.0	9.90	99.0	24.4	9.3	42.9
20.0	21.3	106	10.3	13.9	41.3
50.0	52.3	105	3.2	9.7	44.4
100	113	113	11.5	10.9	47.2
500	505	101	3.8	4.6	43.0
Mean		98.6	14.2	11.9	41.9

Table 2
Validation of quetiapine analysis in human plasma quality control and dilution samples[a]

Concentration (ng ml^{-1})	Pooled mean (ng ml^{-1})	Accuracy (%)	Intra-assay precision (% RSD)	Inter-assay precision (% RSD)
8.00	7.27	90.9	7.8	9.0
25.0	22.2	88.8	8.0	8.1
60.0	64.5	108	3.4	16.2
150	151	101	7.8	10.3
400	422	106	8.7	14.8
Mean		98.9	7.1	11.7
2000	2370	119	9.0	NC
5000	5900	118	16.4	NC
Mean		119	12.7	NC

[a] NC, not calculated

Table 3
Validation of ICI 214,227 analysis in human plasma calibration standards

Concentration (ng ml^{-1})	Pooled mean (ng ml^{-1})	Accuracy (%)	Intra-assay precision (% RSD)	Inter-assay precision (% RSD)	Recovery (%)
2.50	3.32	133	8.5	27.0	60.9
5.00	5.56	111	28.4	8.6	49.8
10.0	10.5	105	20.7	14.3	49.1
20.0	20.3	102	23.9	6.2	45.5
50.0	52.5	105	8.4	15.8	48.3
100	112	112	10.6	13.9	47.7
500	465	93.0	8.8	9.7	41.8
Mean		109	15.6	13.6	49.0

Table 4
Validation of ICI 214,227 analysis in human plasma quality control and dilution samples[a]

Concentration (ng ml^{-1})	Pooled mean (ng ml^{-1})	Accuracy (%)	Intra-assay precision (% RSD)	Inter-assay precision (% RSD)
8.00	7.79	97.4	15.8	16.5
25.0	22.8	91.2	19.0	15.2
60.0	58.5	97.5	28.3	13.5
150	146	97.3	44.2	19.0
400	367	91.8	11.2	13.5
Mean		95.0	23.7	15.5
2000	2150	108	13.2	NC
5000	5220	104	7.8	NC
Mean		106	10.5	NC

[a] NC, not calculated

Table 5
Validation of M 236,303 analysis in human plasma

Concentration (ng ml^{-1})	Pooled mean (ng ml^{-1})	Accuracy (%)	Intra-assay precision (% RSD)	Inter-assay precision (% RSD)	Recovery (%)
2.50	2.64	106	8.4	14.4	36.5
5.00	5.09	102	21.0	14.9	34.7
10.0	9.40	94.0	15.2	13.7	33.5
20.0	17.2	86.0	22.8	10.4	30.1
50.0	52.5	105	18.2	21.8	35.5
100	118	118	15.7	23.3	40.8
500	576	115	9.8	15.2	39.3
Mean		104	15.9	16.2	35.8

Table 6
Validation of M 236,303 analysis in human plasma quality control and dilution samples[a]

Concentration (ng ml^{-1})	Pooled mean (ng ml^{-1})	Accuracy (%)	Intra-assay precision (% RSD)	Inter-assay precision (% RSD)
8.00	6.22	77.8	16.6	12.8
25.0	19.8	79.2	21.5	14.3
60.0	57.9	96.5	23.3	7.4
150	141	94.0	44.2	19.2
400	381	95.3	14.0	10.9
Mean		88.6	24.0	12.9
2000	2260	113	14.9	NC
5000	5510	110	9.3	NC
Mean		112	12.1	NC

[a] NC, not calculated

2. Experimental

2.1. Materials

Quetiapine (ICI 204,636), internal standard (M 214,652) and metabolite standards (ICI 214,227 and M 236,303) were synthesized at Zeneca Pharmaceuticals (Wilmington, DE). High purity methanol, ethyl acetate and acetonitrile were purchased from Burdick & Jackson (Muskegon, MI). Ammonium hydroxide, hydrochloric acid, phosphoric acid, sodium phosphate and potassium chloride were from J. T. Baker (Phillipsburg, NJ). HPLC-grade water was prepared with a Nano-Pure water purification system.

2.2. Preparation of reagents and solutions

Stock solutions of quetiapine, ICI 214,227 and M 236,303 were prepared by dissolving 2.0 mg of each compound in 2.0 ml of 50% acetonitrile:methanol to give a concentration of 1.0 mg ml^{-1}. A 100 μl aliquot of each of the three stock solutions was combined with 3.7 ml of 50% acetonitrile:methanol to give a concentration of 25 μg ml^{-1} of each analyte. This solution was further diluted with 50% acetonitrile:methanol to give spiking solutions of 5.00, 2.50, 1.00, 0.500, 0.250 and 0.125 μg ml^{-1}. M 214,652 internal standard solution was prepared by dissolving 2.0 mg into 10 ml 50% acetonitrile:methanol to give a concentration of 200 μg ml^{-1}, and further diluting with 50% acetonitrile:methanol to a working concentration of 2.50 μg ml^{-1} for use in the assay. Plasma standards for generating the standard curves were prepared by adding 8 μl of each spiking solution to 400 μl of plasma, to give final plasma concentrations of quetiapine and metabolites of 500, 100, 50.0, 20.0, 10.0, 5.00 and 2.50 ng ml^{-1}. Additionally, 10 μl of working internal standard solution was added to each calibration standard. Pipettings of these volumes are rou-

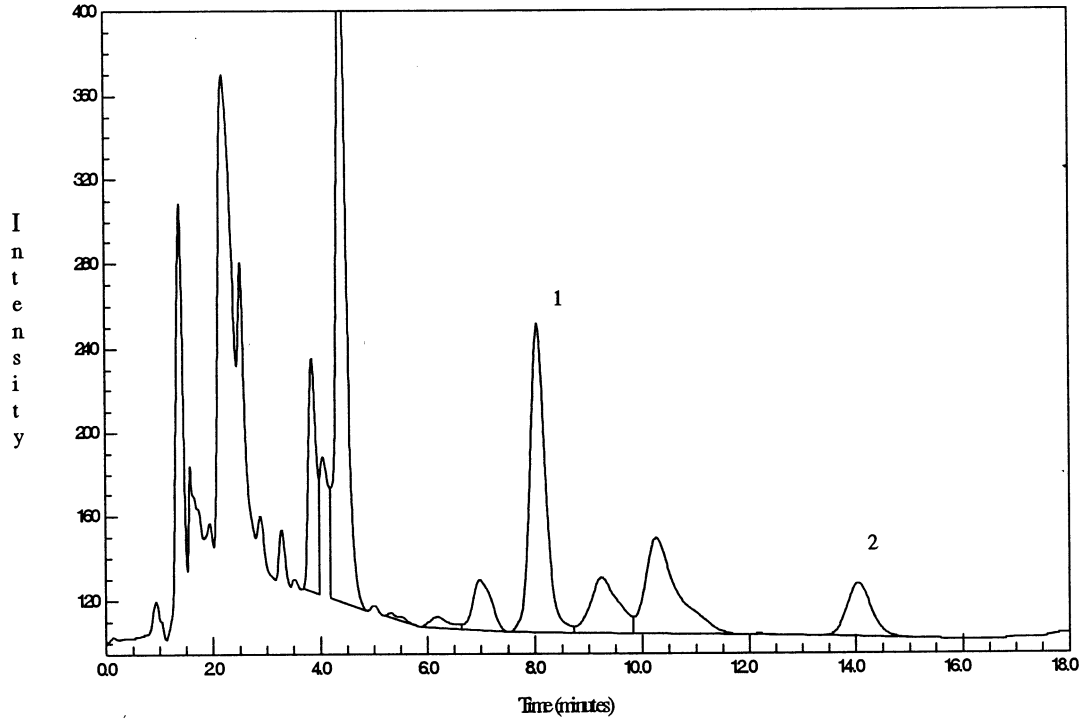


Fig. 4. Chromatograms of extracted plasma collected from a patient 2 h after receiving a 150 mg dose of quetiapine fumarate containing 228 ng ml^{-1} quetiapine (1) and M 214,652 (2) (internal standard).

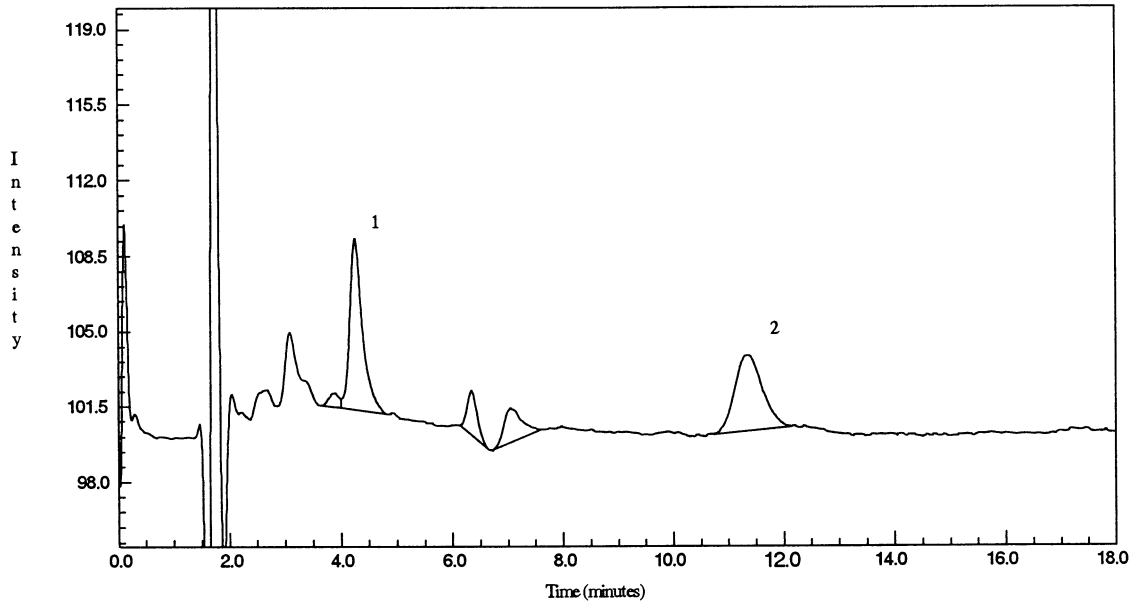


Fig. 5. Chromatograms of extracted plasma collected from a patient 2 h after receiving a 150 mg dose of quetiapine fumarate containing 11.3 ng ml^{-1} ICI 214,227 (1) (7-hydroxylated metabolite of quetiapine) and 9.76 ng ml^{-1} M 236,303 (7-hydroxylated, *N*-dealkylated metabolite of quetiapine).

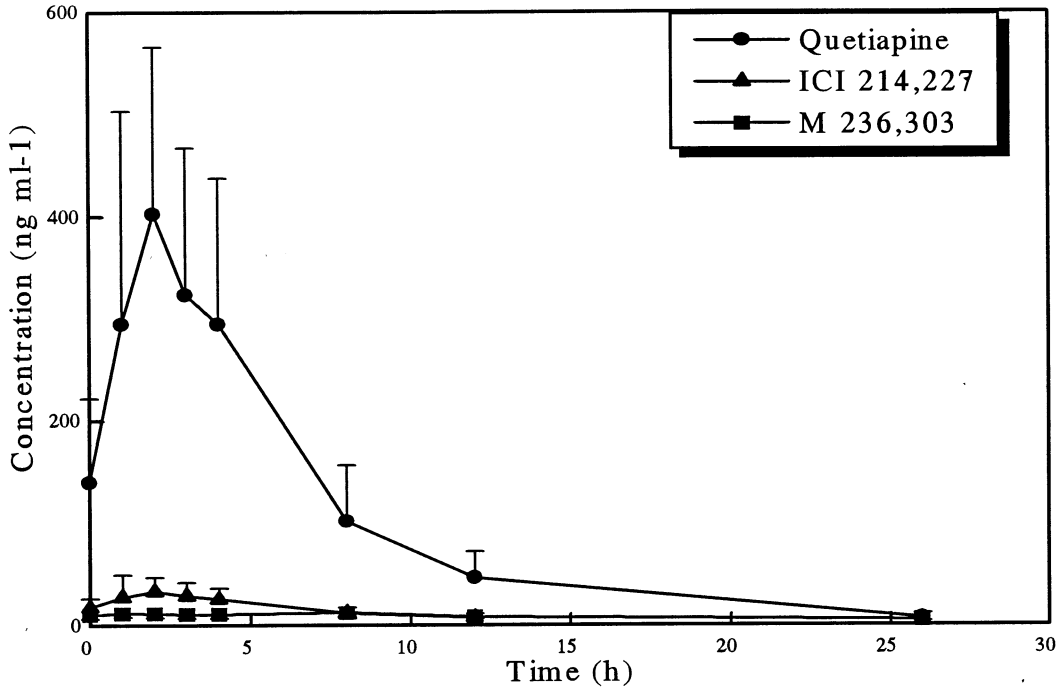


Fig. 6. Mean (S.D.) concentrations of quetiapine, ICI 214,227 and M 236,303 following a 150 mg dose at steady state.

tinely performed in our laboratories with precision of approximately 1% coefficient of variation (CV%). Calibration standards were prepared daily with each set of plasma samples.

The mobile phase consisted of a mixture of 20 mM phosphate buffer (containing 30 mM potassium chloride and adjusted to pH 7.4 with 1 M phosphoric acid), methanol and acetonitrile (40:50:10, v/v/v). The injection solvent was composed of the same components in a ratio of 60:30:10, v/v/v.

2.3. Instrumentation

The HPLC system included a Hewlett Packard model 1090 solvent delivery system and autosampler, a Shimadzu SPD-6A UV spectrophotomteric detector (225 nm, 0.001 AUFS) and a Bioanalytical Systems LC-44 electrochemical detector with a dual cell (+ 0.25 V precell, + 0.55 V analytical cell, 200 nA) and Ag/AgCl reference electrode. Separation was achieved on a narrow bore ZORBAX Stablebond phenyl (SB-Ph) column (150 × 2.1 mm, 5 μm) at a flow of 0.25 ml min^{-1}. Detection of quetiapine and M 214,652 internal standard was via UV absorption at 225 nm, while ICI 214,227 and M 236,303 were detected by oxidation at + 0.55V. Peak heights were measured using VG Multichrom software (VG Laboratory Systems, Altrincham, Cheshire, UK).

3. Procedures

3.1. Blood sample collection and processing

Venous blood samples (7 ml each) were collected in tubes containing sodium heparin (green-top Vacutainer BD-6483, Becton–Dickinson, Cockeysville, MD). Immediately after collection, each blood sample was gently inverted a few times for complete mixing with the anticoagulant and then centrifuged at room temperature within 30 min of collection to separate the plasma. The resulting plasma samples were transferred, using glass transfer pipettes, to polypropylene tubes, then frozen immediately. The samples were maintained at − 20°C or colder until used in the assay.

3.2. Assay of plasma samples

Quetiapine, ICI 214,227, M 236,303 and the added internal standard M 214,652 were extracted from 400 μl of human plasma, made basic with 25 μl of 15% ammonium hydroxide, into 3 ml ethyl acetate. After vortexing for 30 s and centrifuging

Table 7
Plasma concentration and pharmacokinetic parameters (Mean ± S.D., n = 8) of quetiapine and its metabolites, ICI 214,227 and M 236,303 following a 150 mg dose at steady state[a]

Time (hours)	Quetiapine (ng ml^{-1})	ICI 214,227 (ng ml^{-1})	M 236,303 (ng ml^{-1})
1	298 ± 208	28.1 ± 22.0	11.9 ± 3.6
2	403 ± 163	33.2 ± 14.0	11.7 ± 4.2
3	324 ± 143	29.3 ± 13.0	11.2 ± 4.0
4	295 ± 143	25.7 ± 11.1	11.0 ± 3.6
8	102 ± 54.6	12.4 ± 5.60	12.6 ± 5.7
12	47.0 ± 25.5	7.6 ± 4.2	8.50 ± 2.0
26	7.2 ± 4.6	NC	5.20 ± 0.9
C_{max}^{SS} (ng ml^{-1})	437 ± 173	38.3 ± 18.9	14.7 ± 5.3
t_{max} (hour)[b]	2 (1.2–4.0)	2 (1.2–4.0)	3.5 (1.2–8.1)
AUC_{τ}^{SS} (ng h ml^{-1})	1980 ± 947	187 ± 82.1	NC
$t_{1/2}$ (h)	5.3 ± 1.9	6.2 ± 3.1	NC
CL/f (l h^{-1})	101 ± 65.8	NC	NC
V_z/f (l)	671 ± 39.3	NC	NC

[a] NC, not calculated
[b] Median (range)

for 5 min, the upper ethyl acetate layer was transferred into a clean tube, and the analytes were back-extracted into 1 ml of 0.2 N hydrochloric acid. Again, after vortexing for 30 s and centrifuging for 5 min, the upper ethyl acetate layer was aspirated to waste, and the remaining aqueous layer made basic with 0.5 ml of 15% ammonium hydroxide. The analytes were re-extracted into 3 ml ethyl acetate, vortexed and centrifuged as above. The upper ethyl acetate layer was transferred to a clean polypropylene tube, and the sample extracts were evaporated under nitrogen at approximately 35°C, reconstituted in 30 μl injection solvent, of which 25 μl was injected on the narrow-bore chromatographic system following transfer by positive displacement pipette to a 300 μl polypropylene autosampler vial.

3.3. Quantitation of quetiapine and metabolites

Quantitation of quetiapine was accomplished by determination of peak height ratios of quetiapine to internal standard. A linear relative response factor was calculated from the spiked plasma calibration standards from 2.50 to 500 ng ml^{-1}, with quantitation to an upper limit of 5000 ng ml^{-1} by sample dilution with de-ionized water. The measurement of ICI 214,227 and M 236,303 was achieved using peak heights determined from their respective standard curves following oxidation at +0.55 V, with the same applicable concentration range as that for quetiapine.

3.4. Assay validation

Human plasma calibration standards containing quetiapine, ICI 214,227 and M 236,303 were prepared in duplicate on 3 validation days, and in quadruplicate on the fourth at 500, 100, 50.0, 20.0, 10.0, 5.00 and 2.50 ng ml^{-1}. Corresponding unextracted standards were prepared on the fourth day for recovery determinations. Human plasma quality control samples containing the same analytes were prepared and frozen on a day prior to analysis at 400, 150, 60.0, 25.0 and 8.00 ng ml^{-1} and analyzed with each chromatographic run.

The stability of quetiapine, ICI 214,227 and M 236,303 was evaluated using the quality control samples described above stored frozen at approximately −20°C, after repeated freezing and thawing, at room temperature and in injection solvent.

The specificity of the method was demonstrated by injecting solutions of other known or theorized metabolites of quetiapine and known or anticipated concomitant medications, and by analyzing plasma from a minimum of 20 drug-free volunteers, including a smoker and caffeine consumer.

3.5. Human pharmacokinetics

Eleven men who met the criteria for chronic or subchronic schizophrenia according to DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders, 3rd edition) entered this study. Patients had a mean age of 34 years (range 20–43) and a mean weight of 82 kg (range 69–111). Eight patients completed this study. Patients received quetiapine fumarate tablets three times daily (TID, 07:00, 15:00 and 23:00 h). The initial dose regimen (25 mg TID) was increased in increments to 150 mg TID in 7 days. This regimen was maintained from day 8 to day 28. Following the morning 150 mg dose on day 29, blood samples were collected at 1, 2, 3, 4, 8, 12 and 26 h, and plasma was stored at −20°C prior to analysis by the HPLC assay as described above.

4. Results

4.1. Chromatography

A chromatogram of extracted control human plasma and that of control human plasma spiked with 20.0 ng ml^{-1} quetiapine and M 214,652 are compared in Fig. 2. Detection was by UV absorption at 225 nm. While the two hydroxylated metabolites also absorbed at this wavelength, they eluted in regions of the chromatogram which contained endogenous plasma components, which interfered with their quantitation at their expected plasma concentrations. An electrochemical detector was placed in series with the UV detector to oxidize these metabolites at +0.55V, which re-

sulted in suitable response for these compounds, as shown for ICI 214,227 and M 236,303 in Fig. 3. However, neither quetiapine nor the internal standard M 214,652 could be oxidized at these, or any reasonable potentials. While a single extraction for sample clean-up would have been preferable, the interference from endogenous plasma components, a large number of which also absorb at 225 nm, made detection of quetiapine untenable. However, following the sample extraction described, no interferences were observed from endogenous plasma constituents, known metabolites of quetiapine, common over the counter medications or potential concomitant medications.

Linear calibration curves were obtained from a quantitation limit of 2.50 to 500 ng ml^{-1} for all three analytes. The applicable range was extended to 5000 ng ml^{-1} by sample dilution with de-ionized water. Quetiapine concentrations were determined by calculating the peak height ratio of parent to internal standard; metabolite concentrations were calculated by their electrochemical peak height response, as the internal standard could not be detected electrochemically. Relative response factors across the linear range averaged 15.2 , 21.8 and 19.1% relative standard deviation (RSD) for quetiapine, ICI 214,227 and M 236,303, respectively, over 4 days of validation.

4.2. Accuracy and precision

The validation data for quetiapine are presented in Tables 1 and 2; results for ICI 214,227 and M 236,303 are shown in Tables 3–6. Calculated quetiapine, ICI 214,227 and M 236,303 concentrations averaged 98.6, 109 and 104% of theory, respectively, across the linear calibration range. Intra-assay precision for quetiapine averaged 14.2% RSD, while inter-assay precision averaged 11.9% RSD across 4 validation days. Intra-assay precision for ICI 214,227 and M 236,303 averaged 15.6 and 15.9% RSD, respectively, while inter-assay precision averaged 13.6 and 16.2% RSD, respectively, across 4 validation days. Calculated quetiapine, ICI 214,227 and M 236,303 quality control sample concentrations averaged 98.9, 95.0 and 88.6% of theory, respectively, across the linear calibration range. Intra-assay precision for quetiapine averaged 7.1% RSD, while inter-assay precision averaged 11.7% RSD across 4 validation days. Intra-assay precision for ICI 214,227 and M 236,303 averaged 23.7 and 24.0% RSD, respectively, while inter-assay precision averaged 15.5 and 12.9% RSD, respectively, across 4 validation days. All dilution samples were within 20% of the theoretical value.

4.3. Recovery and stability

Recovery of quetiapine and its metabolites from plasma was calculated by comparing the peak height of the analyte from extracted plasma standards to peak heights obtained from unextracted standards at the same concentrations. Absolute recovery ($\pm$S.D.) across the linear concentration range of 2.50 to 500 ng ml^{-1} averaged 41.9 (11.6), 49.0 (13.4) and 35.8% (7.9) for quetiapine, ICI 214,227 and M 236,303, respectively. Recovery of the internal standard from extracted plasma averaged 42.4% (9.8).

Calculated concentrations of quetiapine, ICI 214,227 and M 236,303 from plasma frozen for 15 months at approximately -20°C averaged 96.4, 109 and 112% of theory, respectively, across four concentrations (8.00, 24.0, 240 and 750 ng ml^{-1}). The concentrations of quetiapine, ICI 214,227 and M 236,303 measured from plasma frozen and thawed eight times were 98.2, 98.9 and 98.1% of theory, respectively, across five concentrations (8.00, 25.0, 60.0, 150 and 400 ng ml^{-1}).

4.4. Human pharmacokinetics

Chromatograms of extracted plasma collected from a patient 2 h after receiving a 150 mg dose of quetiapine fumarate are shown in Fig. 4 (quetiapine and internal standard) and Fig. 5 (ICI 214,227 and M 236,303. Fig. 6 shows the plasma concentration–time profiles for quetiapine and its metabolites, ICI 214,227 and M 236,303). The mean ($\pm$S.D.) concentrations and pharmacokinetic parameters of these three compounds are presented in Table 7. Quality control samples for quetiapine, ICI 214,227 and M 236,303 assayed during this study averaged 93.0, 92.2 and 95.4%

of theory, respectively. Inter-assay precision for these samples averaged 12.5, 4.7 and 6.7% RSD for quetiapine, ICI 214,227 and M 236,303, respectively.

5. Discussion

A sensitive and specific HPLC method, with both UV absorption and electrochemical detection, was developed and validated for the determination of quetiapine and its metabolites in human plasma. This method offers a significant improvement in sensitivity, specificity and reproducibility over the previous HPLC method [3], and enhanced robustness compared to the previous GC/MS procedure [3].

The use of narrow-bore chromatography necessitates the use of small injection volumes, and the sensitivity requirements of the assay demand concentration of the sample. The low recoveries observed in this validation were initially thought to be the result of incomplete redissolution of dry residue in 30 μl of injection solvent. However, recovery in this step was virtually complete when compared to directly injected solutions. The reconstituted samples were transferred to autosampler vials using positive displacement pipettes, with little or no loss of sample. In addition, various organic solvent systems, bases and pHs were evaluated, as well as variation in extraction times and methods, glass adsorption or adsorption during the heated evaporation phase, all without improvement in recovery. Subsequent determinations of these analytes by LC/MS/MS utilizing a single, basic ethyl acetate extraction (in publication) yielded higher, reproducible recoveries, suggesting that recovery loss occurs during the subsequent extractions necessary for detection at 225 nm.

The intra-assay precision, expressed as the percentage RSD exceeded 20% at the three lowest concentration levels evaluated for quetiapine and its metabolites during validation. This variability is due in part to the three extractions required during the sample cleanup procedure, as well as the lack of a suitable internal standard for the metabolites. These validation data also include one or two spurious results which would be rejected from what would otherwise be an acceptable standard curve. However, the overall mean calculated value at each quetiapine concentration was within $\pm 20\%$ of the theoretical value. The variability above was accepted with the constraint that no sample concentration values were reported below the lowest accepted standard (where at least one replicate back-calculated to within (20% of the theoretical value) on each day of analysis. All samples whose concentrations fell below a raised quantitation limit were repeated in a subsequent analytical run where the quantitation limit of 2.50 ng ml^{-1} was achieved. The use of additional plasma volume to increase sensitivity also increased endogenous interferences, with no gain in quantitation limit. For both quetiapine (with an internal standard) and its metabolites (without an internal standard), while neither recovery nor precision during validation were as robust as hoped, the very strict controls placed on the acceptance of assays at lower concentrations ensured that the data generated by the assay was reliable, as supported by the accuracy of the quality control data. Both runs used to generate the pharmacokinetic data for this study successfully achieved a quantitation limit of 2.50 ng ml^{-1}, which easily permitted calculation of AUCs out to 26 h post-dose and no repeat sample analysis was required.

Quetiapine was rapidly absorbed after oral administration with a mean t_{max} of about 2 h. Plasma quetiapine concentration was variable and the CV% for C^{SS}_{max} and AUC^{SS}_{τ} were about 40%. The mean terminal half-life ($t_{1/2}$) of quetiapine was 5.3 h, which predicts that quetiapine will attain steady state in less than 48 h following multiple dosing, and confirms that the pharmacokinetics parameters derived from this study are steady-state parameters. The mean apparent volume of distribution was (V_z/f) 681 l. This indicates that quetiapine is widely distributed throughout the body. The mean values of $t_{1/2}$, CL/f and V_z/f obtained in the present study are consistent with those reported for quetiapine in schizophrenic men and women in the clinical dose range [4].

Although ICI 214,227 and M 236,303 are active at the dopamine and serotonin receptor sites, the

plasma concentrations of these two compounds were lower than that of quetiapine as evidenced by the difference in the mean C_{max}^{SS} and AUC_{τ}^{SS} of these three compounds. The concentrations of M 236,303 at most of the time points were below the limit of quantitation of the assay. As a result, the AUC_{τ}^{SS} and $t_{1/2}$ values were not reported for this compound. The plasma half-life of ICI 214,227 (6.2 h) was similar to that of quetiapine.

6. Conclusions

Improvements in sensitivity, specificity and reproducibility were achieved using an HPLC procedure with UV and electrochemical detection for the quantification of quetiapine and two of its active metabolites. An experienced operator can comfortably prepare 80 plasma extracts a day for automated chromatographic analysis. The wide linear range of the assay can support both low-dose studies in normal volunteers and higher dose studies in subjects with schizophrenia and other psychotic disorders. The present assay method was used to characterize the pharmacokinetic profile of quetiapine with the time course of dopamine D_2 and serotinin 5-HT_2 receptor occupancy in the brain using PET.

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