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Use of liquid chromatography–tandem mass spectrometry for the quantitative and qualitative analysis of an antipsychotic agent and its metabolites in human plasma and urine

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Abstract

The use of liquid chromatography–tandem mass spectrometry (LC–MS–MS) for the quantitative analysis of an 'atypical' antipsychotic agent in human plasma is described. The method uses atmospheric pressure chemical ionisation, and offers increased sensitivity, selectivity and speed of analysis over an existing high-performance liquid chromatography method using fluorescence detection. This method enabled same day turn around of results for in excess of 100 samples, including sample preparation, data acquisition and processing. LC–MS–MS was also used to detect and characterise known and unknown in vivo metabolites of the drug in human urine and plasma, using electrospray ionisation. © 1998 Elsevier Science B.V.

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1. Introduction

Schizophrenia is a chronic syndrome characterised by *positive* psychotic symptoms (delusions, hallucinations, thought disorder) and *negative* symptoms (social isolation, blunted affect, poverty of speech, marked lack of initiative) [1]. The causes remain unknown, although the prevailing neurochemical hypothesis is that the dopamine neurotransmitter system is dysfunctional [2,3]. All currently available antipsychotics antagonize dopamine D_2 receptors [3]. 'Typical' antipsychotics such as haloperidol, are widely accepted as being effective against the *positive* symptoms of schizophrenia, but do not benefit all patients. Furthermore, they are relatively ineffective against *negative* symptoms and cognitive disorders, and produce extrapyrimidal side effects (EPS) at therapeutic doses. EPS are dysfunctions of the motor system such as acute dystonia, parkinsonism, and akathisia that result from D_2 receptor antagonism. 'Atypical' antipsychotics such as clozapine are effective against *positive* and *negative* symptoms [4,5]. A rapidly growing body of data suggests that dysfunction in serotonergic (5-HT) function may be involved in the pathophysiology of schizophrenia, and that pharmacological agents for this illness have their therapeutic effects mediated through 5-HT mechanisms [6–8]. Clozapine's unusual clinical profile may result from the combination of potent serotonin 5-HT_2 antagonism with moderate D_2 antagonism [4]. Unfortunately, clozapine induces agranulocytosis in about 1% of

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patients and seizures in up to 5%. A drug with an atypical profile yet without clozapine's limiting side effects would be an important advance.

The compound, 2-amino-*N*-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl) benzamide (**I**, see Fig. 1), is a potent serotonin 5-HT_2 and dopamine D_2 receptor antagonist [9], and may have potential as an 'atypical' antipsychotic agent. Its ratios of EPS dose to therapeutic dose are equal to or better than the ratios for currently available antipsychotics in mouse, rat and macaque. The compound is also a 5-HT_{1a} receptor agonist and therefore could relieve the anxiety associated with stress that often triggers psychotic episodes and relieve the depression that is often associated with schizophrenia [10,11]. This potent 5-HT_{1a} agonism is not shared by other antipsychotics and should give **I** an improved clinical profile. As part of drug safety evaluation studies it was necessary to obtain rapid metabolic information on this compound. Initial in vitro metabolic studies showed extensive metabolism of the drug, which underwent oxidation at the benzisothiazol sulphur atom to generate sulphoxide (**II**) and sulphone (**III**) metabolites (Fig. 1). In addition, ring hydroxylations at either the 3 or 5 position of the benzamide ring gave rise to 3- and 5-hydroxyl metabolites, IV and V, respectively (unpublished data).

The preclinical development of **I** was initially supported by a reversed-phase high-performance liquid chromatography using fluorescence detection (HPLC-FLD) following liquid–liquid extraction. This method provided a lower limit of quantitation (LLOQ) of 1 ng/ml for **I** in rat and dog plasma. As the development of this compound has progressed into clinical phase it has become apparent that a more sensitive method is required. The combination of high-performance liquid chromatography with tandem mass spectrometry (LC–MS–MS) has previously been applied to the rapid and convenient quantification of drugs in biological matrices [12]. This report describes an LC–MS–MS method for the rapid and sensitive quantitative determination of **I** in human plasma following oral administration of the compound. LC–MS–MS is also used to detect and characterise in vivo metabolites of **I**, in urine and plasma.

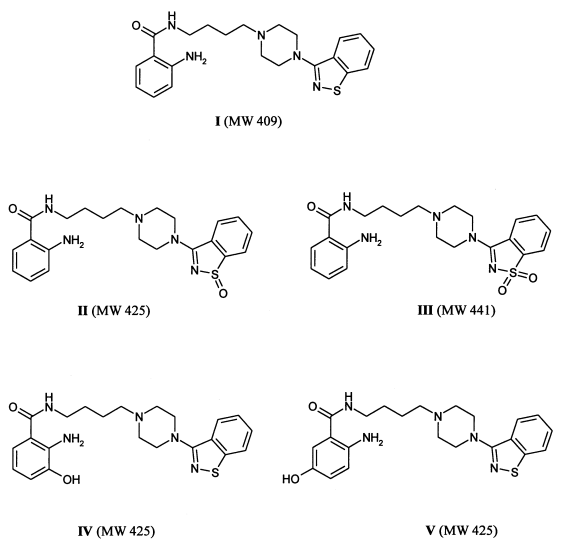

Fig. 1. **I** and its known metabolites.

2. Experimental

2.1. Materials

Compounds **I**–**V** (see Fig. 1) and internal standard, $^{13}C_6$-labelled **I**, were synthesised by GlaxoWellcome Research Laboratories (Research Triangle Park (RTP), NC, USA). Analytical solutions of all compounds were prepared in 50% (v/v) aqueous methanol. HPLC-grade acetonitrile was obtained from Rathburn (Walkerburn, UK). Analytical-grade ammonium formate, formic acid, methanol and HPLC-grade water were all purchased from BDH (Poole, UK).

2.2. Dosing and sample collection

Human plasma and urine samples were taken from a double-blind, placebo-controlled, parallel design study of the safety and pharmacokinetics of increasing single doses of **I**. Eighteen healthy male volunteers received single doses of 1, 2, 5, 10, 30, and 40 mg **I** or placebo at weekly intervals. Twelve received **I** and six received placebo. During the study, safety and dose tolerance was assessed via adverse experience reports, vital sign measurements, clinical laboratory tests, physical examinations, ECGs, chest X-rays and ophthalmological examina-

tions. Serial blood samples were collected pre-dose, and at 0.5, 1, 1.5, 2, 4, 6, 8 and 12 h post-dose. At each dose, urine passed was also collected and pooled. All samples were frozen at −20°C prior to quantitative LC–MS–MS analysis. For qualitative metabolite profiling, plasmas and urines were taken from the 10- and 40-mg dose levels.

2.3. Sample preparation

All unknown plasma samples, calibration standards and quality controls were centrifuged at 1000 *g* (3500 rpm) for 10 min to remove precipitated solids. Five hundred μl of each sample, 500 μl 20 m*M* ammonium formate buffer and 25 μl $^{13}C_6$-labelled **I** internal standard solution (ca. 100 ng/ml) were added to 10-mm test tubes and vortex mixed.

The samples were loaded onto 100-mg C18 solid-phase extraction cartridges (Varian) previously conditioned with 1 ml methanol and 1 ml 20 m*M* ammonium formate buffer, pH 4.0. After washing with 1 ml water and 500 μl acetonitrile, the analytes were eluted from the cartridges with two 75-μl aliquots of acetonitrile–20 m*M* ammonium formate (75:25 v/v) buffer. Both the wash and elution stages were extracted applying a low pressure at the cartridge outlet. For quantitative analysis, extracts were analysed directly by LC–MS–MS. For qualitative analysis, extracts were taken to dryness under a stream of nitrogen, and reconstituted in 200 μl of 0.1% (v/v) aqueous formic acid.

Urine samples (200 μl) were centrifugally filtered through 0.45-μm membrane filters (Millipore) prior to direct analysis.

2.4. Quantitative LC–MS–MS method

All quantitative LC–MS–MS experiments were conducted on a Hewlett-Packard (Cheshire, UK) Model 1090 liquid chromatograph coupled to a PE-Sciex (Ontario, Canada) API-III$^+$ triple quadrupole mass spectrometer, with an atmospheric pressure chemical ionisation (APCI) source. A 20-μl aliquot of the plasma extract was injected onto a 30×4.6-mm I.D. SCX column (Phenonenex, Macclesfield, Cheshire, UK) operated at 40°C using an isocratic mobile phase of 75% (v/v) acetonitrile in 20 m*M* ammonium formate buffer (pH 4.0) at 1 ml/min. The flow from the column was directed to a Heated Nebuliser (HN) interface operating in positive-ion mode, at 500°C, using nitrogen as both the nebulizer (50 p.s.i.) and auxiliary gas (2 l/min). Analyte and internal standard were detected by tandem mass spectrometry (MS–MS) using selected reaction monitoring (SRM) of the transitions m/z 410⇒120 and m/z 416⇒126 for **I** and $^{13}C_6$-labelled **I**, respectively, with a dwell time of 200 ms per transition. Argon was used as the collision gas at an indicated target thickness of 300. A collision energy of 20 eV was used.

2.5. Preparation of calibration curve

Duplicate calibration standards at 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, 7.0 and 10.0 ng/ml were prepared by spiking control human plasma with appropriate volumes of standard solutions containing **I** to achieve the required concentration. Quality control (QC) samples were prepared independently (i.e. separate weighing) by spiking batches of control plasma at 0.1, 1.0 and 8.0 ng/ml. Additional, independent spiked controls at 0.05, 0.5, 5.0 and 10.0 ng/ml were prepared for the initial validation of the method. The one-way analysis of variance (ANOVA) of the validation data was performed by an Excel macro (MicroSoft Corp.). LC–MS–MS SRM peaks were integrated by the PE-Sciex MacQuan processing software. Calibration curves were constructed by plotting peak area ratios of **I** to internal standard against concentration using a weighted ($1/x$) linear regression model. Concentrations of the analyte in unknown and QC samples were subsequently interpolated from these curves.

2.6. Qualitative LC–MS–MS method

All qualitative LC/MS and LC–MS–MS experiments were conducted on an HP1090 liquid chromatograph coupled to a Finnigan-MAT (San Jose, CA, USA) TSQ-700 mass spectrometer, with an API source. Separations were achieved on a 250×4.6-mm I.D. Zorbax Rx-C8 column (Dupont) operated at 40°C, using a mobile phase consisting of 0.1% (v/v) formic acid in water–acetonitrile at 1 ml/min. The proportion of acetonitrile was maintained at 5% for the first 5 min of the run, and then

programmed to increase linearly to reach 50% (v/v) at 30 min. The effluent from the column was directed to a pneumatically assisted electrospray (ESI) interface without splitting. ESI ionisation was performed in the positive-ion mode with a probe capillary potential of 4.6 kV. For all experiments the heated capillary of the TSQ 700 was maintained at 250°C.

Full-scan positive-ion spectra were acquired over a range of m/z 250–700 and analysed on a DEC station 5000/33 computer using Finnigan-MAT ICIS® software (version 6.0). Dwell times of approximately 4 and 200 ms per ion were used for full-scan and selected ion monitoring (SIM) experiments, respectively. MS–MS experiments were based on collision-induced dissociations (CID) occurring in the RF-only collision cell (Q2) of the triple quadrupole, with a collision energy of 20 eV and argon collision gas at a pressure of approximately 1.8 mTorr. Additional structural information was obtained by collisional activation within the high-pressure region of the TSQ 700 interface, via an accelerating voltage (40 V) applied to the first octapole, followed by CID within Q2.

An automated post-column Rheodyne (Cotati, CA, USA) 7010 switching valve installed in the HP1090 liquid chromatograph, was used to divert the first 5 min of each chromatographic run to waste.

3. Results and discussion

3.1. Quantitative LC–MS–MS

The full-scan total ion current trace of **I** (data not shown) consisted of a single chromatographic peak, a detailed examination of which indicated the absence of any significant impurities. A background subtracted full scan (50–500 a.m.u.) spectrum of **I** is presented in Fig. 2(a). Under APCI **I** gives rise to an abundant protonated molecule (MH^+) at m/z 410. Similarly the internal standard, $^{13}C_6$-labelled **I**, was shown to produce an MH^+ ion at m/z 416 (data not shown). APCI was chosen in preference to ESI due to significantly lower levels of chemical noise and also due to its compatibility with conventional HPLC columns (i.e. 4.6-mm I.D.) using flow-rates of 1 ml/min and above (at the time of method development the PE-Sciex TurboIonspray interface was not available). The APCI MS–MS product-ion spectra of these MH^+ ions are presented in Fig. 2(b) Fig. 2(c), respectively, and both yield abundant product ions suitable for use as selected reaction monitoring (SRM) transitions. The base peak at m/z 120, owing to the ion $[C_6H_4NH_2CO]^+$, and the corresponding higher-mass ion at m/z 126, were selected as the Q3 transition for **I** and its $^{13}C_6$-labelled analogue, respectively. The 6-a.m.u. difference between the chosen Q3 transition ions confirms the site of ^{13}C-labelling to be on the benzoyl ring.

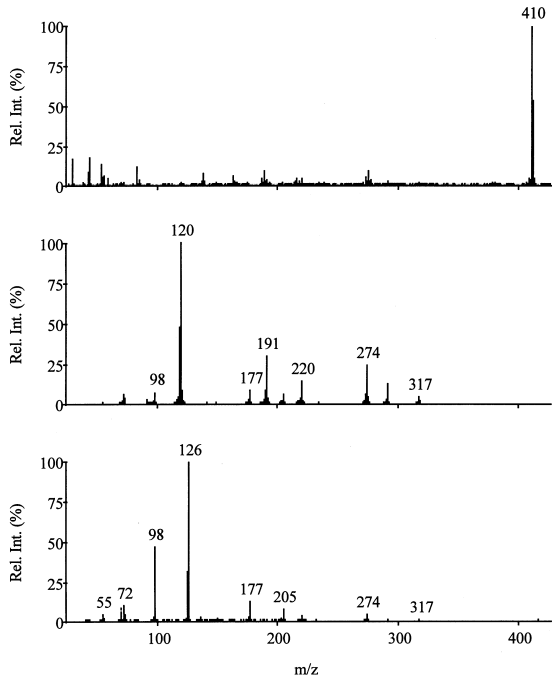

Fig. 2. Positive-ion APCI spectra of (a) **I** and positive-ion MS–MS product-ion spectra of (b) **I** and (c) the internal standard $^{13}C_6$-labelled **I**.

The selectivity of MS–MS reduces the need for complete chromatographic resolution of individual components, and the emphasis in quantitative LC–MS–MS method development was shifted more to reducing the analytical run time to provide high sample throughput and to use API-compatible mobile phases. In our laboratory, we have had some success in the development of 'generic' methods for LC–MS–MS. One such method is based on short, strong cation-exchange (SCX) columns which, although not operated in the true ion-exchange mode, have pro-

vided retention characteristics suitable for fast LC–MS–MS analysis for several series of compounds and their metabolites [13]. By operating the SCX column in a reversed-phase mode, careful optimization of the mobile phase can enable resolution of the parent drug and metabolites of varying polarities without significantly increasing run times. The LC–MS–MS SRM chromatograms, resulting from the analysis of an extract of a 50-pg/ml calibration standard, are presented in Fig. 3. The signal-to-noise ratios, and lack of interference in the chromatograms, illustrate the sensitivity and specificity of LC–MS–MS. With a mobile phase of 75% (v/v) acetonitrile in 20 m*M* ammonium formate at pH 4.0, the analyte of interest and the internal standard elute in under 2 min with good chromatographic peak shape.

3.2. Method validation

The LC–MS–MS method was formally validated prior to the analysis of study samples. The following

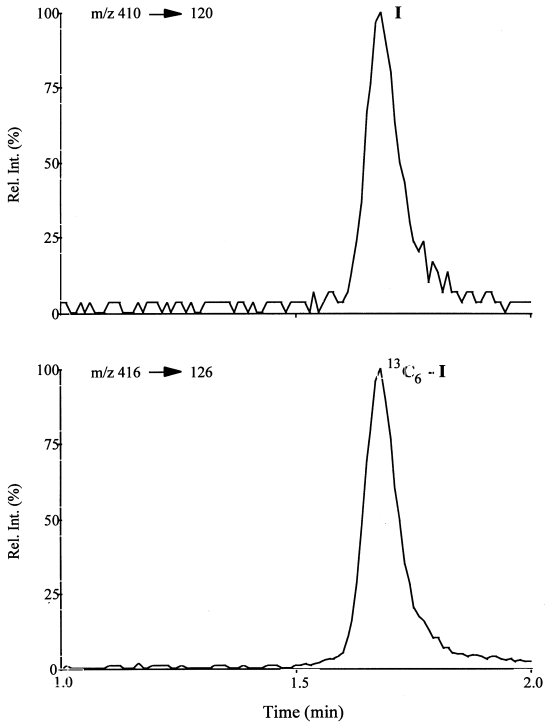

Fig. 3. LC–MS–MS SRM chromatograms of (a) **I** (50 pg/ml) and (b) the internal standard $^{13}C_6$-labelled **I** (ca. 2 ng/ml) resulting from a 20-μl injection.

parameters were assessed: calibration model, precision and accuracy, specificity, recovery and the stability of the analyte in analytical stock solutions, frozen human plasma (including freeze–thaw cycles) and in processed extracts.

The suitability of a weighted ($1/x$) linear regression model for the analyte of interest was confirmed by examining standardized residual plots of calibration data from four inter-assay experiments. The plot for the unweighted least-squares regression (not shown) indicated a large increase in variance with increasing concentration (heteroscedasticity), indicating that some form of weighting was necessary. The distribution of residuals in the $1/x$ weighted plot was improved over the unweighted fit, although true homoscedasticity (equal variance with concentration) was not achieved. A similar distribution was observed in the $1/x^2$ weighted plot, however the regression was overweighted with variance now increasing at lower concentrations. While the appropriate weighting would appear to lie somewhere between $1/x$ and $1/x^2$, in the absence of a user-defined weighting being available with the data system the simpler $1/x$ weighted model was chosen.

The precision of the method (%C.V.) was determined by assessing the agreement between replicate measurements of mixed spiked control samples prepared independently from the calibration standards. Spiked control samples at four concentrations were analysed in replicates of four, on four separate occasions. The data was examined by one-way analysis of variance (ANOVA) to give estimates of the inter- and intra-assay precision of the method. The results are presented in Table 1 and show that, at all spiked concentrations, the inter- and intra-assay precision (%C.V.) are less than 8% over the limits of quantification (0.05–10 ng/ml). The accuracy of the

Table 1
LC–MS–MS accuracy and precision data from the pre-study validation

	Spiked control concentrations			
Nominal (ng/ml)	0.050	0.500	5.000	10.000
Mean (ng/ml)	0.051	0.514	5.189	10.016
Accuracy (% bias)	2.0	2.8	3.8	0.2
Precision (%CV)				
intra-assay	7.2	6.2	3.0	1.8
inter-assay	5.7	0.8	negligible	negligible

method (% bias) was determined by assessing the agreement between the measured and nominal (weighed) concentrations of spiked control samples, the measured concentration being the mean of the concentrations obtained during the precision assessment above. The results are presented in Table 1 and show that, at all spiked control concentrations, the bias does not exceed ±4%.

The specificity of the method was assessed by the visual examination of SRM chromatograms generated in the analysis of control human plasma from several volunteers, pre- and post-dose samples from **I** clinical trials, and of control plasma spiked with metabolites (**II**–**V**, Fig. 1) of **I**. Metabolites **II**, **III** and **V** were chromatographically resolved from the parent drug, **I**, thus eliminating any risk of interference. Metabolite **IV** was shown to co-elute with **I**, however, the SRM transitions for the two analytes were unique and no interference was observed.

The analyte, **I**, was shown to be stable in analytical stock solutions and frozen human plasma for at least 28 days, including up to three freeze–thaw cycles. In addition, **I** was found to be stable in processed extracts for at least 24 h. A comparison of extracted and unextracted sample areas gave estimated recoveries of 37 and 34% at concentrations of 0.05 and 2 ng/ml, respectively, suggesting that recovery was consistent across the quantifiable range. While the recovery is relatively low, the analyte is eluted in a small volume (150 μl) resulting in a highly concentrated sample. A higher recovery could be achieved by using a larger elution volume but at the expense of sensitivity due to a dilution of the sample extract. The recoveries of individual metabolites was not investigated.

The LC–MS–MS method described has been used to support early clinical studies, including an acute dose tolerance and pharmacokinetic study involving the analysis of some 1600 samples. Same day turnaround of results for in excess of 100 samples was possible, including sample preparation, data acquisition and processing.

3.3. Qualitative LC–MS

In order to profile for potential systemic metabolites, selected plasma samples from several volunteers from the aforementioned study were analysed by positive-ion full-scan LC-MS using ESI ionisation. ESI ionisation was chosen in preference to APCI for qualitative analysis, in order that any potential phase II conjugates (e.g. glucuronides or sulphates), would also be detected in addition to **I** and any phase I metabolites present. The thermal instability associated with this class of metabolites often renders it difficult to obtain molecular weight information using APCI, which is often crucial in confirming the presence or otherwise of a phase II conjugate. The use of ESI has enabled, for example, glucuronic acid conjugates to be analysed much more readily, with the resultant mass spectra providing molecular weight information [14]. In addition, the use of a longer column and gradient elution, provides a means of resolving drug-related material from the large number of endogenous compounds in biological matrices, such as urine and plasma, thereby assisting identification as opposed to purely confirmation of drug-related material.

Putative drug-related peaks were assigned by their absence from the corresponding pre-dose sample and by comparison of their retention and mass spectral characteristics with those of authentic materials. Extracted-ion chromatograms of a 1-h post-dose plasma sample, following a 10-mg oral dose of **I** are given in Fig. 4. Unchanged parent drug was detected as its protonated molecule, MH^+, at m/z 410, eluting with a retention time of 24:24 min:s. The sulphone metabolite, **II**, was the only detectable plasma metabolite of **I** indicated by the presence of its MH^+ ion at m/z 426 and significant $[M+Na]^+$ and $[M+K]^+$ adduct ions at m/z 448 and 464, respectively.

Full-scan ESI-LC–MS analysis of selected urine samples following a 40-mg oral dose, revealed the presence of **I**, and its metabolites **II**, **III** and **V**. Metabolites **III** and **V** gave rise to abundant MH^+ ions at m/z 442 and 426, respectively. Extracted-ion chromatograms showing parent drug and these metabolites identified in human urine are given in Fig. 5. An additional drug-related peak was detected in human urine (denoted **VI**) which did not co-chromatograph with any of the known metabolites (**II**–**V**) of **I**, and which gave rise to an MH^+ ion at m/z 426 with a retention time of 14:13 min:s. Based on these characteristics, alone, **VI** was rationalised as a novel hydroxyl metabolite of **I**, i.e. hydroxylation is not at the 3- or 5-positions (**IV** and **V**, respective-

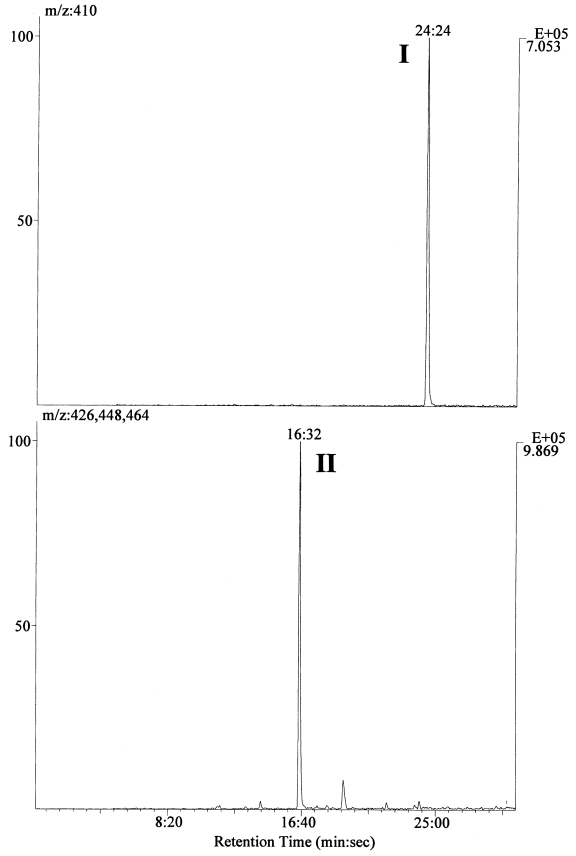

Fig. 4. Extracted ion chromatograms of (top) **I** (m/z 410) and (bottom) sulphoxide metabolite, **II** (m/z 426), from the full-scan (m/z 250–600) positive-ion LC–MS analysis of 1-h post-dose human plasma, following a 10-mg oral dose of **I**. Conditions: 250×4.6 mm I.D. column packed with Zorbax Rx-C8 stationary phase, 0.1% formic acid (v/v) in water–acetonitrile mobile phase. The proportion of acetonitrile maintained at 5% for the first 5 min, and then increased linearly to reach 50% at 30 min, at a flow-rate of 1 ml/min: 100 μl injected.

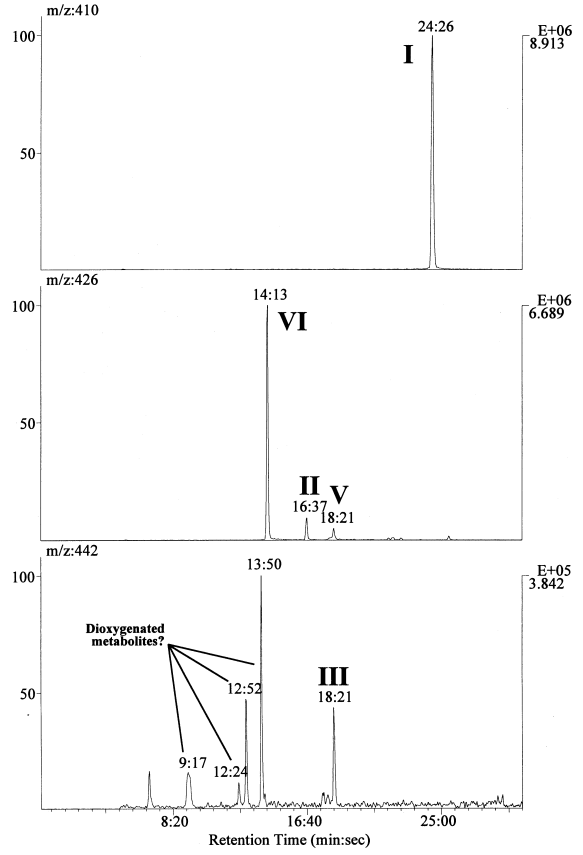

Fig. 5. Extracted ion chromatograms of (top) m/z 410, (middle) m/z 426 and (bottom) m/z 442, from the full-scan (m/z 250–600) positive-ion LC–MS analysis of human urine, following a 50-mg oral dose of **I**. Conditions as for Fig. 4.

ly). Several additional peaks, visible in the m/z 442 extracted-ion chromatogram at 9:17, 12:24, 12:52 and 13:50 min:s, were also shown to be absent from the corresponding pre-dose sample. Based on molecular weight alone, these peaks were rationalised as isomeric dioxygenated metabolites of **I**. No evidence was obtained for the formation of phase II conjugates.

Analysis by selected ion monitoring (SIM) of m/z 410 and 426, was used to estimate the relative amounts of **I**, **II**, **V** and **VI** in selected urines from all volunteers based on a single point calibration mixture containing **I**, **II** and **V** (prepared by spiking control human urine with standard solutions) at nominal concentrations of 10 ng/ml. Concentrations of the novel hydroxyl metabolite, **VI**, were estimated using **V** as a reference and was shown to consistently be the major urinary drug-related substance (data not shown).

3.4. Qualitative LC–MS–MS

Confirmation of **I**, **II** and **V** in human urine was provided by LC–MS–MS, using their MH^+ ions as the precursor masses. ESI-MS–MS product-ion spectra of **I** and its metabolites **II** and **V** in urine, compared favourably to those of authentic material (see Fig. 6). In all three cases, cleavage of the amide

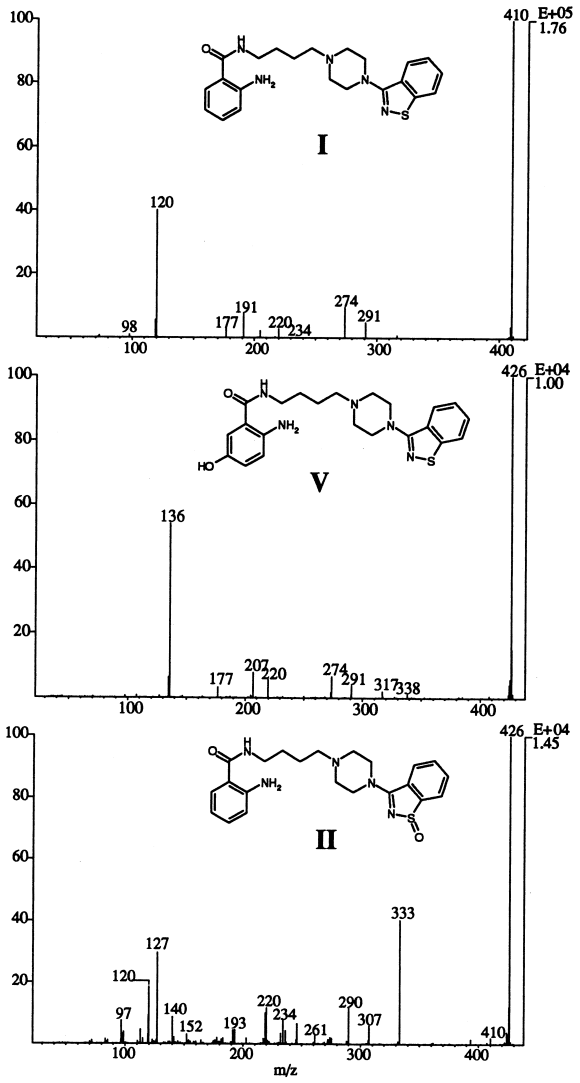

Fig. 6. LC–MS–MS product-ion spectra of authentic reference material of (top) **I**, (middle) the sulphoxide metabolite, **II** and (bottom) the 5-hydroxyl metabolite, **V**. Conditions: collision energy of 25 eV and argon collision gas at a pressure of approximately 1.8 mTorr.

bond β to the terminal-subsituted phenyl ring gives rise to a characteristic $ArC{\equiv}O^+$ fragment, resulting in product-ions at m/z 120, for **I** and **II**, and m/z 136, in the case of **V**. Cleavage of the bond adjacent to the ring to form a fragment of the type $RC{\equiv}O^+$ is also apparent in the case of **II**, and gives rise to the product-ion at m/z 333. Cleavage either side of the secondary amine within the butyl linker chain, with concomitant charge retention on the benzisothiazol half of the molecule, generates the product ions at m/z 274 and 291, respectively, from both **I** and **V**. Equivalent fragmentation gives rise to corresponding ions at m/z 290 and 307 in the case of **II**.

ESI-MS–MS product-ion analysis was also used to obtain structural information on the novel metabolite, **VI**. Rationalisation of the resulting product-ion spectrum, shown in Fig. 7, was assisted by comparison with those of authentic **I** and **II**–**V**. The major product-ion at m/z 277 is not observed in the corresponding spectra of **I** or any of its known metabolites. However, the ion at m/z 120 is common with **I**, whilst the ions at m/z 219 and 234 are common with **II**. The intensity of the m/z 120 ion, characterised as a fragment of the type $ArC{\equiv}O^+$ (see above), was proportional to the collision energy, and its presence indicates that the substituted phenyl ring is not the site of biotransformation. The product-ions from **VI**, can instead be rationalised in terms of a hydroxyl metabolite (as predicted), with the tentative site of oxidation being the benzisothiazol moiety (see Fig. 8).

Collisional activation in the high-pressure region of the TSQ 700 interface, via an accelerating voltage applied to the first non-mass-selective focusing octapole, may also be used to generate fragmentation. In combination with more conventional MS–MS, this enables second-generation product-ion spectra to be obtained. Using these techniques, further inves-

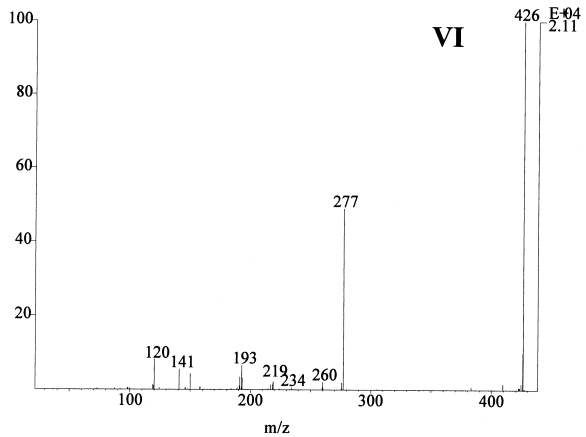

Fig. 7. LC–MS–MS product-ion spectrum of metabolite **VI**. Conditions as for Fig. 6.

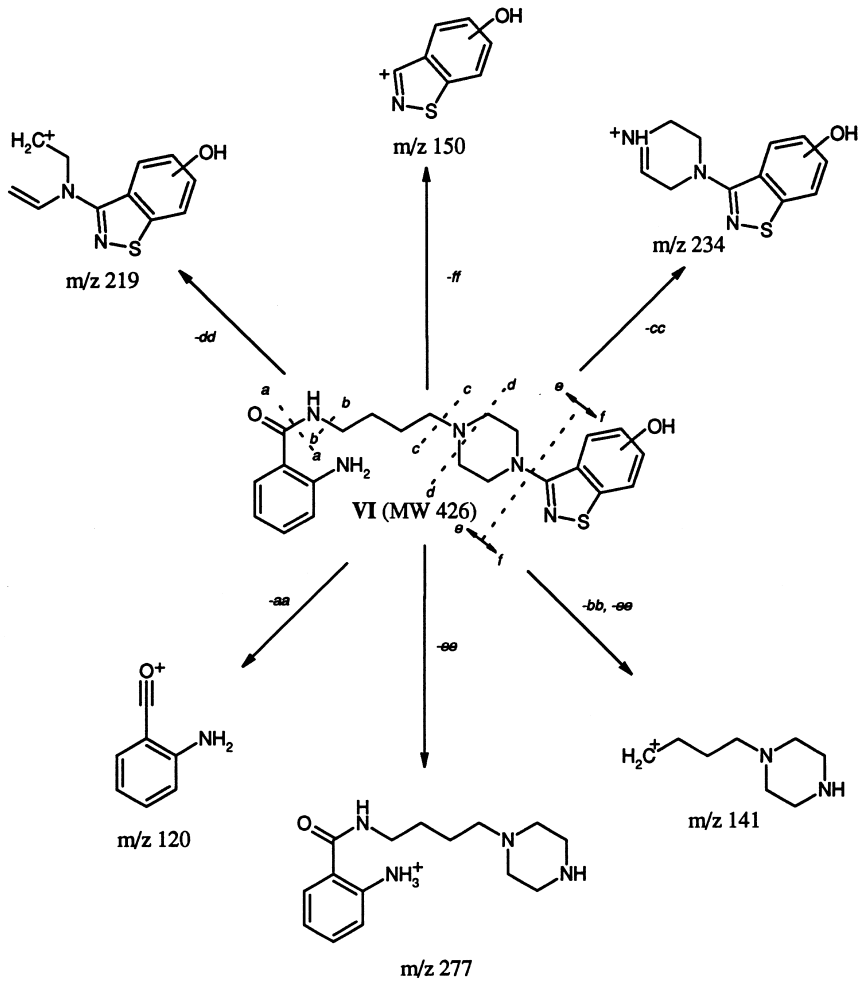

Fig. 8. Interpretation of CID fragments, produced from metabolite **VI**.

tigation of the *m*/*z* 120 product-ion formed from both **I** and **VI**, and the *m*/*z* 277 product-ion formed from **VI** alone, was possible. The resulting second-generation product-ion spectra of *m*/*z* 120 presented in Fig. 9, are identical, being dominated by the sequential loss of C=O and CH_2=$CH^{\cdot}$ giving rise to the ions at *m*/*z* 92 and 65, respectively, and therefore providing unequivocal confirmation that **VI** is drug-related. The second-generation product-ion spectrum of *m*/*z* 277 (data not shown) exhibited ions at *m*/*z* 120 and 141, indicating that both these product ions are derived ultimately from *m*/*z* 277.

Product-ion spectra were not obtained for **III**, or the putative dioxygenated metabolites detected in human urine, due to the low concentrations of these substances.

4. Conclusions

A sensitive LC–MS–MS method has been developed for the antipsychotic agent **I**, allowing rapid determination of the analyte in complex biological matrices, such as plasma, with quantifiable levels as low as 50 pg/ml. This study has also demonstrated the power of LC–MS–MS as a technique for the detection and identification of metabolites in biological matrices.

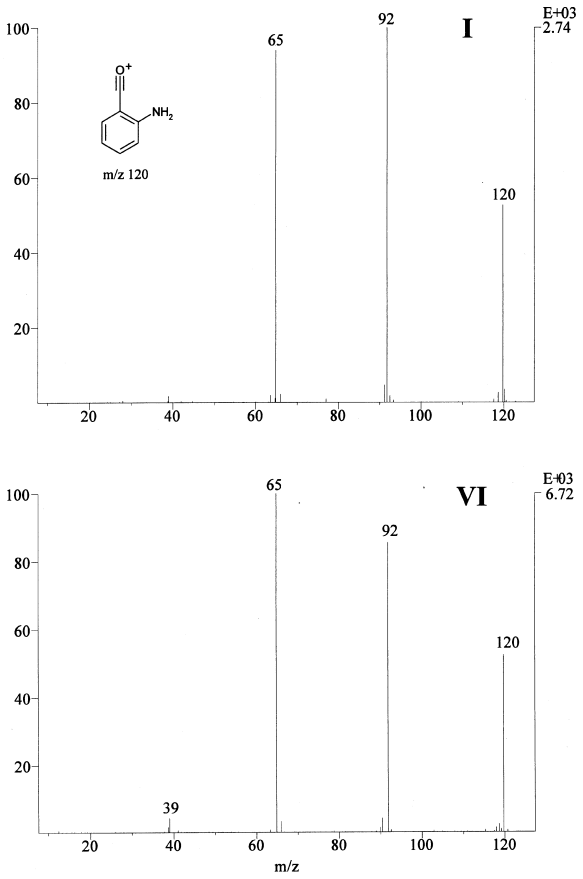

Fig. 9. LC–MS–MS second generation product-ion spectra of the m/z 120 product-ion formed from (a) **I** and (b) metabolite **VI**. Conditions as for Fig. 6, with an acclerating voltage of 40 V applied to the first octapole of the TSQ 700.

Urine samples from humans following oral administration of **I**, were analysed with minimal sample preparation, by LC–MS for metabolites of parent drug. In addition to the expected metabolites of **I**, a previously undetected urinary hydroxyl metabolite was identified. Based on LC–MS–MS experiments using CID in both the RF-only quadrupole and in-source, the site of hydroxylation was tentatively assigned to the benzisothiazol moiety of parent drug.

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