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Electrospray ionisation–mass spectrometric characterisation of selected anti-psychotic drugs and their detection and determination in human hair samples by liquid chromatography–tandem mass spectrometry

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Abstract

Electrospray ionisation quadrupole ion-trap mass spectrometric (ESI–MS) characterisation of the anti-psychotic drugs chlorpromazine, trifluoperazine, flupenthixol, risperidone and the antidepressant/internal standard trimipramine is presented and possible mechanisms for the observed MS^n fragmentation patterns proposed. A validated liquid chromatography (LC)–MS–MS method is then applied to the detection and determination of these drugs in the hair of a patient under clinical treatment for schizophrenia. Chlorpromazine, trifluoperazine and flupenthixol are identified and determined in this hair sample following alkaline degradation of the matrix, solvent extraction and LC–MS–MS using trimipramine as internal standard.

Keywords: Anti-psychotic drugs

1. Introduction

The phenothiazine class of pharmaceutical compounds has long been used for the treatment of mental disorders such as schizophrenia. Chlorpromazine has been particularly useful in this regard from the time of its release onto the US market by Smith Kline and French in 1954 under the trade name Thorazine [1]. The phenothiazines are thought to exhibit their anti-psychotic effects via interference with dopaminergic pathways in the mesolimbic chemoreceptor trigger areas of the brain but as yet the exact mechanism of their action has not been established [2].

These compounds are of interest in forensic and clinical toxicological fields and have been included in comprehensive reviews by Maurer on their liquid chromatography–mass spectrometry (LC–MS) [3] and gas chromatography–mass spectrometry (GC–MS) [4] behaviour. Chlorpromazine, the most commonly known phenothiazine, has been determined in chemically fixed organ tissue [5] and biological fluids [6] by GC–MS and in liquid oral pharmaceutical formulations by LC–UV [7].

One drawback of the phenothiazines is that chemically they are unstable and decompose to give

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monosulphoxides which themselves may be psychoactive. For that reason an LC method for the determination of trifluoperazine, trifluopromazine and perphenazine and their corresponding monosulphoxides has been developed [8]. A comprehensive monograph on the action of trifluoperazine is available [9].

LC with thermospray tandem mass spectrometric detection has been used for the determination of the thioxanthene derivatives chloroprothixene, flupenthixol, thiothixene and zuclopenthixol in whole blood [10]. LC with NMR and MS detection was employed for the identification of in vitro and in vivo metabolites of the benzisoxazole derivative iloperidone in urine and bile salts [11].

Risperidone is a relatively new atypical benzisoxazole derivative that has high binding affinity to serotonin-5HT$_2$ and dopamine-D$_2$ receptors [12]. A sensitive method for the determination of risperidone in human plasma by LC with electrochemical (EC) detection has been presented by Aravagiri et al. [13]. More recent studies have described the simultaneous determination of risperidone and 9-OH risperidone in human plasma again using LC–EC [14,15].

To date only a few references exist in the literature describing the determination of anti-psychotics in human hair. Couper et al. have investigated the LC–UV determination of haloperidol, chlorpromazine and thioridazine in human hair samples following alkaline degradation of the matrix. For 1 *M* NaOH treatment at 70°C for 30 min, haloperidol, chlorpromazine and thioridazine had acceptable recoveries of 88, 103 and 96%, respectively [16]. In a further publication the same authors detected concentrations of the three drugs mentioned above in hair of post-mortem cases by initial screening with GC followed by confirmation with GC–MS [17]. Kinzt has catalogued a total of eight methods for the determination of haloperidol and in some cases its metabolite in hair. Usually NaOH dissolution of the hair is used before analysis by radioimmunoassay, LC–EC, LC–UV, GC–MS or GC with nitrogen/phosphorus detection [18].

Of the drugs of interest in this study, risperidone, flupenthixol, trifluoperazine and chlorpromazine only the latter has appeared in the literature with regard to its determination in human hair. Sato et al. have developed a method for the determination of chlorpromazine in hair as an index of dosage history and compliance compared with simultaneously measured haloperidol. Hair was dissolved in 2 *M* NaOH by heating at 80°C for 30 min. Following solvent extraction the drugs were determined by LC–EC using the internal standard trimipramine for quantification purposes [19].

For this study a similar dissolution/extraction procedure to that described above was used to determine the levels of anti-psychotic drugs present in the hair of a schizophrenic patient under clinical treatment for the condition but compliance testing was not an aim of the work. LC–MS–MS was the chosen analytical tool and electrospray ionisation–mass spectrometry (ESI–MS^n) characterisation of the drug compounds was also carried out. Total confirmation of the presence of a particular compound was therefore possible.

2. Experimental

2.1. Instrumentation

MS^n characterisation and detection of the drug compounds took place using an LCQ™ quadrupole ion-trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) utilising electrospray ionisation (ESI). Samples of the drugs were infused into the MS by means of a syringe pump located on the instrument.

The LC system used was supplied by ThermoQuest (San Jose, CA, USA) and comprised a P4000 pump, AS 3000 autosampler, 20 μl injector loop, on-line UV 1000 UV detector and an SCM 1000 vacuum membrane degasser. The column used for chromatography separations was a Phenomenex (Macclesfield, Cheshire, UK) Luna C$_{18}$ column, 15 cm long with an internal diameter of 4.6 mm. A guard column of similar characteristics but 30 mm in length was positioned just before the analytical column. For mass spectrometric studies and quantitation of the drugs in hair samples the LC system was interfaced with the LCQ™ instrument.

Dissolution of the hair samples took place in a Grant waterbath (Grant Instruments, Cambridge, UK). Any pH adjustments were measured using an Orion 410A pH meter (Beverly, MA, USA) fitted with a BDH Gelplas combination electrode (BDH, Poole, Dorset, UK). Centrifugation of dissolved hair

samples took place using a Sanyo MSE MicroCentaur centrifuge (Sanyo, Uxbridge, UK) while centrifugation of the drugs following extraction into hexane was carried out on a Beckman J2-H5 centrifuge (Beckman Instruments Inc., Fullerton, CA, USA).

2.2. Reagents

All solvents were of high-performance liquid chromatography (HPLC) grade while other chemicals used were of analytical reagent quality. Methanol, acetonitrile, hexane, acetic acid, concentrated hydrochloric acid and sodium hydroxide were obtained from BDH (Poole Dorset, UK). Milli-Q 18 MΩ water (Millipore) was used throughout. Chlorpromazine hydrochloride salt was obtained from Aldrich (Poole, Dorset, UK) while trifluoperazine and trimipramine (used as internal standard) were obtained from Sigma (Poole, Dorset, UK) as their dihydrochloride and maleate salts, respectively. Flupenthixol was donated to us by the Northern Ireland Forensic Science Laboratory (Carrickfergus, Northern Ireland) as was a 1 mg tablet of risperidone. Standard stock solutions of the drugs were prepared by dissolving an appropriate mass in methanol to provide concentrations of 1.0×10^{-3} mol l^{-1}. In the case of risperidone a 1 mg tablet was dissolved in methanol providing a concentration of 9.74×10^{-4} mol l^{-1} and this used as the standard stock solution. Nitrogen gas for the LCQ™ was delivered from a Whatman nitrogen generator (Whatman Inc., Haverhill, MA, USA) while helium damping gas, present in the ion-trap was obtained from BOC Medical Gases (Guildford, Surrey, UK).

2.3. Hair samples

A positive hair sample was voluntarily donated by a patient under clinical treatment for schizophrenia. Blank hair samples were collected from workers in the laboratory or from members of their families.

2.4. Procedures

2.4.1. MS^n characterisation

MS^n characterisation of the compounds was carried out using 1.0×10^{-5} mol l^{-1} standards in methanol infused individually into the mass spectrometer at a flow-rate of 10 μl min^{-1}. In the ESI source nitrogen sheath and auxiliary gas flows were maintained at 50 and 5 units, respectively and refer to arbitrary values set by the software. The heated capillary temperature was 220°C and the spray voltage set to 4.5 kV. Positive ion mode was used throughout. In order to establish the MS^n fragmentation pattern for each compound the precursor $[M+H]^+$ ion was chosen in the MS scan and fragmentation of it initiated. The major peak observed in the resulting MS–MS scan was then chosen for the next stage of fragmentation and the process continued until no further ions were observed. Collision energy was optimised for each stage of fragmentation using LCQ™ software.

2.4.2. LC–MS–MS analysis

LC–MS–MS detection was used to obtain RSD values, correlation coefficients, LODs and to determine the levels of the drugs in hair samples. The mobile phase utilised binary elution of two components A and B in the proportions 35 and 65%, respectively. Solvent A consisted of 0.02 mol l^{-1} ammonium acetate and 0.1% acetic acid in water while solvent B contained only acetonitrile. A flow-rate of 0.5 ml min^{-1} was used and a 20 μl injection made of each sample. Mass spectrometer settings were similar to those described above except that the sheath and auxiliary gas flows were raised to 70 and 40 units, respectively to account for the increased solvent flow-rate. Using MS–MS data generated from the above studies the product ions were investigated for each compound thereby providing a selective method of determination for each drug.

2.4.3. Preparation of analytical standards for recovery studies

NaOH is commonly used in the procedure to extract drugs from hair, probably because of its ability to completely dissolve the hair sample. One drawback to such harsh chemical treatment is the extent to which the drug under investigation is degraded in the process. As mentioned earlier, phenothiazines are known to be unstable, but good recoveries have been reported for chlorpromazine using NaOH dissolution in work carried out by Couper et al. [16,17].

The efficiency of recovery of the analytes from the matrix must also be addressed. In order to investigate

this peak area responses of the five drugs, which had been taken through the digestion/extraction process (Section 2.4.4) in the presence of 500 mg of blank hair, were compared to peak areas of standard solutions of identical concentration. A 4.9×10^{-7} mol l^{-1} sample was used for risperidone while all of the compounds were investigated at the 5.0×10^{-6} mol l^{-1} concentration level. These standard solutions were prepared by appropriate dilution of the stock solutions in methanol.

2.4.4. Hair treatment procedure

Hair samples of 500 mg mass were washed in methanol to remove possible exogenous contamination on the hair surface. The hair was steeped in methanol for approximately 5 min after which the sample plus wash solution were transferred to a Büchner funnel and a further aliquot of fresh methanol poured over the sample. The hair was left to dry on the funnel and the methanol collected. In order to establish the presence of any of the drugs in the wash solution the solvent was evaporated in an air stream prior to being made up accurately to 1 or 5 ml volumes and subjected to LC–MS–MS. The hair sample was cut into 1 mm (approximately) sections and an accurate mass treated with 5 ml of 1 mol l^{-1} NaOH at 80°C in a water bath for 30 min. The solution was then cooled to room temperature and the pH adjusted to between 8 and 10 with 4 *M* hydrochloric acid. The solution was centrifuged in an MSE MicroCentaur centrifuge at 10 000 rpm for 10 min. The resulting solution was decanted from the particulate matter and hexane extractions (2×10 ml) carried out. Centrifugation on a Beckman centrifuge at $10\times g$ for 20 min was used to remove any emulsion which had formed in the hexane layer. The solvent was evaporated off and the residue reconstituted in 2.0 ml methanol prior to autosampler injection. Blank hair samples were treated in a similar fashion. Prior to drug determination in hair samples, the injection system was washed with methanol, the column equilibrated with mobile phase and a number of methanol blanks analysed to ensure that the system was free from all traces of drug standards from previous analyses.

For quantification trimipramine was used as the internal standard. Prior to injection 30 μl of a 5.0×10^{-6} mol l^{-1} solution of trimipramine which had been taken through the dissolution/extraction procedure in the presence of 500 mg hair was added to 270 μl of the methanolic hair extract.

3. Results and discussion

3.1. MS^n characterisation

MS^n characterisation data for the five drugs under investigation is given in Table 1. Where a number of fragment ions were produced the ion used in the next stage of the fragmentation process is shown underlined.

All of the compounds gave $[M+H]^+$ profiles in MS mode. The chlorpromazine spectrum contains

Table 1
MS^n characterisation data for anti-psychotic drugs and the optimised collision energy (CE) for each step

Drug	MS	MS–MS		MS^3		MS^4	
	Peak	Peak	CE (%)	Peak	CE (%)	Peak	CE (%)
Chlorpromazine	319.1	274.0	24	246.1	22	214.3	24
Trifluoperazine	408.1	141.1	26	113.1	16	70.1	16
Flupenthixol	435.2	307.1	30	265.2	24	233.4	30
		362.1					
		390.1					
Risperidone	411.2	191.2	26	163.2	20	120.2	20
				110.0		110.0	
				148.2		82.1	
				82.1			
Trimipramine	295.1	100.1	22	58.1	14	*	*

* No peak observed.

the parent peak at m/z 319.1 and another peak one-third its size at 321.1 indicative of the ^{37}Cl isotope (Fig. 2a). Trimipramine was somewhat anomalous in that two major m/z peaks were observed in MS, one at 208.2 and the other at 295.1. As the latter corresponded to the expected $[M+H]^+$ signal for the compound it was used in further MS^n analyses. Proposed mechanisms for the fragmentation of the compounds and tentative structures for the respective fragments are given in Fig. 1a–e while the corresponding MS^n spectra produced are shown in Fig. 2a–e and refer to chlorpromazine, flupenthixol, trifluoperazine, risperidone and trimipramine in both cases, respectively.

3.1.1. Chlorpromazine

In MS and MS–MS it is believed that protonation occurs at the nitrogen atom in the 10 position on the phenothiazine nucleus. While the tertiary amine nitrogen on the dimethylamino group is more likely to undergo protonation, this group is lost at the MS–MS stage and formation of a propene entity giving an MS–MS product-ion with m/z 274. If protonation did occur on the tertiary amine nitrogen then 'charge hopping' would have to occur from this group to the ring nitrogen to retain charge in the ion-trap for the proposed MS–MS fragment. This is a rather implausible explanation. An ion of m/z 246 at the MS^3 stage may be attributed to shortening of the propene group to methyl to give a carbocation. (Carbocations are also reported by Nedved et al. [20] in the fragmentation of the 1,4-benzodiazepine chlordiazepoxide, albeit not the relatively unstable primary carbocation proposed here). In MS^4 loss of 32 a.m.u. is attributed to the removal of sulfur (Fig. 1a). The MS^n spectra produced are shown in Fig. 2a.

3.1.2. Flupenthixol

Loss of 45 a.m.u. in the MS–MS stage may correspond to removal of an alcohol group giving a peak at m/z 390.1. The major peak in MS–MS, 307.1, may be attributable to loss of the entire piperazineethanol group and with it the two nitrogen sites likely to undergo protonation. For that reason it is proposed that protonation in MS and MS–MS occurs at the sulfur on the thioxanthene nucleus. This is the only plausible explanation that can be given under these circumstances apart from proposing some type of 'charge-hopping' mechanism in which a nitrogen is protonated in MS but during fragmentation in MS–MS the charge 'hops' onto the unprotonated S group. Loss of the propyl group and one further hydrogen atom in MS^3 causes a net positive charge to reside on the 3-position carbon atom of the thioxanthene nucleus. It is proposed that charge remains in the same position in MS^4 where a contraction of the ring by loss of S explains an overall net loss of 32 a.m.u. (Figs. 1b and 2b).

3.1.3. Trifluoperazine

While chlorpromazine retains the phenothiazine nucleus throughout the fragmentation procedure, it appears that trifluoperazine loses it the MS–MS stage as a major loss of 267 a.m.u. is observed. For that reason it is proposed that protonation in MS occurs at the methyl-substituted nitrogen atom in the 1-position of the piperazinyl group and that charge can be retained there throughout the rest of the fragmentation steps. In MS–MS the protonated piperazinyl propyl group is retained with formation of a propene substituent. Shortening of the propene substituent to methyl and formation of a double bond in the six-membered ring in MS^3 accounts for an overall loss of 28 a.m.u. Further loss of 43 a.m.u. in MS^4 comes about through contraction of the six-membered ring. This results in ejection of the N–CH_3 group and simultaneous loss of the methyl group on the remaining nitrogen to provide a protonated pyrrole-type entity of m/z 70 (Figs. 1c and 2c). However, a second MS^3–MS^4 pathway may also be proposed based upon carbocation formation. In MS^3 the propene chain is shortened to a methyl carbocation on which charge now resides rather than on the 1-position nitrogen atom. Contraction of the ring and loss of both methyl groups to provide a pyrrole-type carbocation may explain the observed m/z 70 in MS^4. It is believed that the former pathway based upon nitrogen protonation provides more stable entities and is preferred.

3.1.4. Risperidone

It is proposed that protonation of risperidone occurs at the nitrogen on the 1-position of the pyrimidinone group rather than at the 5-position nitrogen which belongs to a cyclic amide. It is therefore possible to speculate structures for frag-

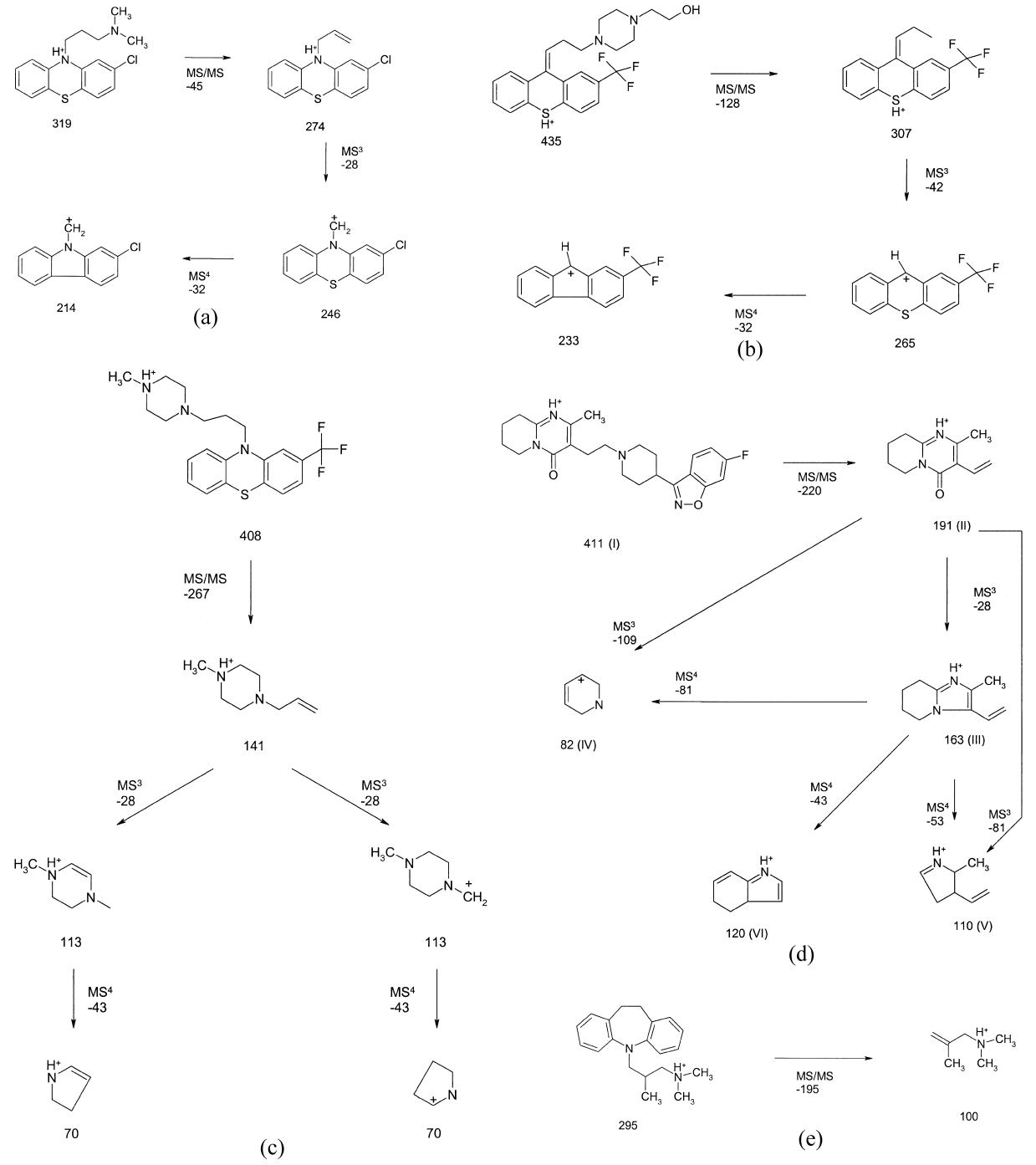


Fig. 1. Proposed MS^n fragmentation pathways for (a) chlorpromazine; (b) flupenthixol; (c) trifluoperazine; (d) risperidone; and (e) trimipramine.

(a)

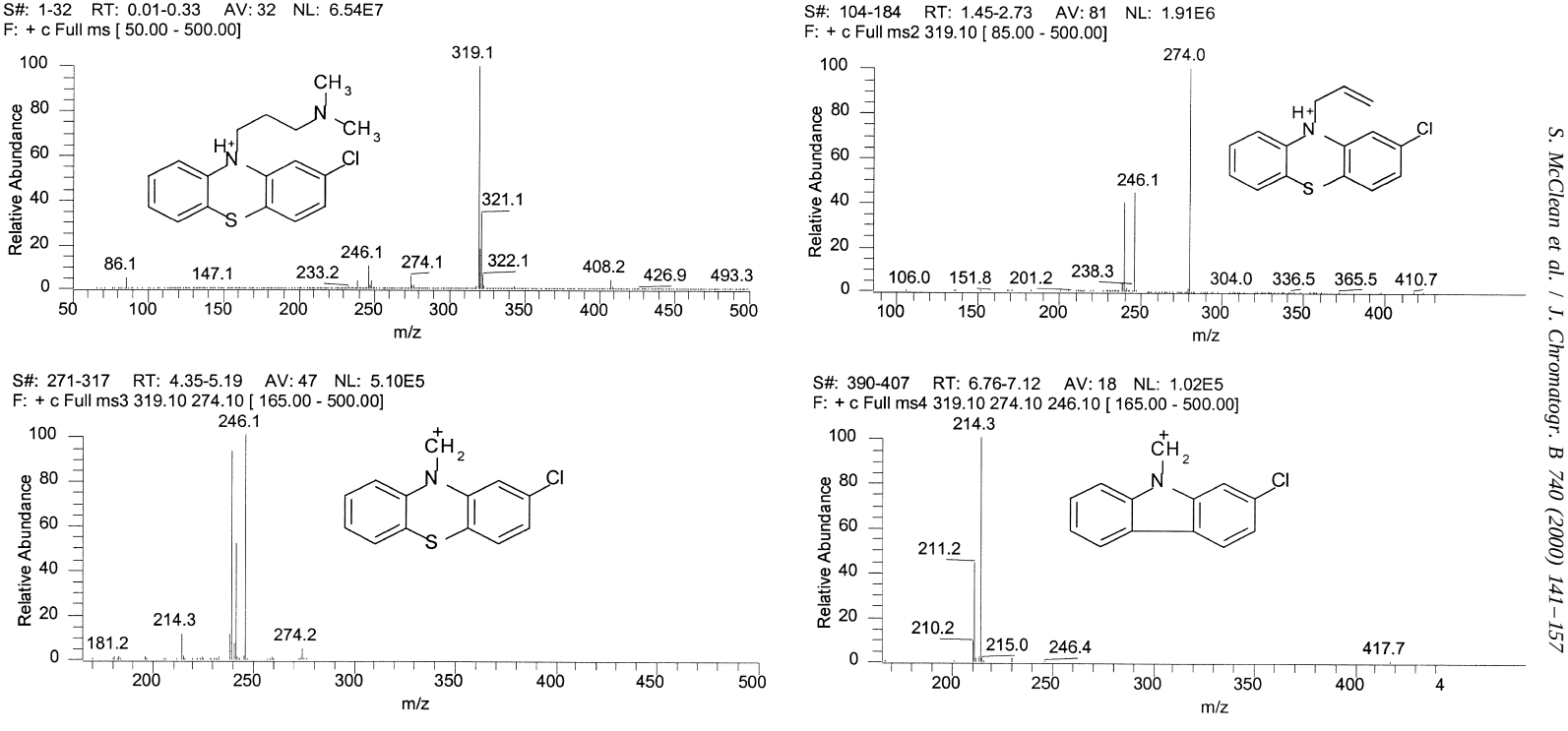


Fig. 2. MS^n fragmentation spectra for proposed structures of fragments formed from (a) chlorpromazine; (b) flupenthixol; (c) trifluoperazine; (d) risperidone; and (e) trimipramine.

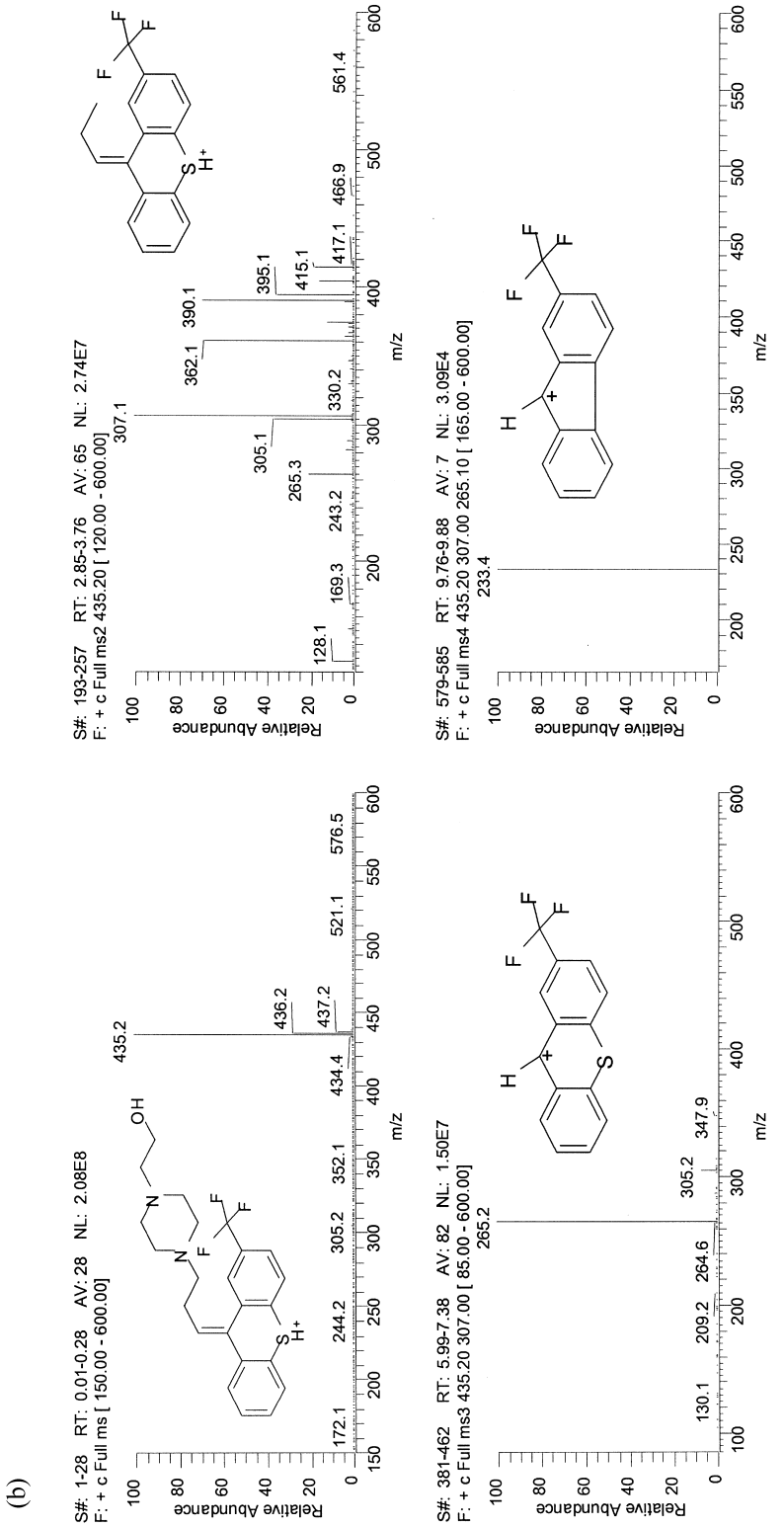
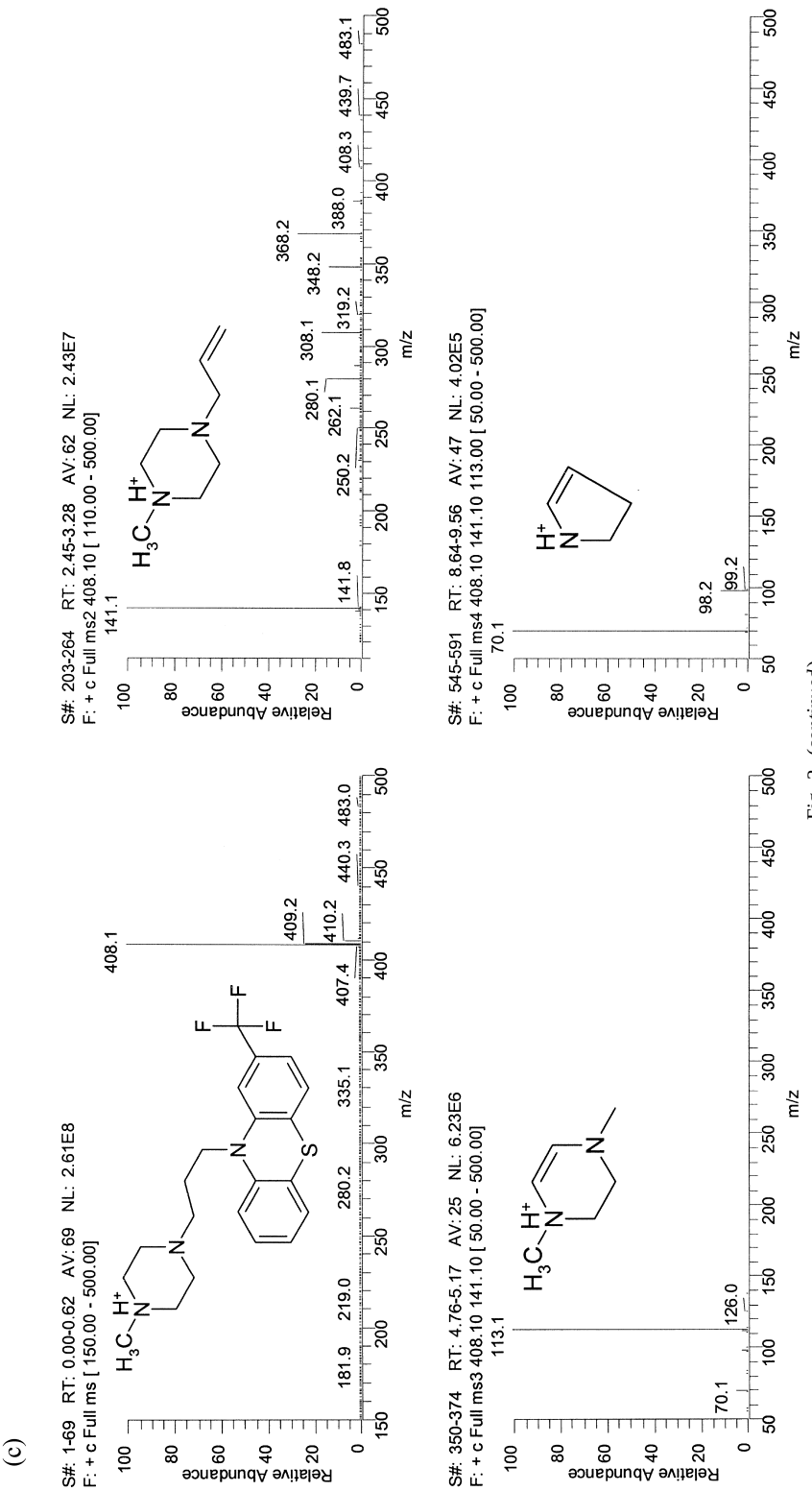


Fig. 2. (*continued*)



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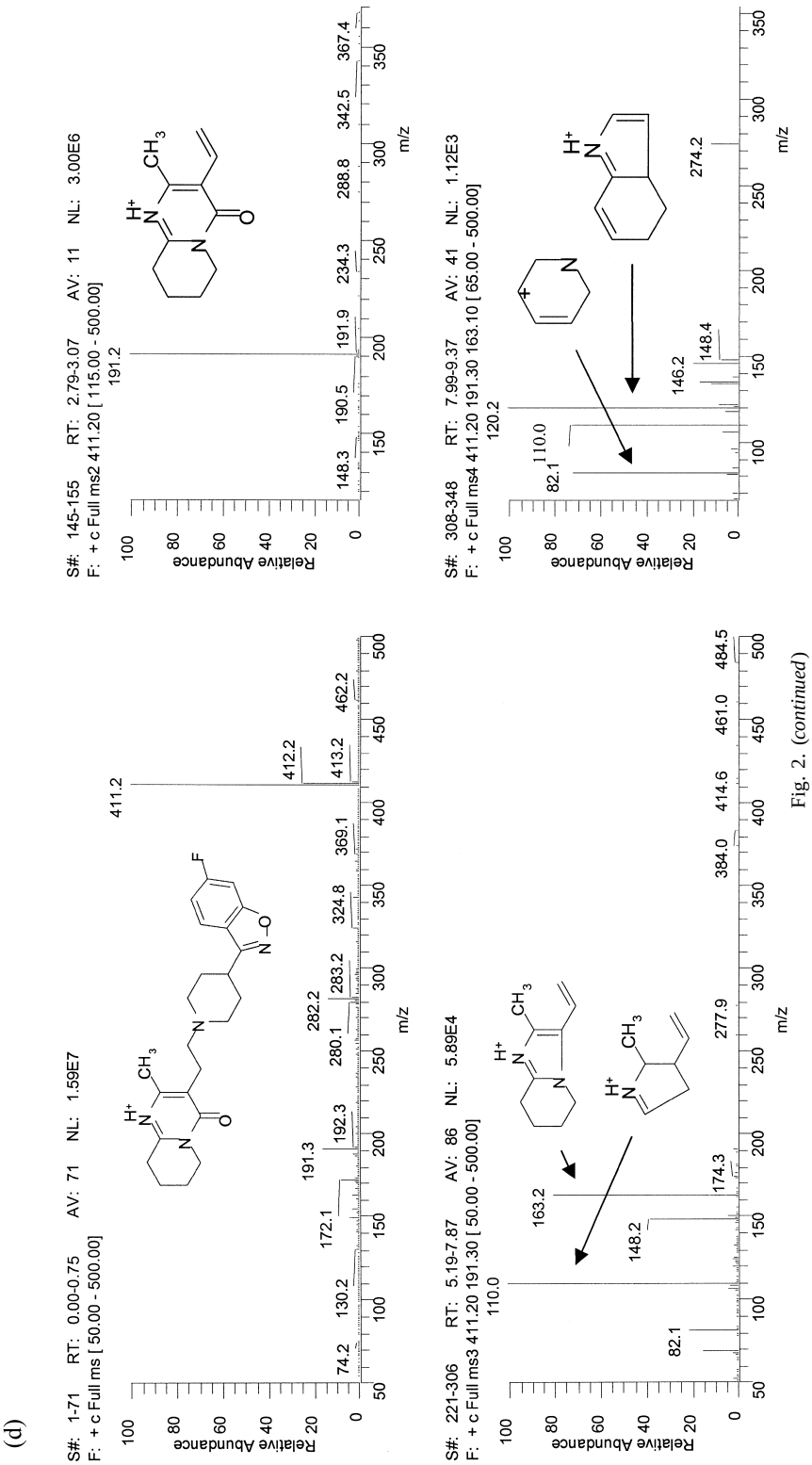

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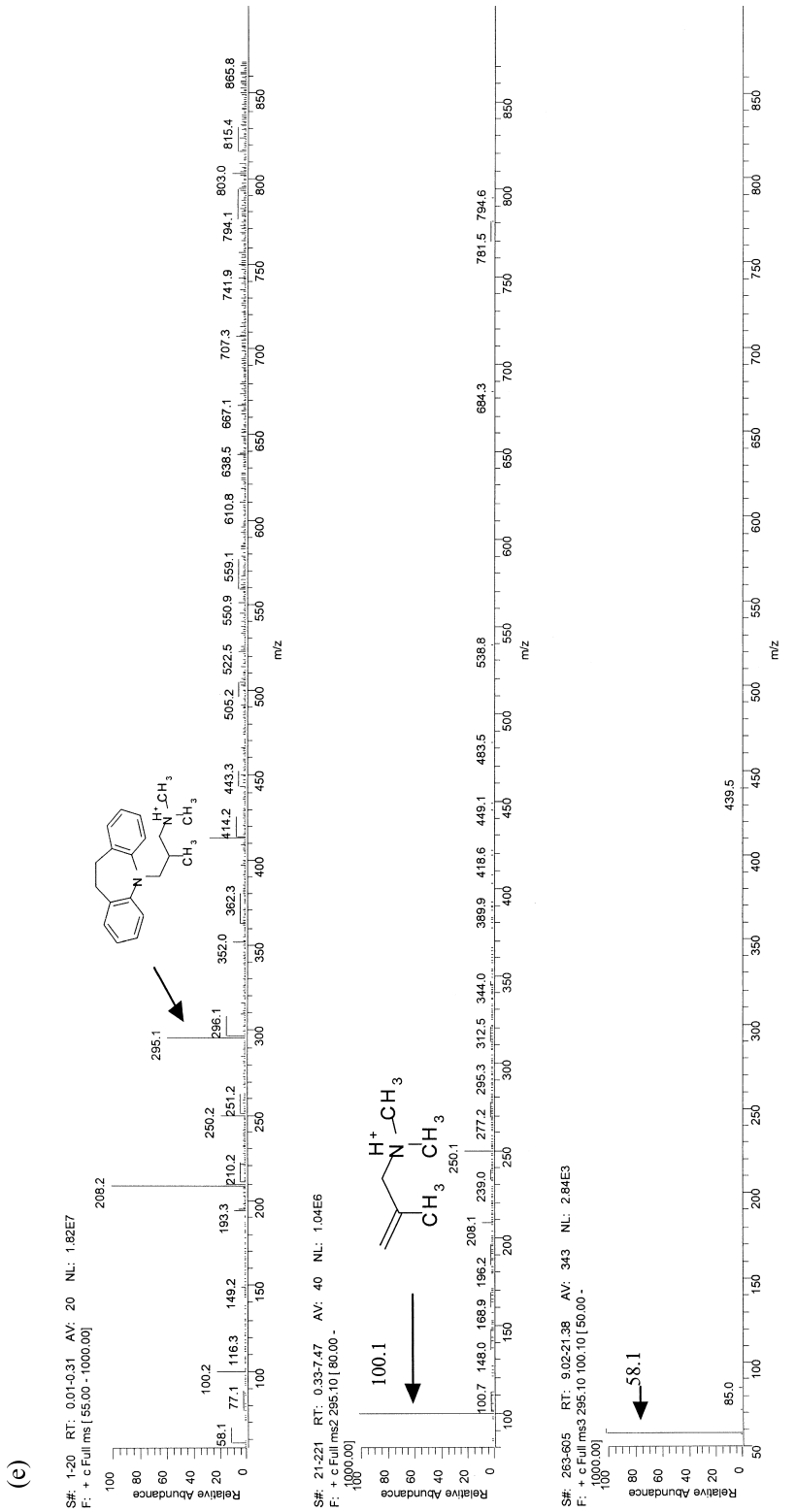

Fig. 2. (*continued*)

ments in the MS^n processes which retain charge at this same position throughout. Fig. 1d shows all possible fragments resulting from the MS^n characterisation of risperidone. MS–MS fragmentation involves a major loss of 220 a.m.u. due to cleavage of the ethyl–piperidinyl nitrogen bond and subsequent loss of the benzisoxazole group with its piperidine substituent. This results in formation of an ethene group at the 3-position of the 2-methyl-4H-pyrido pyrimidin-4-one (Fig. 1d, structure II). The MS^3 spectrum is somewhat complicated in that peaks are observed at m/z 163, 110 and 82. Some of these are also observed in MS^4. For MS^3 using Fig. 1d structure II as the precursor, loss of the 4-position carbonyl and subsequent ring contraction accounts for an overall net loss of 28 a.m.u. giving structure III (Fig. 1d). The m/z 82 ion in MS^3 and MS^4 may be due to a pyridine/piperidine-type carbocation shown as structure (IV) in Fig. 1d. MS^4 of structure III provides a pyrrole entity which has retained the methyl and ethene groups intact (structure V). This too is a possible fragment in the MS^3 spectrum of structure II, caused by a loss of 81 a.m.u. A further fragment of m/z 120 is observed in MS^4 which is indole-like in structure (VI) and formed by rearrangement of structure III.

3.1.5. Trimipramine

For trimipramine it would appear that protonation in MS occurs on the nitrogen atom of the dimethylamino–2-methylpropyl substituent group that is the only remaining fragment in MS–MS. Charge resides in the same position and a carbon double bond terminates the original point of fusion to the nitrogen atom on the aminodibenzyl structure. A plausible structure cannot be proposed for the MS^3 product ion with m/z 58.1.

3.2. Analytical data

All analytical determinations were carried out using MS–MS information as presented above. Calibration data and RSD values were calculated on standard solutions of the compounds. As NaOH dissolution of the hair was used prior to analysis, standards, treated by the hair extraction procedure, were also analysed. This involved spiking a digestion mix of blank hair with a known concentration of the drugs. This would give an indication of the effect of the extraction procedure on linearity of response, RSD of peak area and limit of detection and recovery from the matrix. Fig. 3 shows a typical LC–MS–MS chromatogram of the five compounds under investigation.

3.2.1. RSD values

Table 2 describes RSD values for peak area and retention time. RSD values for retention time are less than 2% for all compounds irrespective of the standard used.

3.2.2. Calibration data

Correlation coefficients (as R^2) greater than 0.9888 were achieved for all drugs using conventional standards while those treated with NaOH gave poorer linearity, risperidone being the worst case having a correlation coefficient of 0.9616. Throughout the analyses risperidone gave consistently poor, non-gaussian peak shape and broad peak width. Despite that it is still possible to use its analytical signal in these determinations. These results are shown in Table 3.

3.2.3. Limits of detection (LODs)

LODs were established by serially diluting standard solutions until a predetermined S/N ratio was achieved. The LOD then corresponded to the concentration giving that detection ratio. Acceptable LODs as shown in Table 4 were in the range 10^{-9} to 10^{-8} mol l^{-1}. Lower LODs may be possible by fully optimising the level of volatile acid and other modifiers in the mobile phase which affect the sensitivity of the ESI signal, as this was not attempted in these studies.

3.2.4. Recovery studies

Chlorpromazine gave the best recovery at 86% while for risperidone only 15% recovery was achieved. These results are summarised in Table 5. It is therefore apparent that while the present method is sensitive to the determination of these compounds, a less degradative extraction method such as Soxhlet (as described by McClean et al. for the extraction of 1,4-benzodiazepines from hair [21]) may provide a higher efficiency of extraction and thereby lower LOD values even further.

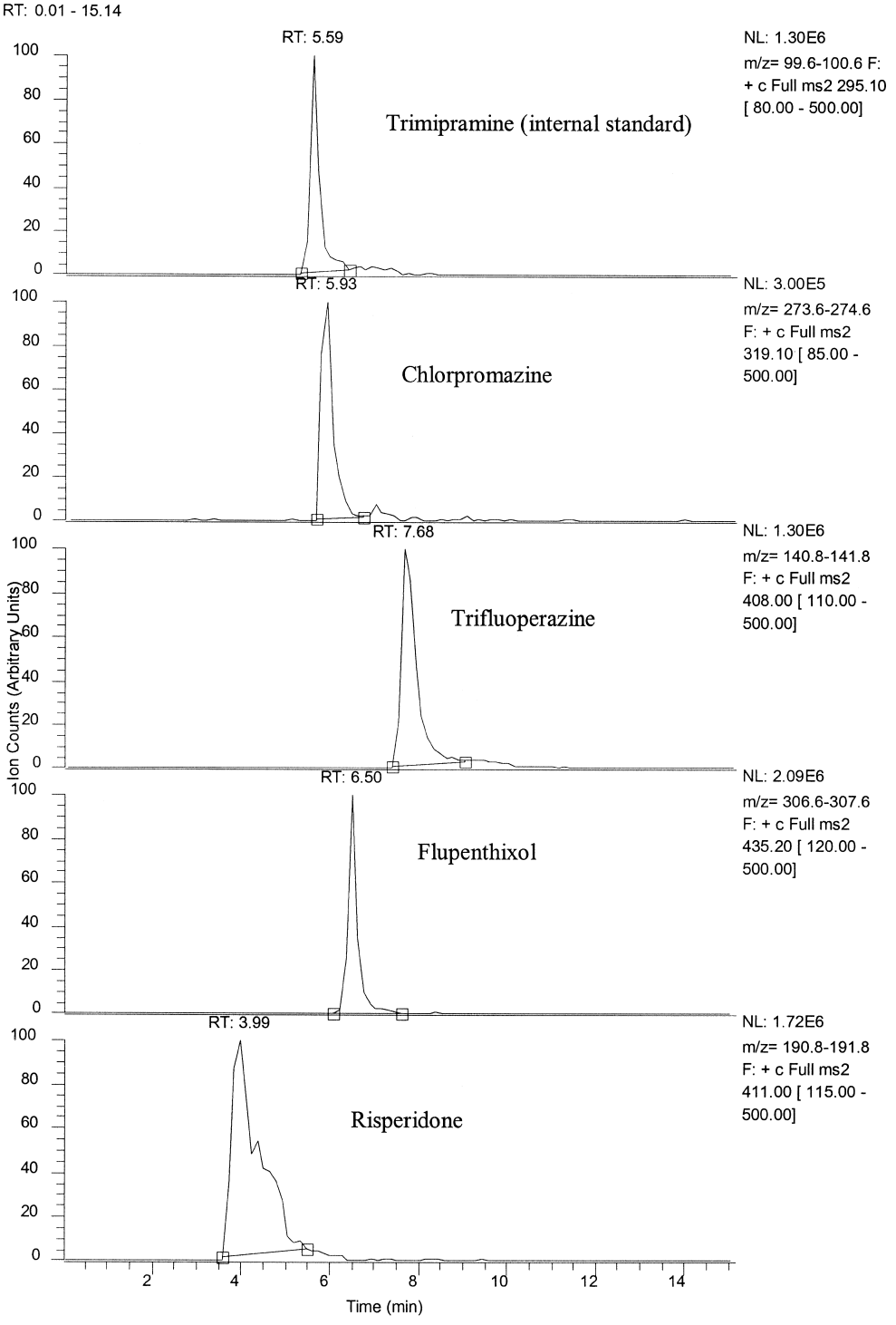

Fig. 3. LC–MS–MS chromatogram of the five drugs under investigation at the 5.0×10^{-6} mol l^{-1} concentration level. Note the poor peak shape for risperidone. The mobile phase consisted of two components A and B in the proportions 35% and 65%, respectively. Solvent A consisted of 0.02 mol l^{-1} ammonium acetate and 0.1% acetic acid in water while solvent B contained only acetonitrile. A flow-rate of 0.5 ml min^{-1} was used and a 20-μl injection made of the sample prepared in methanol.

Table 2
Comparison of RSD values for absolute retention time and peak area for five anti-psychotic drugs by LC–MS–MS

Drug[a]	Peak area						Retention time					
	Conventional standards			Treated standards			Conventional standards			Treated standards		
	RSD (%)	Conc. $\times10^{-6}$ mol l^{-1}	n	RSD (%)	Conc. $\times10^{-6}$ mol l^{-1}	n	RSD (%)	Conc. $\times10^{-6}$ mol l^{-1}	n	RSD (%)	Conc. $\times10^{-6}$ mol l^{-1}	n
CPZ	2.25	5.0	6	8.83	0.5	6	1.31	5.0	6	1.31	0.5	6
TFP	5.47	5.0	6	10.57	0.5	5	0.94	5.0	6	0.95	0.5	6
FPT	14.07	1.0	6	6.80	0.5	6	1.29	1.0	6	0.06	0.5	6
RSP	10.76	9.7	5	6.50	4.9	6	0.13	9.7	6	0.75	4.9	6
TMP	4.56	5.0	6	5.16	5.0	6	0.93	5.0	6	1.36	0.5	6

[a] CPZ, chlorpromazine; TFP, trifluoperazine; FPT, flupenthixol; RSP, risperidone; and TMP, trimipramine.

Table 3
Correlation coefficients for calibration data of anti-psychotic drugs measured using absolute peak area in both cases[a]

Drug	Conventional standards		Treated standards	
	R^2	n	R^2	n
Chlorpromazine	0.9905	6	0.9894	7
Trifluoperazine	0.9907	6	0.9867	5
Flupenthixol	0.9888	5	0.9802	6
Risperidone	0.9889	5	0.9616	6
Trimipramine	0.9979	5	0.9713	5

[a] The calibration range was 5.0×10^{-7} to 1.0×10^{-5} mol l^{-1} in all cases apart from risperidone where the range was 4.9×10^{-7} to 9.7×10^{-6} mol l^{-1}.

Table 4
LOD's for the determination of anti-psychotic drugs using conventional and treated standards

Drug	Conventional standards		Treated standards	
	LOD/mol l^{-1}	S/N	LOD/mol l^{-1}	S/N
Chlorpromazine	6.0×10^{-8}	3	6.0×10^{-8}	3
Trifluoperazine	7.0×10^{-8}	3	6.0×10^{-8}	2
Flupenthixol	3.0×10^{-9}	4	1.0×10^{-8}	3
Risperidone	4.0×10^{-9}	3	2.0×10^{-8}	4
Trimipramine	7.5×10^{-9}	2	2.0×10^{-8}	2

Table 5
Recoveries for drugs taken through the extraction procedure compared to conventional standard solutions of the same concentration

Drug	Recovery (%)	Concentration level (mol l^{-1})
Chlorpromazine	86	5.0×10^{-6}
Trifluoperazine	54	5.0×10^{-6}
Flupenthixol	20	5.0×10^{-6}
Risperidone	15	4.9×10^{-7}
Trimipramine	33	5.0×10^{-7}

Table 6
Concentration of anti-psychotic drugs found in human hair

Drug	Measured concentration (mol l^{-1})	Concentration in hair (ng mg^{-1})	RSD value (%)
Chlorpromazine	9.72×10^{-7}	1.24	47.91
Flupenthixol	1.26×10^{-6}	0.22	17.59
Trifluoperazine	1.16×10^{-5}	18.88	27.00

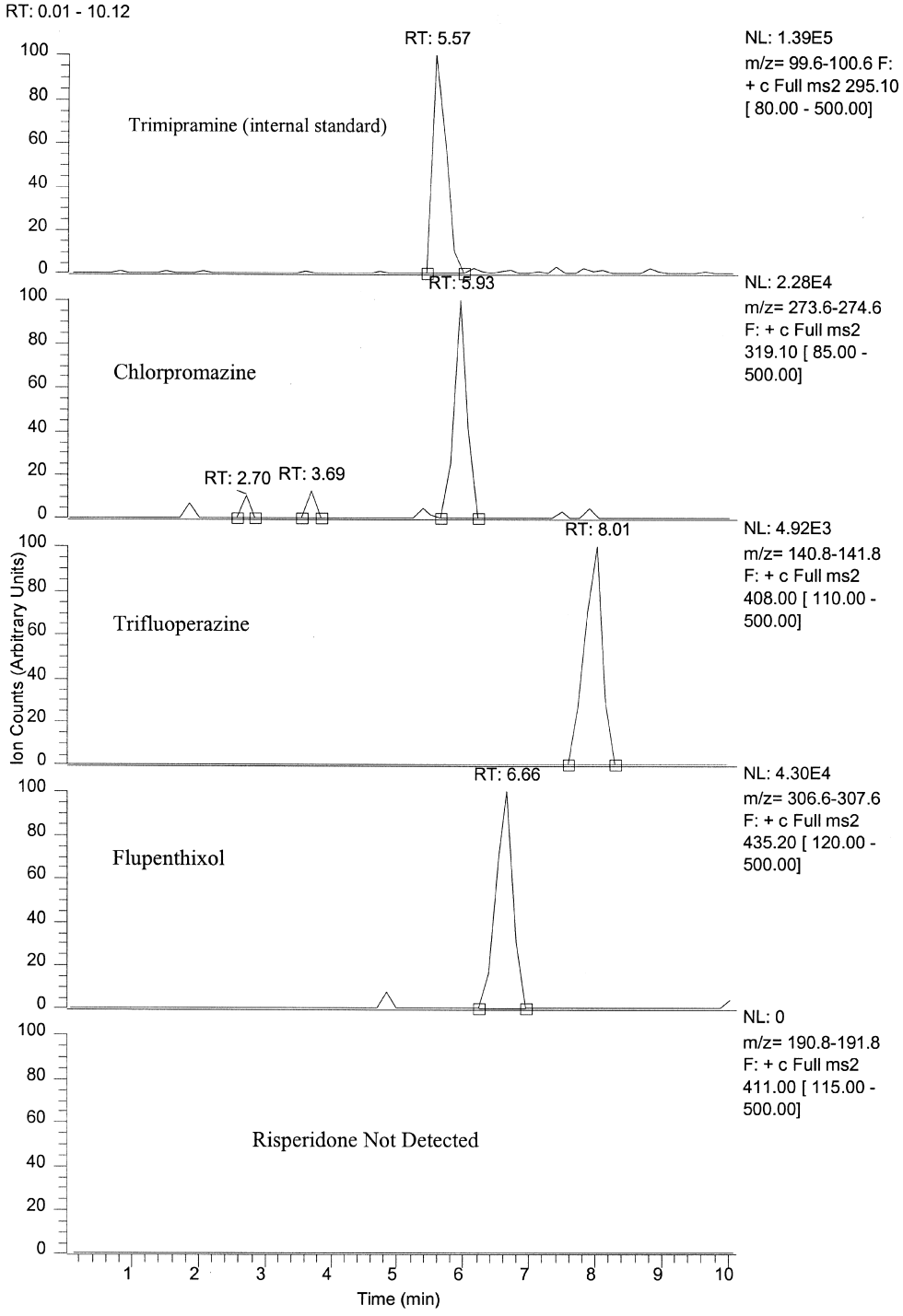

Fig. 4. LC–MS–MS chromatogram of anti-psychotic drugs in a hair sample using trimipramine as the internal standard. The mobile phase consisted of two components A and B in the proportions 35% and 65%, respectively. Solvent A consisted of 0.02 mol l^{-1} ammonium acetate and 0.1% acetic acid in water while solvent B contained only acetonitrile. A flow-rate of 0.5 ml min^{-1} was used and a 20-μl injection made of the sample prepared in methanol.

While complete dissolution of the hair does release much more of the drug from the matrix, the dissolved sludge-like sample requires centrifugation to remove particulate matter. Formation of an emulsion is almost unavoidable when carrying out the liquid–liquid extraction step. Even with long standing times it does not settle out completely and so further centrifugation is required. Due to the relatively long extraction process it is almost certain that losses are incurred at each stage, and these are reflected (along with degradation of the compound) in the results obtained from the recovery studies. Cross-contamination of glassware was readily identified as one area causing false positives and so rigorous washing and use of clean glassware were necessarily adopted.

3.2.5. Hair extraction

Using trimipramine as the internal standard response factors for the drugs were measured at the 5.0×10^{-7} mol l^{-1} level. Determination of chlorpromazine, flupenthixol and trifluoperazine by the internal standard method gave concentrations of 1.24, 0.22 and 18.88 ng/mg hair, respectively. Table 6 shows the measured concentration of these drugs in hair along with RSD values for the measurements. Trimipramine was not detected in this sample and so could confidently be used as the internal standard. Fig. 4 shows a typical LC–MS–MS chromatogram for the determination of chlorpromazine, trifluoperazine and flupenthixol in human hair in the presence of the internal standard trimipramine.

The RSD values produced are rather poor. This is somewhat surprising as the peak shape in relatively distinct in each case and the concentrations involved are far above respective LODs for the compounds.

4. Conclusion

Electrospray ionisation quadrupole ion-trap mass spectrometry (ESI–MS) has been satisfactorily used to characterise the anti-psychotic drugs chlorpromazine, trifluoperazine, flupenthixol, risperidone and the antidepressant/internal standard trimipramine through the use of MS^n fragmentation. The fragmentation patterns can be satisfactorily explained in terms of chemical structures. An LC–MS–MS method has been developed for determining trace concentrations of these drugs. It has been validated with respect to RSD values of R_t and peak area, correlation coefficients for calibration data, LOD values and recovery values.

This LC–MS–MS method is capable of detecting and determining levels of anti-psychotic drugs in a human hair sample following therapeutic administration and a preliminary study to illustrate this is presented. However, some further work should be carried out to improve the RSD values found for the determination of the drugs in the real sample. One parameter that could be investigated is the use of acetonitrile in the mobile phase. It has been shown that methanol based LC mobile phases for positive mode ESI–MS give a small increase in response compared with those containing acetonitrile [22]. This change may effect an improvement in the LOD and perhaps on the RSD values. Another area of concern in this application is the use of NaOH dissolution which appears to have a detrimental effect on the recovery of, in particular, risperidone from the hair matrix. The method is found particularly useful for the determination of the phenothiazines, chlorpromazine and trifluoperazine and the thioxanthene, flupenthixol, in a human hair sample. If these are of particular interest then the low recovery for risperidone is of no consequence. If risperidone is the main analyte then another dissolution procedure would have to be investigated.

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