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RESEARCH PAPER

Validation of a multi-analyte LC–MS/MS method for screening and quantification of 87 psychoactive drugs and their metabolites in hair

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Abstract A multi-analyte method for the detection and quantification of 87 psychoactive drugs (antidepressants, antipsychotics, benzodiazepines, and z-drugs) in human hair has been developed and fully validated using the liquid chromatography–tandem mass spectrometry system. Due to the remarkable increase in requests of hair sample tests (such as for driver's license renewals, child custody, DFA cases, and postmortem toxicology), we focused on the development of a rapid sample preparation. About 20 mg of hair samples, previously washed and cut into snippets, was ultrasonicated with 700 μl of methanol. Samples were then directly analyzed using a 4000 QTRAP (AB SCIEX, Foster City, CA, USA) with an electrospray ionization (ESI) Turbo V^{TM} Ion Source. The validation criteria parameters were satisfactory and in accordance with the international guidelines. All the compounds tested were successfully detected. One important aspect is the LODs in the low picogram per milligram concentration which may suggest a potential use of this method in cases of detection of single drug exposure. However, the LC–MS/MS method has been successfully applied for the analysis of postmortem cases (n=9).

Keywords Psychoactive drugs · Antidepressants · Benzodiazepines · Hair · LC–MS/MS

Introduction

Identification of drug exposure based on a hair test is of great interest in the forensic field especially due to the large window of detection that the keratin matrix guarantees [1, 2]. Drugs of abuse in hair like cocaine, opiates, amphetamines, amphetamine derivatives, cannabis, and many others are routinely detected by laboratories of forensic medicine for many different purposes, such as driver's license renewal, workplace drug testing, organ transplantation, child custody, and divorces [3–5]. In the last years, psychoactive drugs are also under investigation, and the requests for their detection in this alternative matrix are extensively increasing [6–9]. In particular, benzodiazepine determination in hair is routinely requested by addiction service staff, in order to monitor patients under therapy. So far, there are not many screening procedures in the literature. Generally, only methods for the detection of a single substance [6–9], or a single class of drugs in hair [10–13], are developed and reported. Even when an analytical approach that aims at identifying and quantifying different classes of pharmaceutical products and their metabolites is settled, a time-consuming sample preparation is needed [14–17]. The aim of this study was to fully validate a method for the screening and quantification in human hair samples of 87 drugs, among benzodiazepines, hypnotics, antidepressants, and antipsychotics, which are commonly prescribed in different therapies in Italy. The LC–MS/MS assay has proven to be useful not only for the potential detection of a single-dose drug exposure, assessed by the high analytical sensitivity, but also for chronic use monitoring, especially in drug users who are frequently poly-drug consumers. It is also our opinion that a multi-analyte method concerning the detection and quantification of psychoactive drugs could find other possible applications such as in clinical toxicology (for intoxication cases) and in psychiatry especially because new commercial drugs can be easily added to the method. Finally, an interesting case regarding long-term changes in psychoactive drug therapy is described.

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Material and methods

Reagents

Eighty-seven analytes among benzodiazepines, antidepressants, antipsychotics, and their metabolites were analyzed. Amilsulpride, clozapine, quetiapine, and risperidone were purchased by Sandoz (Sandoz Industrial Products, Trento, Italy); amitriptyline, asenapine, halazepam SI, mianserin, mirtazapine, and perphenazine were obtained by Merck & Co. (MSD Italia, Pavia, Italy); bromperidol, clomipramine, desipramine, desmethylclozapine, desmethylvenlafaxine, droperidol, duloxetine, hydroxyzine, imipramine, levomepromazine, meprobamate, olanzapine, paliperidone, phenelzine, pimozide, and trazodone were purchased by LGC Standards (Milan, Italy); buspirone was obtained by Menarini (Gruppo Menarini, Florence, Italy); chlorpromazine, fluoxetine, and haloperidol were purchased by Lusofarmaco (Gruppo Menarini, Milan, Italy); citalopram, desmethylcitalopram, fluphenazine, nortriptyline, and protryptiline were obtained by Lundbeck (Lundbeck Italia SPA, Milan, Italy); clothiapine, dibenzepin, maprotiline, trimipramine, and veralipride were purchased by Novartis (Novartis Farma, Varese, Italy); dixyrazine and tranylcypromine were obtained by Laboratorio Farmacuetico S.I.T. (Pavia, Italy); dothiepin, levosulpiride, pericyazine, sultopride, and tiapride were purchased by Teofarma (Pavia, Italy); fluvoxamine was obtained by Duphar B.V. (Solvay, Weesp, Holland); paroxetine was purchased by SmithKline Beecham Pharmaceuticals (GSK, Verona, Italy); promazine was obtained by Pierrel (Pierrel SPA, Milan, Italy); reboxetine, sertraline, venlafaxine, and ziprasidone were purchased by Pfizer Roerig (Pfizer SPA, Milan, Italy). Alprazolam, bromazepam, camazepam, chlordiazepoxide, clobazepam, clonazepam, 7-amino-clonazepam, demoxepam, diazepam, desmethyldiazepam, flunitrazepam, 7-amino-flunitrazepam, flurazepam, α-hydroxy-ethylflurazepam, desalkylflurazepam, lorazepam, lormetazepam, medazepam, midazolam, nitrazepam, oxazepam, prazepam, temazepam, α-hydroxy-triazolam, triazolam, zolpidem, and zopiclone were purchased by Lipomed (Nova Chimica, Milan, Italy); clotiazepam, estazolam, and etizolam were obtained by Formenti (Formenti SPA, Milan, Italy); brotizolam and chlordesmethyldiazepam were purchased by Ravizza (Ravizza Farmaceutici SPA, Milan, Italy); and ketazolam and pinazepam were obtained by Ciba Geigy (Basel, Switzerland). Water was purified by filtering deionized water on a Milli-Q Simplicity 185 filtration system from Millipore (Bedford, MA, USA). Formic acid for mass spectrometry was obtained from Sigma-Aldrich (St. Louis, MI, USA). HPLC-grade methanol and acetonitrile were purchased from Mallinkrodt Baker (Milan, Italy).

Instrumentation

LC–MS/MS analyses were performed with an Agilent 1100–1200 Series system (Agilent Technologies, Palo Alto, CA, USA) interfaced to a 4000 QTRAP (AB SCIEX, Foster City, CA, USA) with an electrospray ionization (ESI) Turbo V™ Ion Source. The LC instrumentation was composed of a vacuum degasser, a binary pump, an isocratic pump, and an autosampler maintained at 4 °C. The injector needle was externally washed with methanol prior to any injection. A Hypersil GOLD column (100 mm, 2.1 mm i.d., 3 mm particle size) (Thermo Scientific, MI, Italy) was kept at 25 °C during the analysis. The mobile phase consisted of formic acid 0.1 % (A) and methanol (B). Chromatographic gradient elution was the following: constant flow of 0.2 ml/min; 95 % phase A maintained for 2.5 min, then decreased up to 30 % A in 0.5 min and successively declined to 5 % A in 2.5 min, maintained at 5 % A for 7 min and re-equilibrated for 8 min.

The 87 substances monitored were then divided into four groups (antidepressants, antipsychotics, and two subgroups for benzodiazepines). Each sample was then injected four times, in order to maintain a high analytical sensitivity. The ESI source settings were ion spray voltage, +5,500 V; source temperature, 350 °C; and nebulization and heating gas (air), 20 and 25, respectively. Multiple reaction monitoring was optimized using nitrogen as collision gas (with pressure set at level 8) and a dwell time of 30 ms. Two transitions for each substance were chosen for identification; the most intense was used for quantification purposes. All the transitions and MS parameters are listed in Table 1. Data acquisition and elaboration were performed by the Analyst software (version 1.5, AB SCIEX).

Sample treatment

Procedure

About 20 mg of hair was washed twice with organic solvent (dichloromethane, methanol), taken to dryness under a nitrogen stream, and cut into small pieces. Then, 20 μl of halazepam (I.S. 100 ng/ml) was added to the sample, together with 700 μl of methanol. After 1 h of ultrasonication, 5 μl was directly injected in the LC–MS/MS system.

Calibration standards and quality control samples

Standards were prepared by dissolution of each compound in methanol at the concentration of 1 mg/ml. Working solutions were prepared in methanol at five different concentrations ranging from the limit of quantification (LOQ) to 1,000 ng/ml by independent dilution.

Table 1 MRM parameters

Analyte	Q1 (amu)	Q3 (amu)	DP (V)	CE (eV)	Analyte	Q1 (amu)	Q3 (amu)	DP (V)	CE (eV)
7-Amino-clonazepam	286.2	121.2–222.2	90	42–35	Hydroxyzine	376.1	202.2–166.2	64	26–70
7-Amino-flunitrazepam	284.1	135.1–226.1	92	40–42	Imipramine	280.9	86.2–58.3	82	25–65
α-Hydroxy-ethylflurazepam	333.3	109.3–211.3	90	41–52	Ketazolam	285.1	193.3–154.3	91	46–40
α-Hydroxy-triazolam	359.1	331.1–250.1	94	38–53	Levomepromazine	330.0	100.3–243.2	72	30–33
Alprazolam	309.4	205.4–281.4	93	58–35	Levosulpiride	342.1	112.2–214.2	94	38–47
Amisulpride	369.9	242.2–112.5	101	39–39	Lorazepam	321.4	275.4–303.4	78	30–22
Amitriptyline	278.1	233.3–91.2	102	25–35	Lormetazepam	335.3	289.3–177.3	74	30–58
Asenapine	286.2	165.9–215.0	96	46–40	Maprotiline	278.2	250.3–219.3	148	28–36
Bromazepam	316.4	182.4–209.4	90	46–36	Medazepam	271.1	91.4–207.4	70	45–38
Bromperidol	419.8	165.2–402.3	84	37–30	Meprobamate	219.3	158.2–97.3	59	12–20
Brotizolam	395.3	314.3–316.3	102	34–33	Mianserin	264.9	208.3–58.3	103	30–47
Buspirone	385.9	122.2–222.3	110	42–42	Midazolam	326.1	291.3–249.3	102	39–54
Camazepam	372.4	255.4–283.4	47	33–19	Mirtazapine	265.9	72.2–195.2	100	35–36
Chlordesmethyldiazepam	305.1	140.2–206.2	92	56–81	Nitrazepam	282.4	180.5–207.5	96	52–48
Chlordiazepoxide	300.1	227.1–192.1	61	36–56	Nortriptyline	263.9	233.3–91.2	80	22–35
Chlorpromazine	318.8	86.2–246.2	78	29–34	Olanzapine	312.9	256.3–282.3	90	34–34
Citalopram	325.1	109.2–262.3	100	35–28	Oxazepam	287.2	241.3–269.3	85	32–24
Clobazam	301.2	259.3–224.3	87	30–46	Paliperdione	427.3	207.2–110.1	122	40–60
Clomipramine	314.9	86.2–58.2	80	26–64	Paroxetine	330.4	192.3–70.2	144	30–49
Clonazepam	316.2	270.4–214.4	89	36–52	Pericyazine	365.9	142.2–114.2	98	34–42
Clothiapine	343.9	287.2–255.3	101	30–44	Perphenazine	403.9	171.3–143.2	106	35–42
Clotiazepam	319.4	291.4–154.4	90	33–41	Phenelzine	137.3	105.1–91.1	53	21–23
Clozapine	327.0	270.1–296.3	100	34–36	Pimozide	462.1	109.2–328.4	126	88–43
Demoxepam	287.2	269.2–180.2	93	40–34	Pinazepam	309.3	241.3–269.3	83	48–45
Desalkylflurazepam	288.8	140.2–226.2	99	44–40	Prazepam	325.4	271.4–140.4	80	34–53
Desipramine	266.9	72.2–208.3	71	28–33	Promazine	284.8	86.2–212.3	65	29–35
Desmethylcitalopram	310.9	262.3–109.2	94	24–35	Protriptyline	264.9	192.2–156.2	110	34–29
Desmethylclozapine	313.0	270.2–253.2	110	36–32	Quetiapine	384.5	253.5–279.1	96	31–34
Desmethyldiazepam	271.1	140.3–165.3	96	40–41	Reboxetine	313.8	176.0–91.1	81	31–43
Desmethylvenlafaxine	264.0	107.1–58.2	70	47–40	Risperidone	411.4	191.3–163.5	95	42–66
Diazepam	285.2	154.2–193.2	93	37–47	Sertraline	306.0	275.2–159.2	63	18–37
Dibenzepin	295.9	250.9–209.2	90	35–47	Sultopride	354.9	227.2–112.2	99	46–39
Dixyrazine	427.9	229.3–187.3	104	37–39	Temazepam	301.3	255.4–193.4	70	30–48
Dothiepin	295.9	223.2–218.3	75	33–33	Tiapride	328.9	256.0–313.3	118	26–48
Droperidol	380.0	194.3–165.2	81	25–37	Tranylcypromine	134.2	117.1–91.1	50	16–38
Duloxetine	298.2	154.1–188.0	38	8–8	Trazodone	372.2	176.3–148.2	97	35–48
Estazolam	295.3	205.3–267.3	86	57–35	Triazolam	343.4	239.4–308.4	93	59–37
Etizolam	343.3	314.3–259.3	110	36–47	Trimipramine	295.0	100.3–58.1	69	23–59
Flunitrazepam	314.4	239.4–268.4	95	48–36	Venlafaxine	279.2	58.2–261.4	64	45–18
Fluoxetine	310.1	148.3–310.1	63	13–7	Veralipride	383.8	124.3–244.1	102	39–44
Fluphenazine	437.9	171.3–143.3	109	38–45	Ziprasidone	413.3	194.2–177.3	121	39–39
Flurazepam	388.4	315.4–287.4	85	35–47	Zolpidem	308.2	235.4–263.4	96	51–38
Fluvoxamine	319.0	258.3–71.2	69	16–29	Zopiclone	389.3	245.1–217.1	55	24–46
Haloperidol	375.9	165.2–123.1	83	34–57					

QC samples were prepared by a different operator by independent dilution at three concentration levels: LOQ, 20 ng/ml, and 100 ng/ml. All standard solutions were stored at −20 °C.

Validation

The method was initially tested for sensitivity. Limits of detection (LOD) and LOQ were measured by evaluating the signal/noise (*S*/*N*) ratio of three replicates for each compound at proper concentrations. LOD was fixed at the concentration with a *S*/*N*>3 while concentrations of analytes with a *S*/*N*>10 were chosen as LOQ.

Fifteen blank hair samples from ten adults and five children were analyzed for possibly interfering peaks during the early validation phase of the method. Linearity was evaluated through the analysis of five replicates for each calibration level. The calibration curve was estimated by least-squares regression.

Intra-day imprecision, expressed as relative standard deviation (RSD), was calculated analyzing the QC samples (20/LOQ and 100 pg/mg) in five replicates, while inter-day precision was measured analyzing the QC samples in duplicate on five different days over a 3-week period. The concentration of the analytes in the QC samples was calculated versus the daily calibration curves. Accuracy was determined for all analytes as the percentage deviation of the average of results from the corresponding nominal value.

Five different blank hair samples and five methanolic solutions were spiked at two levels (20/LOQ and 100 pg/mg), processed separately with the described procedure, and the absolute peak areas were compared. Experiments were carried out in triplicate. Results were calculated as the percentage of the mean deviation of drug response in hair samples from the response measured in methanol at the same concentration level. Matrix effects were then expressed as ion suppression or enhancement.

Several postmortem hair samples of known diazepam users were collected together, and a homogeneous sample of washed and cut hair was created. This sample was analyzed over a 5-week period to monitor reproducibility and robustness of the method.

Application on real samples

The procedure was applied to healthy volunteers and to patients under treatment with psychoactive drugs who have given an informed consent before sample collection, as well as to four different hair samples collected from autopsy cases. For healthy volunteers, 15 hairs were analyzed. Six were collected among the authors' colleagues and friends. All the subjects did not consume any psychoactive substances during the 9 months before sample collection. The 9-cm proximal hair segment was used for the analysis. Nine samples were collected from the patients under pharmacological treatment. All the substances consumed in the last 9 months were registered, and the 9-cm proximal segment was submitted to the analytical procedure. In one case, hair was divided in three aliquots of 3 cm length. Finally, analyses of four hair samples collected from autopsy cases were carried out.

Results and discussion

A multi-analyte method in hair for simultaneous screening and quantification of psychoactive drugs frequently prescribed in Italy was developed by LC–MS/MS and fully validated according to international guidelines [18, 19]. Specifically, 87 substances were identified through one extraction procedure and the direct injection of the methanolic solution using the same chromatographic conditions over four consecutive injections. The liquid chromatographic system is equipped with a secondary isocratic pump and a Valco valve. This is not used during routine analysis, but it could be of interest in case of a great batch of samples. In fact, using a secondary isocratic pump, the run time of a single injection lasted 12 min, allowing to save the column equilibration and to obtain a total run time of 48 min, as already observed in previously published methods [20].

During the development of the method, zuclopenthixol and flupenthixol have also been evaluated, but they did not fulfill the validation acceptance criteria, and they have been removed from the validation procedure, but still remaining on the list of transitions detected. Olanzapine provided a good sensitivity and specificity, but it is rather stable on the solution and was validated only for qualitative purposes. The quantification for this molecule is performed, whenever necessary, using an ad hoc method. The phenothiazine group generally provided the worst sensitivity, mainly due to the bad chromatographic retention. However, all the validation parameters were within the acceptance range, and they were maintained in the list of the detected compounds. All the LODs and LOQs are listed in Table 2. The method showed a very high sensitivity for the majority of the molecules evaluated and provided LODs generally lower than those previously published by other authors [15, 21].

The procedure was validated using a five-point calibration curve ranging from LOQ to 1,000 pg/mg. A good linearity was assessed by a regression coefficient always higher than 0.99. A weighted regression (1/*X*) was used to calculate the concentrations. Accuracy and imprecision were measured at two different quality control levels (20 pg/mg, except for the molecules with higher LOQ, and 100 pg/mg) and fulfilled the acceptance criteria. Ion suppression and enhancement were found to be negligible for all the compounds. The quantitative determination of diazepam in a real pool sample was found to be reproducible over a period of 5 weeks.

The method was entirely validated using halazepam as internal standard. Halazepam is no more prescribed and used in Italy; therefore, there is no chance to have false-positive and

Table 2 Limits of detection and limits of quantification

Analyte	LOD (pg/mg)	LOQ (pg/mg)	Analyte	LOD (pg/mg)	LOQ (pg/mg)
7-Amino-clonazepam	5.0	15.0	Hydroxyzine	10.5	35.0
7-Amino-flunitrazepam	0.1	0.3	Imipramine	0.2	0.7
α-Hydroxy-ethylflurazepam	5.0	15.0	Ketazolam	0.2	1.0
α-Hydroxy-triazolam	1.0	5.0	Levomepromazine	3.5	11.7
Alprazolam	0.2	1.0	Levosulpiride	12.6	42.0
Amisulpride	1.7	5.8	Lorazepam	5.0	15.0
Amitriptyline	1.4	4.6	Lormetazepam	1.5	5.0
Asenapine	4.1	13.8	Maprotiline	1.8	6.0
Bromazepam	5.0	20.0	Medazepam	1.0	3.0
Bromperidol	2.7	9.1	Meprobamate	4.9	16.3
Brotizolam	2.0	4.0	Mianserin	1.1	3.8
Buspirone	5.6	18.7	Midazolam	0.5	3.0
Camazepam	0.1	0.5	Mirtazapine	2.9	9.6
Chlordesmethyldiazepam	0.5	2.0	Nitrazepam	3.0	10.0
Chlordiazepoxide	5.0	20.0	Nortriptyline	0.6	2.1
Chlorpromazine	0.6	2.1	Olanzapine	3.2	10.5
Citalopram	1.9	6.3	Oxazepam	1.5	5.0
Clobazam	1.0	3.0	Paliperdione	1.3	4.5
Clomipramine	0.6	2.2	Paroxetine	4.1	13.8
Clonazepam	1.0	3.0	Pericyazine	7.0	23.4
Clothiapine	2.7	8.9	Perphenazine	2.8	9.3
Clotiazepam	0.1	0.3	Phenelzine	11.7	39.0
Clozapine	1.8	6.1	Pimozide	2.4	8.2
Demoxepam	3.0	10.0	Pinazepam	0.5	3.0
Desalkylflurazepam	0.5	2.0	Prazepam	0.5	2.0
Desipramine	0.3	0.9	Promazine	0.4	1.3
Desmethylcitalopram	0.3	1.1	Protriptyline	8.8	29.2
Desmethylclozapine	5.1	17.0	Quetiapine	9.3	31.1
Desmethyldiazepam	0.3	1.5	Reboxetine	1.4	4.5
Desmethylvenlafaxine	2.7	9.0	Risperidone	1.1	3.7
Diazepam	0.3	1.5	Sertraline	0.6	2.0
Dibenzepin	0.1	0.3	Sultopride	5.8	19.2
Dixyrazine	1.0	3.3	Temazepam	1.0	3.0
Dothiepin	0.4	1.4	Tiapride	2.8	9.2
Droperidol	1.2	3.9	Tranylcypromine	1.6	5.4
Duloxetine	0.4	1.3	Trazodone	0.4	1.5
Estazolam	0.3	1.0	Triazolam	1.0	5.0
Etizolam	1.5	5.0	Trimipramine	0.4	1.4
Flunitrazepam	0.5	2.0	Venlafaxine	2.7	9.1
Fluoxetine	0.8	2.8	Veralipride	6.7	22.2
Fluphenazine	9.9	32.9	Ziprasidone	2.6	8.6
Flurazepam	1.5	5.0	Zolpidem	0.1	0.3
Fluvoxamine	4.4	14.6	Zopiclone	13.0	43.4
Haloperidol	0.5	1.6			

real interferences. However, before any calibration curve, the blank hair samples were tested for the presence of this substance. Recently, we have purchased d5-diazepam, and we are using both the compounds as internal standards.

Table 3 Concentrations measured in real samples

Sample	Case	Analyte	Concentration (pg/mg)
Subject no. 1	Autopsy	Diazepam	588.0
		Desmethyldiazepam	1,998.0
		Midazolam	85.0
		Haloperidol	3,235.0
Subject no. 2	Autopsy	Venlafaxine	35.9
Subject no. 3	Autopsy	Mirtazapine	8,300.0
		Citalopram	2,072.0
		Desmethylcitalopram	388.0
		Quetiapine	4,289.0
		Diazepam	102.8
		Desmethyldiazepam	560.8
Subject no. 4	Autopsy	Tiapride	5,762.3
Subject no. 5	Patient under treatment	Fluoxetine	1,129.0
		Citalopram	2,333.3
		Desmethylcitalopram	1.6
		Haloperidol	<LOQ
Subject no. 6	Patient under treatment	Citalopram	270.7
		Desmethylcitalopram	181.1
		Diazepam	16.7
		Haloperidol	29.0
Subject no. 7	Patient under treatment	Amitriptyline	9.5
		Citalopram	42.0
		Diazepam	2.7
Subject no. 8	Patient under treatment	Clotiapine	332.9
		Quetiapine	2,607.6
Subject no. 9	Patient under treatment	Sertraline	128.0
Subject no. 10	Patient under treatment	Citalopram	69.7
		Desmethylcitalopram	22.7
		Amitriptyline	24.2
		Nortriptyline	57.8
		Mirtazapine	20.2
		Diazepam	23.7
		Desmethyldiazepam	12.9
Subject no. 11	Patient under treatment	Citalopram	95.5
		Desmethylcitalopram	8.7
		Clotiapine	17.8
		Haloperidol	9.0
		Bromperidol	144.0
		Diazepam	13.7
		Desmethyldiazepam	4.2
Subject no. 12	Patient under treatment	Sertraline	536.8
Subject no. 13A	Patient under treatment (segment 0–3 cm)	Olanzapine	35.4
		Diazepam	4.1
		Desmethyldiazepam	2.7
Subject no. 13B	Patient under treatment (segment 3–6 cm)	Negative	Negative
Subject no. 13C	Patient under treatment (segment 6–9 cm)	Citalopram	32.0

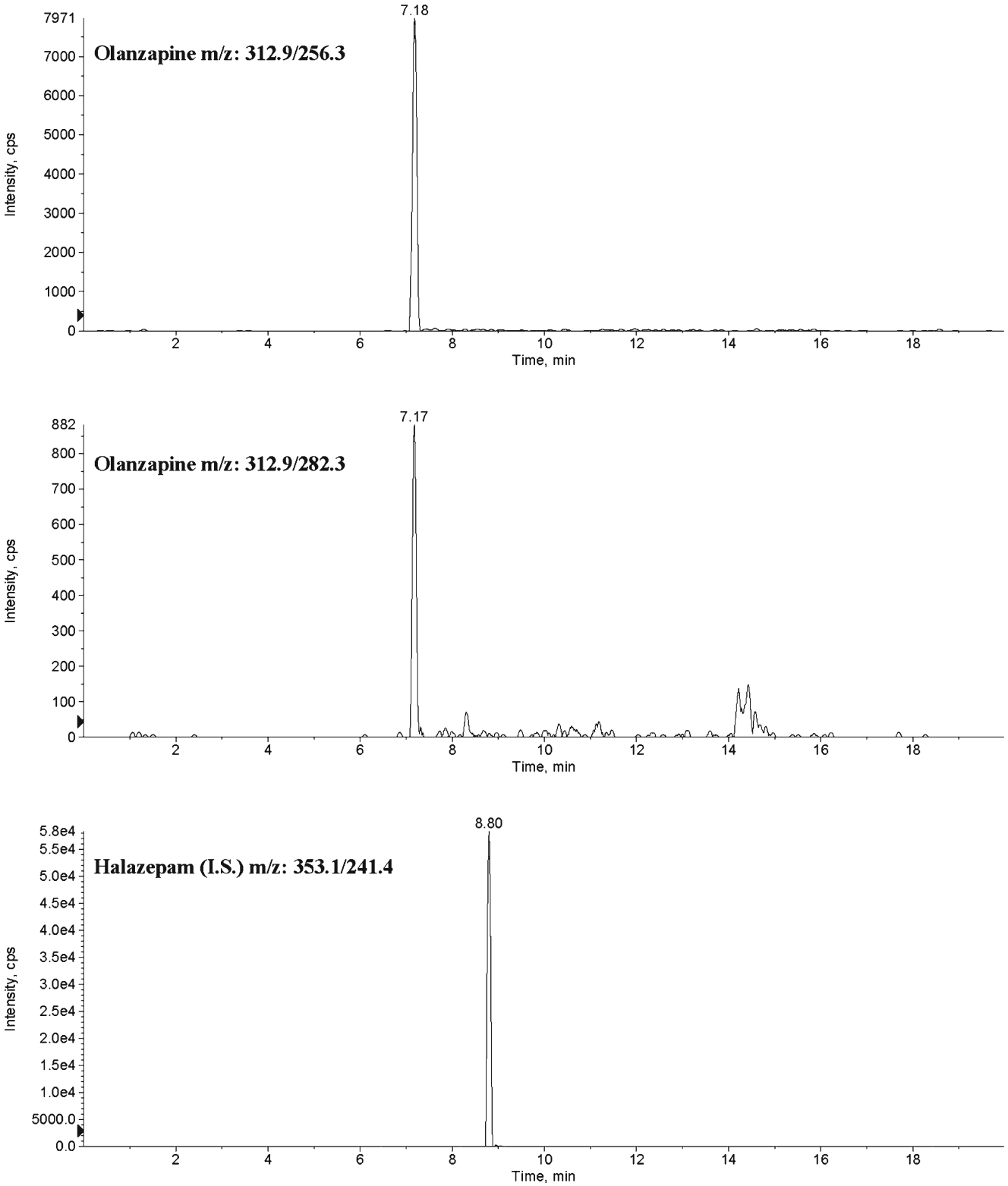

Fig. 1 MRM transitions of olanzapine in a real sample (35.4 pg/mg)

The procedure was applied to several hair samples, collected from autopsies, healthy volunteers, and the patients under pharmacological treatment. The four postmortem cases regarded subjects that were under therapeutic treatment with psychoactive substances before death, and their blood samples provided positive results. The first hair sample was collected from a woman, 47 years old, who was suffering from anxiety and psychosis and who committed suicide by precipitation.

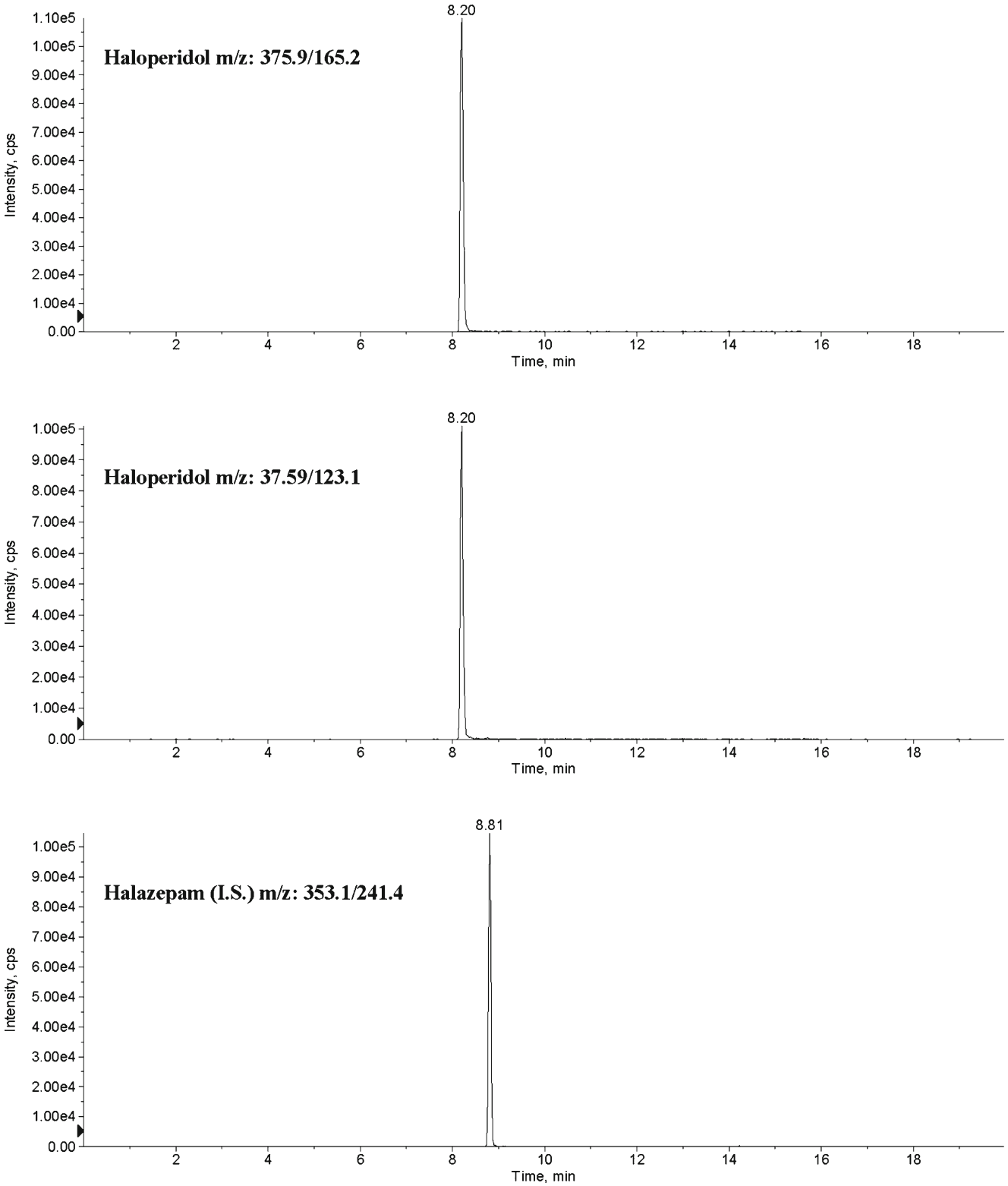

Fig. 2 MRM transitions of haloperidol in a real sample (5,500.0 pg/mg)

Benzodiazepines and haloperidol were found in the keratin matrix. The second case regarded a man, 33 years old, found dead after a fatal intoxication with methadone and heroin. Venlafaxine was detected in hair. The third postmortem case concerned a man, 52 years old, found dead in his apartment, apparently from natural causes, and that provided positive

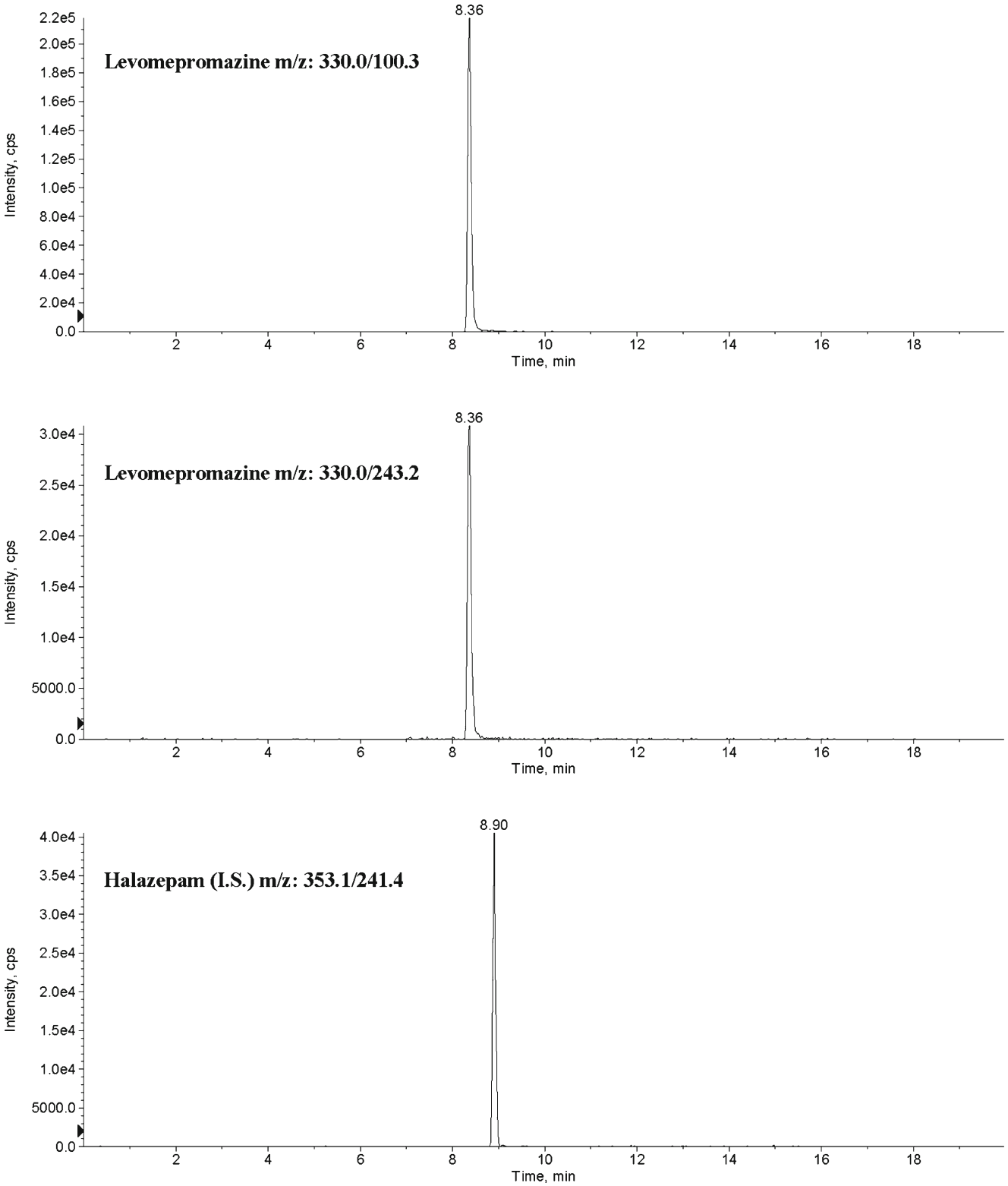

Fig. 3 MRM transitions of levomepromazine in a real sample (1,021.0 pg/mg)

results for several psychoactive substances. The fourth sample was collected from a man, 40 years old, found dead after a fatal alcohol intoxication; hair tested positive for tiapride. All the 15 hair samples collected from volunteers provided negative results. All the psychoactive drugs used by nine patients under treatment were identified in hair samples. All the

molecules and concentrations measured are listed in Table 3. Of considerable interest is the case reported as subject no. 13. This patient declared that he has been under citalopram therapy until 5 months before sample collection, but after a period of abstinence, not properly specified, he has been taking olanzapine and diazepam within the 3 months before sample collection. Hair samples were cut to 9 cm proximal hair segment and then divided in three segments of 3-cm length. The results assessed the patient's changing of therapy. Figures 1, 2, and 3 show (MRM) transitions of olanzapine in real samples.

Conclusion

The LC–MS/MS method here presented allows the identification and quantification of 87 psychoactive drugs in hair. The assay was successfully developed and fully validated. The direct injection of the sample after one single extraction step has been proven to be the best in the evaluation of a therapeutic use of these medicaments. The procedure was successfully applied to hair collected from autopsy samples and from the patients under treatment, where all the substances prescribed were easily detected. Due to the high sensitivity of the method, it is our opinion that it also could be used in cases of drug-facilitated crime related with the use of these substances efficient to incapacitate the victim. Referring to these specific cases, a future perspective would be to develop a screening method in keratin matrix including drugs of abuse and other molecules of potential interest in the forensic field.

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