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HPLC analysis of the antidepressant trazodone and its main metabolite *m*-CPP in human plasma

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ABSTRACT

The present paper deals with the development of a rapid and feasible high-performance liquid chromatographic method for the determination of trazodone and its main active metabolite 3-(1-clorophenyl)piperazine (*m*-CPP) in human plasma. Trazodone is a second-generation antidepressant with serotonin antagonist activity. The metabolite seems to be involved in the onset of some side effects of trazodone therapy, thus its determination is very important during therapeutic drug monitoring. Separation was achieved using a C8 reversed-phase column and a mobile phase composed of aqueous phosphate buffer (70%), containing triethylamine, at pH 3.5 and acetonitrile (30%). The UV detector was set at 255 nm and loxapine was used as the internal standard. An original pre-treatment procedure of plasma samples was developed, based on solid-phase extraction with C8 reversed phase cartridges (50 mg, 1 mL). The obtained extraction yields values were higher than 90% and precision, expressed as R.S.D., was lower than 5.6%. The method was successfully applied to plasma samples from depressed patients undergoing therapy with trazodone; accuracy results were satisfactory (recovery >91%). Thus, the method seems to be suitable for the therapeutic drug monitoring of trazodone and its main active metabolite in depressed patients' plasma.

1. Introduction

Trazodone (2-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-1,2,4-triazole[4,3-a]pyridin-3(2*H*)-one, TRZ, Fig. 1a) is a weak inhibitor of monoamine reuptake and its major mechanism of action seems to be the antagonism at serotonin 5-HT_2/5-HT_{1C} receptors [1]. TRZ is used for the treatment of major depression, sometimes in conjunction with selective serotonin reuptake inhibitors (SSRIs), like fluoxetine [2], or to control sleep disturbance symptoms when using serotonin and norepinephrine reuptake inhibitors (SNRIs) [3].

TRZ (Desyrel, Molipaxin, Trittico, Thombran and Trialodine) is commercially available as tablets, long-acting tablets, oral solutions and solutions for injection [4]. Treatment should be started with a dose of 25–50 mg daily, which may be increased slowly to a maximum of 300 mg daily in ambulatory patients or to 600 mg daily in hospitalised patients.

TRZ is mainly metabolised in the liver by the cytochrome isoform CYP3A4. The most important metabolite thus formed is 3-(1-clorophenyl)piperazine (*m*-CPP, Fig. 1b) [5], which is a serotonergic agonist with a long half-life [6].

Plasma levels in patients treated with TRZ at therapeutic doses usually range between 130 ng mL^{-1} and 2 μg mL^{-1} for the parent drug [7], while *m*-CPP concentrations are typically less than 20% of those of TRZ [8].

The main side effects associated with TRZ administration are: nausea, insomnia, agitation, dry mouth, constipation, headache, hypotension, blurred vision and confusion [9]. Some of these effects can be attributed to *m*-CPP, which has well-known pro-headache activity and hallucinogenic properties; in fact, due to this latter action it is also used on its own as a recreational drug [10]. From short-term studies, TRZ seems to increase the risk of suicide in children and adolescents with depression [11]; though this assertion is still a matter for debate [12], the FDA requires that package inserts of this drug report a warning that TRZ should be used with caution in children and adolescents and the patients kept under appropriate surveillance [13]. For these reasons, it is evident that a reliable therapeutic drug monitoring (TDM) of patients treated with TRZ could greatly improve their quality of life and that the determination of *m*-CPP should be an integral part

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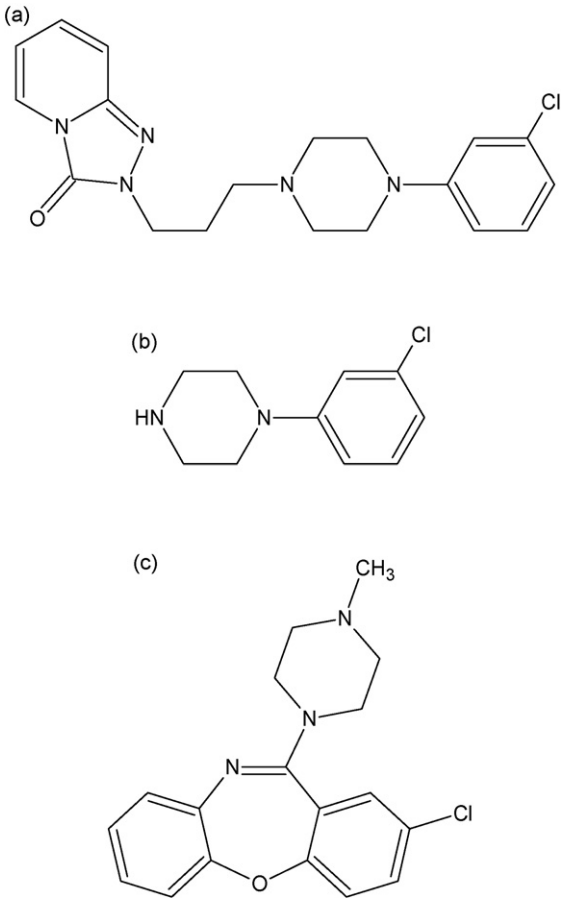

Fig. 1. Chemical structures of (a) trazodone (TRZ), (b) 3-(1-clorophenyl)piperazine (*m*-CPP) and (c) loxapine (IS).

of the TDM. In fact, TDM is currently considered a powerful tool for therapy optimisation and personalisation [14–17]. In the last few years, TDM is acquiring more importance in antidepressant therapy [18], especially when metabolic anomalies or low compliance are suspected, or in case of polypharmacy.

Several methods can be found in the literature for the determination of TRZ plasma levels of depressed patients. Most older methods were based on gas chromatography [19–21]. More recently, HPLC has clearly become the technique of choice for this kind of analysis. In fact, numerous methods have been published for this purpose, and they generally use HPLC coupled with UV detection [7,22–24]. However, these methods do not include *m*-CPP determination [22,24], or require a different (coulometric) detector for the metabolite [23]. The only HPLC–UV method, which simultaneously analyses TRZ and *m*-CPP, uses a two-step and time-consuming liquid–liquid extraction procedure and requires quite long analysis times (more than 13 min for a complete chromatographic run) [7]. A recent paper reports the analysis of TRZ by HPLC with fluorescence detection and direct sample injection [25]. However, the method analyses TRZ only and is applied to urine samples. Some HPLC–mass spectrometric (MS) methods [26–28] allow the simultaneous determination of several drugs, which include TRZ, but they also require very expensive instrumentation, which is not always available in the clinical setting for patients' TDM.

The aim of this study was the development of a feasible and reliable HPLC–UV method for the simultaneous determination of TRZ and *m*-CPP in human plasma for TDM purposes. The sample pretreatment step is based on solid-phase extraction (SPE), which is more feasible and reliable than traditional liquid–liquid extraction.

2. Experimental

2.1. Chemicals and solutions

TRZ and *m*-CPP, reference pure compounds, were purchased from Sigma–Aldrich Italia (Milan, Italy). Loxapine (2-chloro-11-(4-methyl-1-piperazinyl)dibenz[b,f][1,4]oxazepine, Fig. 1c), used as the Internal Standard (IS), was kindly donated by Lederle Laboratories (Gosport, Hampshire, UK). HPLC-grade acetonitrile and methanol and 85% (w/w) phosphoric acid, pure for analysis, were purchased from Carlo Erba (Milan, Italy). Triethylamine pure for analysis was purchased from Fluka (Buchs, Switzerland). Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, USA).

Stock solutions of the analytes and the IS (1 mg mL^{-1}) were prepared by dissolving suitable amounts of each pure substance in methanol. Standard solutions were obtained by diluting stock solutions with the mobile phase and directly injected into the HPLC.

2.2. Instrumentation and chromatographic conditions

Experiments were carried out using a PU-980 chromatographic pump and an UV-975 spectrophotometric detector set at 255 nm (Jasco, Tokyo, Japan).

Separations were obtained on a Jones Chromatography (Hengoed, UK) Genesis C8 reversed-phase column (150 mm × 4.6 mm i.d., 5 μm) kept at room temperature. The mobile phase was composed of a mixture of acetonitrile (30%, v/v) and a pH 3.5, 50 mM phosphate buffer containing 0.3% (v/v) triethylamine (70%, v/v). The mobile phase was filtered through a Phenomenex (Torrance, CA, USA) membrane filter (47 mm membrane, 0.2 μm, NY) and degassed by an ultrasonic bath. The flow rate was 1.2 mL min^{-1} and the injections were done through a 50-μL loop. Data processing was handled by means of a Varian (Walnut Creek, USA) Star Chromatography 4.0 software.

Solid-phase extraction (SPE) was carried out by means of a VacElut (Varian) apparatus.

A Crison (Barcelona, Spain) Basic 20 pHmeter and a Hettich (Tuttlingen, Germany) Universal 32 R centrifuge were used.

2.3. Sample collection and preparation

The blood samples were collected from patients admitted to the Ward of the Institute of Psychiatry (Parma, Italy) and subjected to naturalistic treatment with TRZ for at least 2 weeks at constant daily doses. The patients gave their informed consent for these analyses. The samples were usually drawn 12 h after the last drug administration for general needs related to the therapy. Blood was stored in glass tubes containing EDTA as the anticoagulant, then centrifuged (within 2 h from collection) at 1400 × *g* and 4 °C for 15 min; the supernatant (plasma) was then transferred into polypropylene test tubes and stored at −20 °C until HPLC analysis. "Blank" plasma was obtained in the same way from blood drawn from healthy volunteers not subjected to any pharmacological treatment.

The solid-phase extraction procedure was carried out on IST (Mid Glamorgan, UK) Isolute C8 cartridges (50 mg, 1 mL). Cartridges were activated by passing 1 mL of methanol through the cartridge three times and then equilibrated by passing 1 mL of ultrapure water three times. To 250 μL of plasma, 500 μL of water and 50 μL of IS solution were added and the resulting mixture loaded onto a conditioned cartridge. The cartridge was then washed once with 1 mL of water and once with 1 mL of a water/methanol (80/20, v/v) mixture. The analytes were then eluted with 1 mL of methanol. The eluate was dried under vacuum (rotary evaporator), redissolved in 250 μL of mobile phase and injected into the HPLC system.

2.4. Method validation

Method validation was carried out according to the guidelines of the main regulatory agencies, such as those issued by the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [29], by the United States Pharmacopeia (USP) [30] and by the U.S. Food and Drug Administration (FDA) [31].

2.4.1. Robustness

Some experimental conditions were varied with respect to those reported in Section 2 to test their influence on method performance. The chosen parameters were: mobile phase buffer pH (±0.5 pH units), mobile phase composition (±5% to the acetonitrile percentage) and flow rate (±0.1 mL min^{-1}).

2.4.2. Response function

Aliquots of 50 μL of analyte standard solutions (prepared daily) at seven different concentrations containing the IS at a constant concentration were added to 250 μL of blank plasma. The resulting mixture (IS concentration: 500 ng/mL) was subjected to the previously described SPE procedure and injected into the HPLC. The procedure was carried out in triplicate for each concentration. The analyte/IS peak area ratios (pure numbers) obtained were plotted against the corresponding concentrations of the analytes (expressed as ng mL^{-1}) and the calibration curves set up by means of the least-square method. The values of limit of quantification (LOQ) and limit of detection (LOD) were calculated as the analyte concentrations which give rise to peaks whose heights are 10 and 3 times the baseline noise, respectively.

2.4.3. Extraction yield (absolute recovery)

The procedure was the same as that described under "Response function" above, except the points were at three different concentrations, corresponding to the lower limit, a middle value and an high value of each calibration curve. The analyte/IS peak area ratios were compared to those obtained by injecting standard solutions at the same theoretical concentrations and the extraction yield values were calculated.

2.4.4. Precision

The assays described under "Extraction yield" were repeated six times within the same day to obtain repeatability (intraday precision) and six times over six different days to obtain intermediate precision (interday precision), both expressed as R.S.D.% values.

2.4.5. Selectivity

Blank plasma samples from six different volunteers were subjected to the SPE procedure and injected into the HPLC; the resulting chromatograms were checked for possible interference from endogenous compounds. The acceptance criterion was, that no interference peak is to be higher than an analyte peak corresponding to its LOD. Furthermore, standard solutions of several different drugs active on the Central Nervous System were injected at concentrations higher than the respective therapeutic levels; if the resulting chromatograms contained any interference peak, the potentially interfering compounds were then subjected to the SPE and injected to ascertain if they could be extracted.

2.4.6. Stability

For each of the following assays, different samples were prepared by spiking blank plasma with low and high concentrations of the analytes. Three samples were prepared for each concentration and then subjected to the chosen assay.

2.4.6.1. Freeze and thaw stability. Analyte stability was determined after three freeze and thaw cycles. The samples were stored at −20 °C for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen for 12 h. This cycle was repeated two more times, then the samples were analysed.

2.4.6.2. Short-term temperature stability. The samples were thawed at room temperature and kept at this temperature for 4 h, then analysed.

2.4.6.3. Long-term stability. The samples were stored under the same conditions as the study samples (i.e., at −20 °C in polypropylene plastic vials) and analysed after 1, 2 and 3 months of storage.

2.4.6.4. Stock solution stability. The stability of stock solutions of TRZ, *m*-CPP and the IS was evaluated at room temperature for 6 h. The stability of the stock solutions was also evaluated after 1, 2 and 3 months of storage at −20 °C.

The concentrations of all the stability samples was compared to the mean values for the standards at the appropriate concentrations.

2.4.7. Accuracy

Accuracy was evaluated by means of recovery assays. The assays described under "Extraction yield" were carried out adding standard solutions of the analytes and the IS to real plasma samples taken from depressed patients subjected to therapy with TRZ. The assays were repeated three times during the same day to obtain mean recovery and S.D. data.

3. Results and discussion

3.1. Choice of experimental conditions

Preliminary experiments were carried out selecting a C18 column (250 mm × 4.6 mm, 5 μm) for method development. This choice was made based on the moderate lipophilic properties of TRZ.

However, this column was found to strongly retain TRZ, while *m*-CPP showed low retention. This made it difficult to find conditions which granted enough retention for *m*-CPP while keeping acceptable run times. Thus, a C8 stationary phase was tested. This kind of sorbent reduced the retention differences between the analytes and was deemed more suitable. Different mixtures of acetonitrile (10–50%) and phosphate buffer were tried, with the buffer pH in the acidic range to keep the analytes protonated and to reduce run times. Analyte resolution was achieved at all acetonitrile percentages except 50%. However, when the acetonitrile percentage was higher than 35%, *m*-CPP retention was low and this tended to prevent its correct quantification when biological matrices are involved. For these reasons, 30% acetonitrile was chosen as the best compromise.

Loxapine was chosen as the IS. In fact, from preliminary assays it showed a chromatographic behaviour similar to that of the analytes, however it was completely resolved from them under the chosen conditions. The typical chromatogram of a standard solution containing TRZ, *m*-CPP and the IS is shown in Fig. 2a.

As can be seen, all analytes were baseline resolved.

3.2. Analysis of standard solutions

Seven-point calibration curves were set up over the 10–2000 ng mL^{-1} range for TRZ and over the 10–1000 ng mL^{-1} range for *m*-CPP. A linear response function ($r^2 > 0.9996$) was obtained for both compounds, with values of LOQ equal to

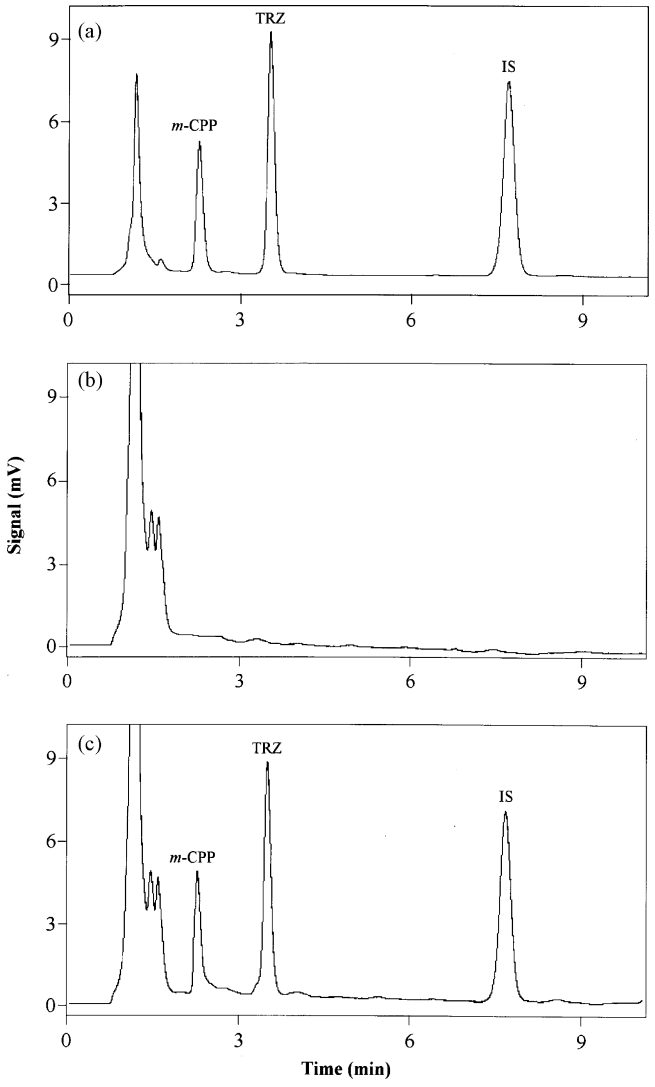

Fig. 2. Chromatograms of (a) a standard solution containing 500 ng mL^{-1} of TRZ, 250 ng mL^{-1} of *m*-CPP and 500 ng mL^{-1} of the IS; (b) a blank plasma sample from a healthy volunteer; (c) the same blank plasma sample spiked with 500 ng mL^{-1} of TRZ, 250 ng mL^{-1} of *m*-CPP and 500 ng mL^{-1} of the IS.

10 ng mL^{-1} and of LOD equal to 4 ng mL^{-1} for both analytes. Precision of peak areas was evaluated at three concentrations (10, 500 and 1000 ng mL^{-1}). R.S.D. values for repeatability (intraday precision) were lower than 2.5% for TRZ and lower than 2.8% for *m*-CPP (2.4% for the IS). R.S.D. values for intermediate precision (interday precision) were lower than 3.0% for TRZ and lower than 3.5% for *m*-CPP (2.9% for the IS).

3.3. Development of the solid-phase extraction procedure

Sample pre-treatment is one of the key steps for the development of a reliable and accurate HPLC–UV procedure. In fact, the pre-treatment procedure has to take into account the relatively low selectivity of the detection means. In this regard, SPE is certainly one of the most effective techniques. In fact, protein precipitation is usually less efficient in eliminating potential interference and also dilutes the sample. On the other hand, liquid–liquid extraction, which can be quite selective, is less feasible, usually more time consuming and also uses higher volumes of polluting organic solvents.

Thus, SPE was chosen for the pre-treatment of plasma samples. Some different kinds of sorbents were tested, such as C18, C8, cyanopropyl (CN), phenyl (PH) and hydrophilic–lipophilic balance (HLB). As expected, C18 cartridges strongly retained TRZ, giving low recovery of the analyte; PH cartridges gave similar results. C8, CN and HLB cartridges gave satisfactory results in terms of both sample cleaning and analyte (and IS) extraction yield. However, the yields obtained with C8 cartridges were slightly better than those obtained with CN and HLB sorbents; thus, C8 was chosen for subsequent assays. Initially, washing was carried out with water. Then, to improve sample clean up, two washing steps with a water/methanol mixture were added. The highest analyte extraction yields were obtained by using an 80:20 (v/v) water–methanol mixture. Experiments carried out using washing mixtures containing methanol and a buffer at different pHs, namely 2.5, 5.0, 6.5 or 8.0, revealed that the best results were those obtained with water/methanol, thus the latter was chosen for its feasibility. Similarly, elution with methanol was found to be suitable (in terms of sample cleaning and extraction yield) and elution with different methanol/acidic buffer mixtures did not significantly improve results.

Fig. 2b shows a chromatogram of a blank plasma sample after SPE C8 clean-up, while the HPLC analysis of the same sample spiked with standard analytes and IS is depicted in Fig. 2c. As can be observed, matrix clean-up is satisfactory and no interference can be seen at the retention times of the analytes.

3.4. Method validation

3.4.1. Robustness

Three important chromatographic parameters (mobile phase buffer pH, mobile phase composition and flow rate) were varied in order to assess their influence on the analysis.

When the buffer pH was modified (by ±0.5 pH units), only small changes of retention times were noticed. Slightly longer run times were observed when increasing the pH, and slightly shorter times when decreasing it, without significant effects on resolution and method applicability. On the contrary, mobile phase composition had a strong influence on the separation. As expected from the method development results, raising the acetonitrile percentage (to 35%) caused a general reduction of retention time, however the *m*-CPP peak tended to be scarcely retained. This can make its quantitation problematic in some instances, given the high variability of the plasma matrix. A lower percentage of acetonitrile prolonged run times, without any other negative effect. Changes to the flow rate (by ±0.1 mL/min) caused inverse changes in run times, without generating problems with respect to resolution, efficiency or peak shape.

3.4.2. Response function

A linear response function ($r^2 > 0.9993$) was obtained for each analyte over the 10–2000 ng mL^{-1} concentration range for TRZ and over the 10–1000 ng mL^{-1} range for *m*-CPP, with values of LOQ and LOD equal to 10 and 4 ng mL^{-1}, respectively. Complete response function parameters can be found in Table 1.

3.4.3. Extraction yield and precision

Extraction yield (absolute recovery) and precision assays were carried out on blank plasma spiked with analyte concentrations corresponding to the lower limit, a middle value and an high value of the calibration curves (i.e., 10, 500 and 1000 ng mL^{-1}). The results of these assays are reported in Table 2. Mean extraction yields were always higher than 93% for TRZ and 90% for *m*-CPP (96% for the IS). Precision results were also satisfactory. R.S.D. values for repeatability were always lower than 4.1% for TRZ and 4.6% for *m*-CPP (3.3%

Table 1
Response function parameters

Compound	Concentration range (ng mL^{-1})	Equation coefficients, $y = a + bx$[a]		r^2	LOQ (ng mL^{-1})[a]	LOD (ng mL^{-1})[a]
		a	b			
TRZ	10–2000	3×10^{-5}	2.44×10^{-3}	0.9995	10	4
m-CPP	10–1000	4×10^{-5}	2.41×10^{-3}	0.9994	10	4

[a] y = analyte/IS peak area ratio; x = analyte concentration, ng mL^{-1}.

Table 2
Precision and extraction yield results

Compound	Concentration (ng mL^{-1})	Repeatability, R.S.D.%[a]	Intermediate precision, R.S.D.%[a]	Extraction yield (%)[a]
TRZ	10	4.0	4.9	95
	500	3.0	3.8	94
	1000	2.8	3.3	94
m-CPP	10	4.5	5.5	94
	500	3.3	4.2	92
	1000	2.9	3.2	91
IS	500	3.3	4.0	96

[a] $n = 6$.

for the IS); R.S.D. values for intermediate precision were lower than 5.0% for TRZ and lower than 5.6% for *m*-CPP (4.0% for the IS).

3.4.4. Selectivity

Selectivity was evaluated by injecting standard solutions of several drugs commonly co-administered during psychiatric therapy (for example, other antidepressants, antipsychotics and anxiolytics-hypnotics). The complete list of these drugs is reported in Table 3. As can be seen, no drug tested and detected within a 20-min run interfered with the determination of TRZ and *m*-CPP. Furthermore, six blank plasma samples were injected after SPE and none of them produced peaks from endogenous compounds which could interfere with the determination.

3.4.5. Stability

Analyte stability was assessed with freeze–thaw, short-term and long-term assays. Stock solution stability was also evaluated.

Freezing and thawing and short-term storage at room temperature did not have any negative effect on TRZ and *m*-CPP stability (R.S.D. < 3%). Long-term storage at −20 °C tended to decrease the TRZ concentration after 3 months (−2%), however the effect does not seem to be significant.

Stock solutions were stable both at room temperature for 6 h and at −20 °C for 3 months.

Table 3
Drugs tested for interference

Therapeutic class	Compound	Retention time (min)
Analytes	*m*-CPP	2.3
	Trazodone	3.6
	Loxapine (IS)	7.7
Antidepressants	Amitriptyline	10.8
	Citalopram	9.6
	Clomipramine	20.1
	Duloxetine	17.4
	Sertraline	12.8
	Venlafaxine	3.0
Antipsychotics	Amisulpride	n.r.[a]
	Aripiprazole	11.5
	Chlorpromazine	17.2
	Clotiapine	10.5
	Clozapine	2.9
	Haloperidol	7.0
	Quetiapine	4.8
	Risperidone	n.r.
Anxiolytics-hypnotics	Clonazepam	12.0
	Diazepam	11.5
	Flurazepam	2.9
	Lorazepam	17.0

[a] n.r.: not retained.

3.5. Analysis of patient plasma samples

Having thus validated the method, it was applied to the analysis of plasma samples from patients admitted to the Ward of the Institute of Psychiatry (Parma, Italy) and undergoing ther-

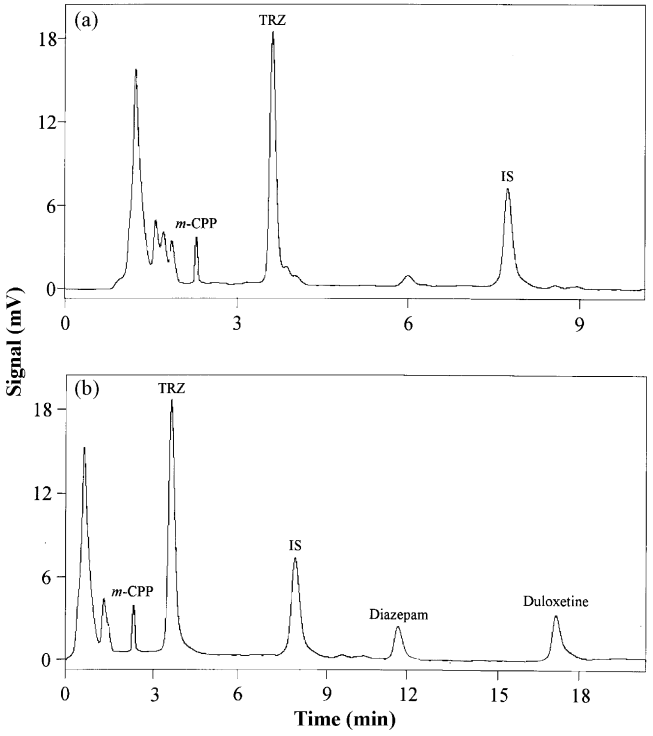

Fig. 3. Chromatograms of (a) a plasma sample from a patient who was subjected to treatment with 150 mg day^{-1} of TRZ, (b) a plasma sample from a patient who was subjected to treatment with 150 mg day^{-1} of TRZ, as well as duloxetine and diazepam.

Table 4
Accuracy results

Compound	Analyte concentration added (ng mL^{-1})	Recovery (%) ±S.D.[a]
TRZ	10	95 ± 5
	200	95 ± 3
	500	94 ± 3
m-CPP	10	95 ± 6
	200	92 ± 4
	500	92 ± 3

[a] $n = 3$.

apy with TRZ (Trittico®). As examples, the chromatograms of plasma samples from patients treated with TRZ are shown in Fig. 3a (patient 1) and b (patient 2), respectively. Plasma levels found in these real samples were the following: patient 1 (150 mg day^{-1} of TRZ), 980 ng mL^{-1} of TRZ and 107 ng mL^{-1} of *m*-CPP; patient 2 (150 mg day^{-1} of TRZ), 930 ng mL^{-1} of TRZ and 95 ng mL^{-1} of *m*-CPP. As can be seen, no interference from endogenous or hexogenous compounds is apparent. Moreover, in addition to TRZ, the second patient was taking duloxetine and diazepam. As expected, none of the coadministered drugs interfered with the determination, thus confirming the selectivity of the method.

Accuracy was evaluated by means of recovery assays. Standard solutions of the analytes at three different concentrations (10, 200 and 500 ng mL^{-1}) and of the IS at a constant concentration (500 ng mL^{-1}) were added to plasma samples containing known amounts of TRZ and *m*-CPP (i.e., patient plasma samples which had been already analysed). Then, the recovery of the analytes was calculated, as well as the standard deviation of the assays. The results thus obtained are reported in Table 4. Mean recovery values were higher than 93% for TRZ and higher than 91% for *m*-CPP.

4. Conclusion

The HPLC method presented here for the analysis of TRZ and *m*-CPP is feasible and reliable. The SPE procedure implemented for the sample pre-treatment, based on C8 cartridges, gives good extraction yields (>90%) and satisfactory precision (R.S.D.% <5.6%). The method is also selective: neither endogenous compounds nor any of the tested Central Nervous System drugs have produced any interference in the analysis of TRZ and *m*-CPP in depressed patients' plasma. The proposed method is also advantageous for other reasons: it has high accuracy and a wide response function range, which allows the determination of the analytes not only at therapeutic doses of TRZ but also in overdose cases and when taken at sub-therapeutic doses (e.g., scarce patient compliance). Compared to HPLC–MS methods [26–28], the proposed method is certainly less expensive and more widely applicable. When compared to existing HPLC–UV methods [22–24], it has the fundamental advantage of simultaneously analysing *m*-CPP with the same system. In comparison to the only one which performs the same function [7], the procedure here presented requires lower volumes of plasma (250 μL instead of 0.5 mL), shorter analysis times (8 min instead of 14 min) and is less laborious than a two-step liquid–liquid extraction. Another method, based on HPLC with fluorescence detection, has been recently published [25]. However, it is only suitable for the determination of TRZ in urine, not in plasma, and still does not allow the simultaneous analysis of *m*-CPP. This method has demonstrated to be suitable for the analysis of TRZ and its main metabolite in plasma samples from some depressed patients undergoing therapy with this drug. Therefore, the method can be applied to the determination of plasma levels of TRZ and *m*-CPP for TDM purposes.

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