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Simultaneous determination of fluvoxamine isomers and quetiapine in human plasma by means of high-performance liquid chromatography

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Abstract

An original HPLC–UV method has been developed for the simultaneous determination of the atypical antipsychotic quetiapine and the geometric isomers of the second-generation antidepressant fluvoxamine. The analytes were separated on a reversed-phase C8 column (150 mm × 4.6 mm i.d., 5 μm) using a mobile phase composed of acetonitrile (30%) and a 10.5 mM, pH 3.5 phosphate buffer containing 0.12% triethylamine (70%). The flow rate was 1.2 mL min^{-1} and the detection wavelength was 245 nm. Sample pretreatment was carried out by an original solid-phase extraction procedure using mixed-mode cation exchange (DSC-MCAX) cartridges; only 300 μL of plasma were needed for one analysis. Citalopram was used as the internal standard. The method was validated in terms of linearity, extraction yield, precision and accuracy. Good linearity was obtained in plasma over the 5.0–160.0 ng mL^{-1} concentration range for each fluvoxamine isomer and over the 2.5–400.0 ng mL^{-1} concentration range for quetiapine. Extraction yield values were always higher than 93%, with precision (expressed as relative standard deviation values) better than 4.0%. The method was successfully applied to human plasma samples drawn from patients undergoing polypharmacy with the two drugs. Satisfactory accuracy values were obtained, with mean recovery higher than 94%.

Keywords: Quetiapine; Fluvoxamine isomers; Human plasma; Liquid chromatography; Solid-phase extraction

1. Introduction

Quetiapine (QUE, Fig. 1a) is one of the most recent "atypical" antipsychotic drugs [1], whose use in schizophrenia is becoming widespread. This drug has also recently been approved for use in the manic phases of bipolar disorder [2]. However, since a single drug often is not sufficient to satisfactorily control the symptoms of psychiatric diseases such as schizophrenia or manic-depressive disorder, polypharmacy is a common occurrence in both outpatients and hospitalised patients of Psychiatric Clinics. Polypharmacy is still a controversial practice and at least some authors are doubtful of its superior efficacy with respect to monotherapy [3]; however, it is widely used by psychiatrists. In fact, an antidepressant drug is often added to an antipsychotic drug in order to keep depressive symptoms of schizophrenia under control; likewise, as previously stated, quetiapine can be used (together with second-generation antidepressants) in the therapy of manic-depressive disorder. The main problem with polypharmacy are the possible pharmacological interactions and toxic effects [4]. Furthermore, fluvoxamine (FLV, Fig. 1b), a second-generation antidepressant drug, is an inhibitor of cytochrome P450 (in particular of cytochrome P450 subtype 1A2, CYP1A2), which is one of the main systems involved in drug biotransformation [5]. Thus, it can cause clinically significant pharmacological interactions with other drugs, increasing their plasma concentrations. On the other hand, this property can willingly be exploited to reduce the administered doses of some CYP1A2 metabolised substrates. In fact, fluvoxamine at sub-therapeutic doses has recently been used in studies of pharmacoeconomy to reduce the doses of olanzapine administered to psychotic patients and consequently the costs of therapy [6].

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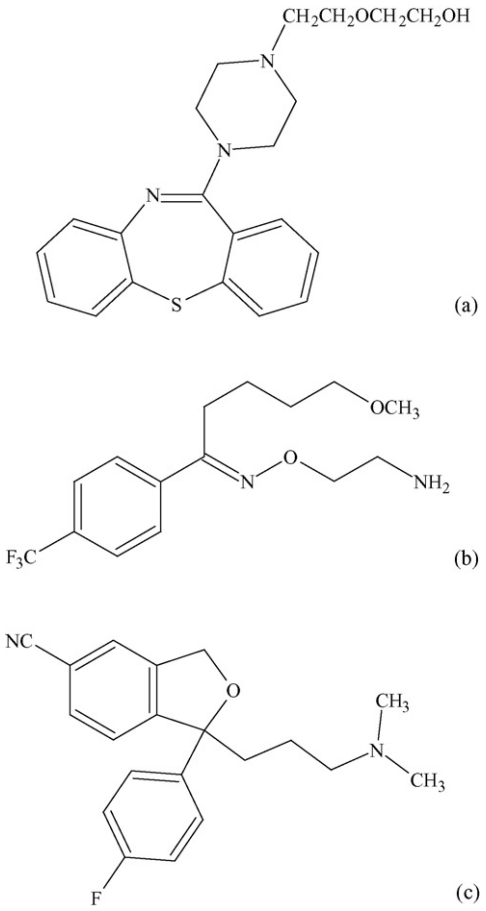

Fig. 1. Chemical structures of (a) quetiapine, (b) fluvoxamine and (c) citalopram (IS).

Chemical–clinical correlations are always useful to increase the efficacy of therapy and decrease the incidence of side effects: comorbidity, uncertain compliance, evaluation and management of pharmacological interactions are good reasons to carry out the therapeutic drug monitoring (TDM) of psychiatric patients. In fact, they are all possible causes of clinically significant alterations of drug plasma levels. Inpatients of hospitals can quite easily be watched during polypharmacy; the outpatients of Mental Health Centre ambulatories, on the contrary, are much more autonomous, and this makes it quite difficult to evaluate the real therapeutic response and to control domiciliary interference. In these latter cases, only the availability of a reliable and timely TDM can enable the psychiatrist to put into practice non-standard, but potentially very effective therapeutic choices, such as the enhancement of antipsychotic therapy using an SSRI antidepressant.

For these reasons, reliable analytical methods are needed which reliably determine plasma levels of psychiatric drugs which are taken simultaneously, in order to detect changes (either desired or otherwise) as early as possible in the plasma concentrations of the drugs themselves. The methods should be as simple as possible and use readily available instrumentation: in fact, only in this way a constant TDM can be carried out even in small research centres or small hospital laboratories. In this paper, an original liquid chromatographic method is described, based on HPLC with UV detection, for the simultaneous analysis of quetiapine and fluvoxamine in human plasma for TDM purposes. Since fluvoxamine can exist as two geometric isomers and only one of them, (*E*)-fluvoxamine, is pharmacologically active [7], the method has also been specifically designed to separate and determine both isomers. The method has been validated and successfully applied to plasma samples drawn from patients undergoing polypharmacy with quetiapine and fluvoxamine; preliminary results of this study have recently been presented at the 5th SAYCS (Sigma–Aldrich Young Chemists Symposium, 2005) [8]. To the best of our knowledge, no analytical method is currently available in the literature for the simultaneous analysis of quetiapine and both fluvoxamine isomers in biological fluids. In fact, some papers determine the active isomer of FLV alone [9,10] or together with other antidepressants [11,12] by HPLC with UV detection [9,11–14,18], gas chromatography [16–18] or HPLC with fluorimetric detection after derivatisation [10,15]. Some papers analyse both isomers of FLV, but not in biological fluids, only in solution [19] or in formulations [20]. Only a few papers regard the determination of quetiapine in biological fluids, including HPLC with UV detection [21–24], HPLC–mass spectrometry [25,26] and gas chromatography–mass spectrometry [21]. None of these, however, simultaneously determines QUE and FLV isomers.

2. Experimental

2.1. Chemicals

The isomers of fluvoxamine, 1-(*E*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-(2-aminoethyl)oxime fumarate (EFLV) and 1-(*Z*)-5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanone *O*-(2-aminoethyl)oxime fumarate (ZFLV), were kindly provided by Solvay Pharmaceuticals (Weesp, The Netherlands). Quetiapine bis(2-[2-(4-dibenzo[*b*,*f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol) fumarate, QUE) was kindly provided by AstraZeneca (Wilmington, USA).

All reagents were analytical grade or better. Methanol and acetonitrile (HPLC grade), 85% (w/w) phosphoric acid, 30% (w/w) ammonia, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, potassium chloride, 2 M hydrochloric acid and 2 M sodium hydroxide were produced by Carlo Erba (Milan, Italy). Citalopram used as the Internal Standard (IS, Fig. 1c) was kindly provided by H Lundbeck A/S (Copenhagen, Denmark). Triethylamine and bovine serum albumin were purchased from Sigma–Aldrich (St. Louis, MO, USA).

Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, MA, USA).

2.2. Apparatus and chromatographic conditions

The chromatographic system was composed of a Jasco (Tokyo, Japan) PU-980 chromatographic pump and a Jasco UV-975 spectrophotometric detector.

Separations were obtained on a Varian ResElut C8 reversed-phase column (150 mm × 4.6 mm i.d., 5 μm). The mobile phase was composed of a mixture of acetonitrile (30%, v/v)

and a 10.5 mM, pH 3.5 phosphate buffer containing 0.12% triethylamine (70%, v/v). The aqueous part of the mobile phase was prepared as follows: a suitable amount of phosphoric acid was dissolved in water, then triethylamine was added; finally, the mixture was brought to pH 3.5 with 2 M hydrochloric acid. 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 apparatus. The flow rate was set at 1.2 mL min^{-1} and the injections were carried out through a 20 μL loop. The detector wavelength was set at 245 nm. Data processing was handled by means of a Varian Star Chromatography software.

Solid-phase extraction (SPE) was carried out on Supelco (Bellefonte, USA) mixed-mode Discovery DSC-MCAX cartridges (100 mg, 1 mL) by means of a Vac Elut (Varian) apparatus.

A Crison (Barcelona–Spain) MicropH 2000 pHmeter and an ALC (Milan, Italy) 4225 centrifuge were used.

2.3. Solutions

Stock solutions of the analytes and the IS (1.00 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. Stock solutions were stable for at least 2 months when stored at −20 °C (as assessed by spectrophotometry and HPLC injections); standard solutions were prepared afresh every day.

2.4. Sample collection

The blood samples were collected from patients subjected to therapy with quetiapine and fluvoxamine for at least 2 weeks. Blood samples were drawn 12 h after the last drug administration. Blood was stored in glass tubes containing EDTA as the anticoagulant, then centrifuged (within 2 h from collection) at 1400 × *g* for 15 min; the supernatant (plasma) was then transferred to polypropylene tubes and stored at −20 °C until HPLC analysis. "Blank" plasma used for method validation was obtained from healthy volunteers not subjected to any pharmacological therapy and prepared in the same way as patient plasma.

"Reconstituted" plasma was used as "blank" plasma for the preliminary assays of the SPE procedure. It was prepared by dissolving 20 mg of KCl, 800 mg of NaCl, 20 mg of KH_2PO_4, 115 mg of Na_2HPO_4 and 4 g of bovine albumin in 100 mL of ultrapure water and then adjusting the pH to 7.4 with 2 M NaOH or 2 M HCl as needed.

2.5. Sample pre-treatment: SPE procedure

The solid-phase extraction cartridges were activated by passing 1 mL of methanol through the cartridge three times, and then conditioned by passing 1 mL of a pH 6.0, 25 mM phosphate buffer three times. To 300 μL of plasma, 600 μL of a pH 6.0, 25 mM phosphate buffer and 50 μL of IS working solution were added, and the resulting mixture was loaded onto the previously conditioned cartridge. The cartridge was sequentially washed with: 1 mL of a pH 6.0, 25 mM phosphate buffer, then 1 mL of methanol and finally 50 μL of a mixture of methanol (95%, v/v) and 7.6 M aqueous ammonia (5%, v/v). The analytes were subsequently eluted with 1 mL of the same methanol/ammonia mixture. The eluate was dried under vacuum (rotary evaporator), redissolved with 100 μL of mobile phase, then injected into the HPLC system.

2.6. Method validation

Method validation procedures were carried out according to FDA [27] and USP [28] guidelines.

2.6.1. Calibration curves

Amounts of 50 μL of analyte standard solutions at seven different concentrations containing the IS at a constant concentration were added to 300 μL of blank plasma. The resulting plasma concentration ranges were: 5.0–160.0 ng mL^{-1} for EFLV, 5.0–160.0 ng mL^{-1} for ZFLV and 2.5–400.0 ng mL^{-1} for QUE; 70.0 ng mL^{-1} (constant) for the IS (the corresponding concentrations in the injected solutions can be obtained by multiplying the plasma concentrations by three). The resulting mixture 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 constructed by means of the least-square method. One stock solution was used for each replicate; different working solutions were prepared from the stock solutions and added to the blank plasma samples to obtain the different concentrations.

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.

2.6.2. Extraction yield (absolute recovery)

The procedure was the same as that described under "Calibration Curve", above, except the points were at 3 different concentrations, corresponding to the upper limit, lower limit and middle point of each calibration curve (i.e., plasma concentrations of 5.0, 80.0 and 160.0 ng mL^{-1} for EFLV and ZFLV and 2.5, 200.0 and 400.0 ng mL^{-1} for QUE). The analyte/IS peak area ratios were compared to those obtained injecting standard solutions at the same theoretical concentrations and the extraction yield values were calculated.

2.6.3. Precision

The assays described under "extraction yield" were repeated six times within the same day to obtain repeatability (intraday precision) and six times over 6 different days to obtain intermediate precision (interday precision) [28], both expressed as R.S.D.% values.

2.6.4. 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 (citalopram) to real plasma samples taken from patients subjected to simultaneous treatment with FLV and QUE tablets (plasma concentrations added: 10.0 and 20.0 ng mL^{-1} for EFLV and ZFLV, 50.0 and 100.0 ng mL^{-1} for QUE). The assays were repeated three times during the same day to obtain mean recovery and standard deviation data.

3. Results and discussion

3.1. Choice of the chromatographic conditions

Chromatographic conditions similar to those already used for the analysis of the SSRI antidepressant sertraline [29] were initially tried for the purpose of separating FLV isomers and quetiapine. In particular, a mixture of a pH 3.0 phosphate buffer and acetonitrile (60/40, v/v) was used as the mobile phase (flow rate 1.0 mL min^{-1}) and a C8 (150 mm × 4.6 mm i.d., 5 μm) column was used as the stationary phase. Under these conditions, the analyte peaks have short retention times and are not baseline separated. Thus, the acetonitrile percentage was lowered in order to have a higher retention of the analytes: it was found that 30% acetonitrile allowed a complete separation of all three analytes within reasonable run times. Then, several compounds were tested as possible ISs; the most suitable was found to be citalopram (Fig. 1c). In fact, citalopram has a relatively short retention time and chemical–physical properties similar to the analytes. When the pH value of the mobile phase buffer was raised from 3.0 to 3.5 and the flow rate was increased to 1.2 mL min^{-1}, baseline separation of all compounds of interest was obtained. Regarding the detection wavelength, each analyte has a UV spectrum with one absorbance maximum very close to 200 nm, which is not suitable for HPLC detection since it is too prone to interference. Since both FLV's and QUE's spectra have an inflection point and good absorbance values at 245 nm, it was decided to set the detector at this wavelength as a compromise between sensitivity, selectivity and reproducibility.

Thus, a reversed-phase C8 (150 mm × 4.6 mm i.d., 5 μm) column was used as the stationary phase, while a mixture of a 10.5 mM, pH 3.5 phosphate buffer containing 0.12% triethylamine and acetonitrile (70/30, v/v) was used as the mobile phase.

The chromatogram of a standard solution containing 125.0 ng mL^{-1} of each FLV isomer, 600.0 ng mL^{-1} of QUE and 210.0 ng mL^{-1} of citalopram (IS) is reported in Fig. 2. As can be seen, the peaks are neat and well-resolved and the chromatographic run lasts 16 min. Retention times (t_R) are: QUE, t_R = 7.3 min; IS, t_R = 8.5 min; ZFLV, t_R = 10.9 min; EFLV, t_R = 15.6 min.

3.2. Analysis of standard solutions

Good linearity ($r^2 > 0.9995$) was obtained over the following concentration ranges: 15.0–480.0 ng mL^{-1} for each FLV isomer, 7.5–1200.0 ng mL^{-1} for QUE. Precision assays carried out at three different levels (15, 240 and 480 ng mL^{-1} for FLV isomers; 7.5, 600 and 1200.0 ng mL^{-1} for QUE) also gave good

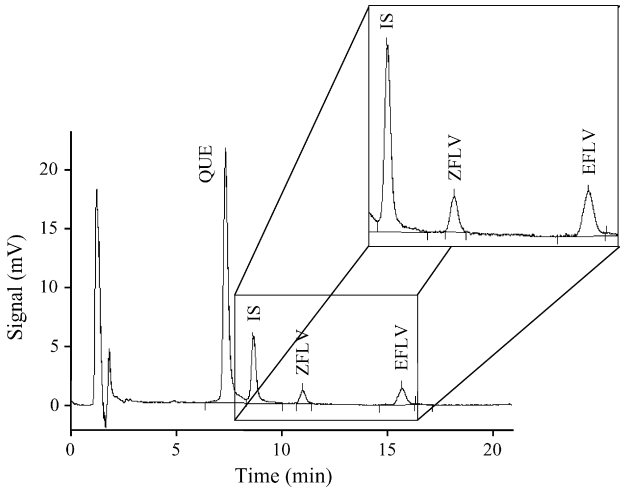

Fig. 2. Chromatogram of a standard solution containing 600.0 ng mL^{-1} of QUE, 125.0 ng mL^{-1} of EFLV, 125.0 ng mL^{-1} of ZFLV and 210.0 ng mL^{-1} of the IS (citalopram).

results: the relative standard deviation (R.S.D.) of repeatability (intraday precision) was always better than 3.3% for all analytes; R.S.D. values for intermediate (interday) precision were always lower than 3.7%. The limits of quantification (LOQ) were 15.0 ng mL^{-1} for FLV isomers and 7.5 ng mL^{-1} for QUE; the limits of detection (LOD) were 5.0 ng mL^{-1} for FLV isomers and 2.5 ng mL^{-1} for QUE.

3.3. Development of a solid-phase extraction procedure

"Reconstituted" plasma was used as blank plasma for all preliminary assays. It is a mixture of salts and albumin which has chemical–physical properties very similar to those of real plasma, and can thus be used as a safe and practical alternative to the latter for the development of analytical procedures. It was decided from an early stage that the sample pre-treatment procedure would be carried out by means of SPE, which is a more efficient and less polluting and time-consuming alternative to traditional liquid–liquid extraction or protein precipitation procedures.

The first choice of sorbent for the SPE procedure was the hydrophilic–lipophilic balance (HLB) sorbent, which is very versatile and can retain a wide range of compounds. However, severe interference from endogenous matrix compounds was detected when using these cartridges, which were thus discarded. Weakly lipophilic sorbents (C1, C2) were also tried: they gave good extraction yields for all compounds of interest, however interference was detected once more at the retention times of FLV isomers. More lipophilic sorbents, such as C8 and C18, gave low extraction yields. Finally, it was decided to use a mixed-mode sorbent, the DSC-MAX. These cartridges contain a sorbent which can establish both hydrophobic and cation exchange interactions with the analytes, by means of C8 chains and benzenesulfonic acid groups, respectively.

The control of the pH is essential for this kind of sorbent: the sample loading and the first step of washing were carried out with acidic buffers (pH 3.0–6.0) in order to keep the ana-

lytes in their protonated form which optimally interacts with the cation exchange part of the sorbent. It was found that a pH 6.0 phosphate buffer gave the best results. A second washing step was carried out with methanol which elutes the most lipophilic interference. Finally, analyte elution was carried out with a mixture of methanol (95%, v/v) and 7.6 M aqueous ammonia (5%, v/v). This basic mixture deprotonates the amines, eliminating the ionic interaction between the analytes and the sorbent, thus eluting the former. In order to obtain optimal purification of the samples, another washing step with 50 μL of the same solution was added before elution: in fact, this small volume of eluent does not contain any appreciable amount of the analytes, however it eliminates a great deal of interference.

When sample pre-treatment is carried out by means of this SPE procedure, clean chromatograms and good sample purification are obtained. To demonstrate this, the chromatogram of a typical blank plasma sample is reported in Fig. 3a, while the chromatogram of the same blank plasma sample spiked with 200 ng mL^{-1} of QUE, 40.0 ng mL^{-1} of EFLV, 40.0 ng mL^{-1} of ZFLV and 70.0 ng mL^{-1} of the IS (plasma concentrations) is reported in Fig. 3b.

3.4. Method validation

Once the SPE procedure was developed, "blank" plasma from healthy volunteers was used for method validation.

Ten-point calibration curves were set up for all analytes; good linearity was found in the 5.0–160.0 ng mL^{-1} range for each FLV isomer and in the 2.5–400.0 ng mL^{-1} range for QUE. When the analyte concentration step introduced by the SPE procedure is taken into account, the LOQ and LOD values were substantially equal to those on standard solutions and namely LOQ = 2.5 ng mL^{-1} for QUE; LOQ = 5.0 ng mL^{-1} for each FLV isomer; LOD = 0.8 ng mL^{-1} for QUE; LOD = 1.7 ng mL^{-1} for each FLV isomer (plasma concentrations). The complete results of linearity assays are reported in Table 1.

Extraction yield and precision assays were carried out at three different concentration levels, corresponding to the lowest level, highest level and middle point of each calibration curve. The results of these assays are reported in Table 2. As one can see, the results are satisfactory: the mean extraction yield was 93.6% for QUE, 96.0% for ZFLV and 95.6% for EFLV; the mean R.S.D. of precision assays was always lower than 4.0% for all analytes. The mean extraction yield of the IS was 97%, with R.S.D. values always lower than 2.5%.

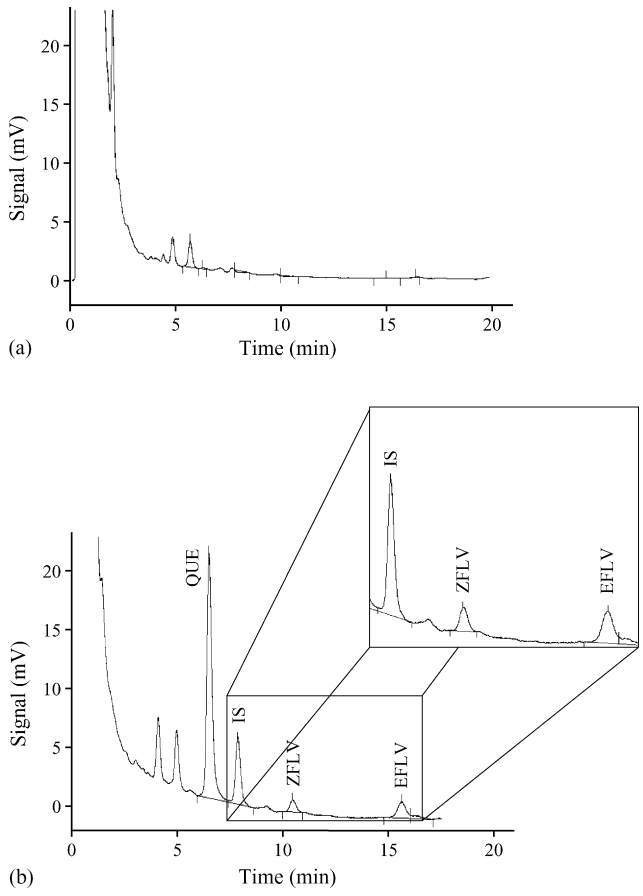

Fig. 3. Chromatograms of (a) a blank plasma sample and (b) the same blank plasma sample spiked with 200 ng mL^{-1} of QUE, 40.0 ng mL^{-1} of EFLV, 40.0 ng mL^{-1} of ZFLV and 70.0 ng mL^{-1} of the IS.

3.5. Selectivity

In order to assess the selectivity of the method, standard solutions of several drugs often administered in psychiatric practice (such as antidepressants, antipsychotics and anxiolytics-hypnotics) were injected into the HPLC. The complete list of drugs tested and the respective k' values are reported in Table 3. As can be seen, several drugs are detected within a 20 min chromatographic run, however only some of them have a potential for interfering in the analyte determination (e.g. clozapine, fluoxetine, mirtazapine). Further assays using blank plasma samples spiked with the potentially interfering drugs showed that none of them is eluted with the analytes from the cartridge during the

Table 1
Linearity parameters

Compound	Linearity range (ng mL^{-1})[a]	Equation coefficients, $y = a + bx$[b]		r^2	LOQ (ng mL^{-1})[a]	LOD (ng mL^{-1})[a]
		a[c]	b[c]			
QUE	2.5–400.0	0.0045 ± 0.0018	0.00430 ± 0.00033	0.9993	2.5	0.8
EFLV	5.0–160.0	−0.0024 ± 0.0010	0.00211 ± 0.00021	0.9991	5.0	1.7
ZFLV	5.0–160.0	−0.0022 ± 0.0012	0.00208 ± 0.00025	0.9991	5.0	1.7

[a] Plasma concentrations; the concentrations of the injected solutions can be found by multiplying the reported values by 3.
[b] y = analyte/IS peak area ratio; x = analyte concentration (ng mL^{-1}).
[c] Value ± standard error.

Table 2
Extraction yield and precision assays

Compound	Concentration (ng mL^{-1})	Repeatability, R.S.D. %[a]	Intermediate precision, R.S.D. %[a]	Extraction yield (%)[a]
QUE	2.5	3.5	3.9	93
	200.0	2.9	3.4	94
	400.0	1.5	2.3	94
EFLV	5.0	3.7	3.9	97
	80.0	3.1	3.6	96
	160.0	2.4	2.9	94
ZFLV	5.0	3.4	4.0	98
	80.0	2.8	3.4	97
	160.0	2.3	2.5	93
IS	70.0	2.3	2.5	97

[a] $n = 6$.

SPE procedure. Thus, none of the tested drugs can interfere with the analysis of plasma samples.

3.6. Analysis of patient plasma samples

Having thus validated the method, it was applied to the analysis of plasma samples from a few patients of Psychiatric Clinics undergoing simultaneous treatment with Seroquel® (quetiapine) and Fevarin® (fluvoxamine). A representative example of the chromatograms obtained when injecting these samples after the SPE procedure is reported in Fig. 4. This sample was drawn from a patient taking 100 mg day^{-1} of QUE and 25 mg day^{-1} of FLV. As can be seen, no interference from endogenous or exogenous compounds is present and peak shapes are identical to those of standard solutions. The analyte concentrations in this sample, obtained by interpolation on the respective calibration curves, were: QUE: 159.3 ng mL^{-1}; EFLV: 26.1 ng mL^{-1}; ZFLV: <LOD.

Table 3
Compounds tested for interference

Compound	Therapeutic class	k'[a]
QUE	–	3.1
IS		3.7
ZFLV		5.1
EFLV		7.7
Clothiapine	Antipsychotics	9.2
Clozapine		3.9
N-Desmethylclozapine		3.6
Clozapine *N*-oxide		5.7
Fluphenazine		n.d.
Haloperidol		5.9
Levomepromazine		9.0
Clomipramine	Antidepressants	n.d.
Fluoxetine		3.3
Norfluoxetine		7.2
Imipramine		0.6
Mirtazapine		3.3
Paroxetine		7.3
Sertraline		n.d.
Trazodone		1.8
Venlafaxine		n.d.
N-Desmethylvenlafaxine		n.d.
Carbamazepine	Antiepileptics	3.5
Lamotrigine		0.4
Phenytoin		3.9
Primidone		n.d.
Clonazepam	Anxiolytics-hypnotics	7.1
Diazepam		1.4
Flurazepam		2.8
Lorazepam		5.5

[a] $k' = (t_R - t_0)/t_0$.

3.7. Method accuracy

The accuracy of the method was evaluated by means of recovery assays: known amounts of the analyte standard solutions were added to plasma samples whose analyte concentration was known (i.e., samples which were already analysed); the samples were analysed again and the percentage recovery of the spiked analytes calculated. The complete results of the accuracy assays are reported in Table 4: as one can see, they are satisfactory, with recovery values always higher than 94%.

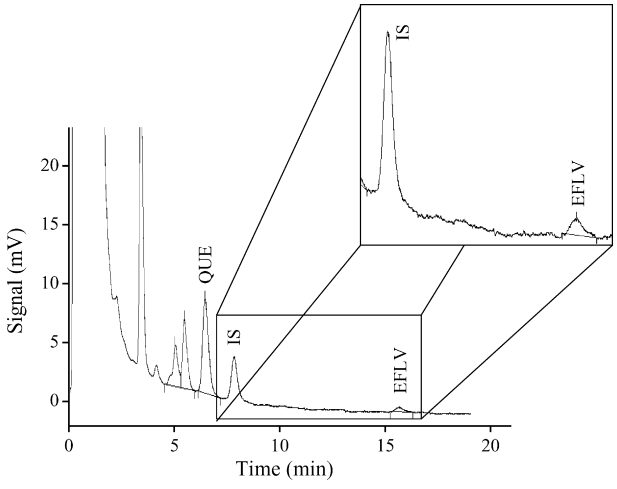

Fig. 4. Chromatogram of plasma sample drawn from a patient taking 100 mg day^{-1} of QUE and 25 mg day^{-1} of FLV.

Table 4
Accuracy assay results

Compound	Concentration (ng mL^{-1})	Mean recovery ± S.D. (%)
QUE	50.0	97.1 ± 1.8
	100.0	100.1 ± 1.0
EFLV	10.0	96.3 ± 2.8
	20.0	95.2 ± 1.5
ZFLV	10.0	95.4 ± 2.7
	20.0	94.8 ± 1.3

4. Conclusion

The method presented, based on HPLC with UV detection, for the simultaneous analysis of QUE and of FLV isomers in human plasma has given good results in terms of sensitivity and accuracy. The developed SPE procedure, which uses DSC-MCAX cartridges, allowed to obtain good extraction yields (>93%) and precision (R.S.D. <4.0%) for all analytes and optimal purification of the plasma samples from endogenous and exogenous interference.

Thus, the method is suitable for the therapeutic drug monitoring (TDM) of patients undergoing simultaneous therapy with QUE and FLV, even when they are subjected to polypharmacy with other SNC drugs; it also has the advantage of using simple, common and reliable instrumentation and techniques.

At the best of our knowledge, this currently is the only method which can determine both quetiapine and each FLV isomer; it could thus be a useful means of investigating pharmacological interactions between QUE and FLV, as well as of detecting and quantitating the undesired inactive isomer of FLV.

In our opinion, this method could give a significant contribution to the field of TDM, which is a very useful, albeit scarcely exploited, tool in the hands of the psychiatrist for the safety of antipsychotic and antidepressant therapy.

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References

[1] M.A. Raggi, R. Mandrioli, V. Pucci, C. Sabbioni, Med. Chem. Rev. Online 1 (2004) 299.
[2] American Psychiatric Association, Am. J. Psychiat. 159 (2002) 1.
[3] S.M. Stahl, M.M. Grady, Curr. Med. Chem. 11 (2004) 313.
[4] J. Ananth, S. Parameswaran, S. Gunatilake, Curr. Pharm. Design 10 (2004) 2231.
[5] L.J. Albers, V. Ozdemir, Curr. Med. Chem. 11 (2004) 297.
[6] L.J. Albers, V. Ozdemir, S.R. Marder, M.A. Raggi, M. Aravagiri, L. Endrenyi, C. Reist, J. Clin. Psychopharmacol. 25 (2005) 170.
[7] G. Miolo, S. Caffieri, L. Levorato, M. Imbesi, P. Giusti, T. Uz, R. Manev, H. Manev Hari, Eur. J. Pharmacol. 450 (2002) 223.
[8] M.A. Saracino, L. Mercolini, G. Flotta, L.J. Albers, R. Merli, M.A. Raggi, in: D. Spinelli, L. Busetto, C. Melchiorre (Eds.), Proceedings of the 5° SAYCS, Sigma-Aldrich Young Chemist Symposium, Sigma–Aldrich, Milano, Italy, 2005, p. 92.
[9] T. Ohkubo, R. Shimoyama, K. Otani, K. Yoshida, H. Higuchi, T. Shimizu, Anal. Sci. 19 (2003) 859.
[10] R.H. Pullen, H. Robert, A.A. Fatmi, J. Chromatogr. 574 (1992) 101.
[11] A.L. Hostetter, Z.N. Stowe, M. Cox, J.C. Ritchie, Ther. Drug Monit. 26 (2004) 47.
[12] G. Tournel, N. Houdret, V. Hedouin, M. Deveaux, D. Gosset, M. Lhermitte, J. Chromatogr. B 761 (2001) 147.
[13] L. Palego, D. Marazziti, L. Biondi, G. Giannaccini, N. Sarno, A. Armani, A. Lucacchini, G.B. Cassano, L. Dell'Osso, Ther. Drug Monit. 22 (2000) 190.
[14] C. Duverneuil, G.L. de la Grandmaison, P. de Mazancourt, J.C. Alvarez, Ther. Drug Monit. 25 (2003) 565.
[15] A. Lucca, G. Gentilini, S. Lopez-Silvia, A. Soldarini, Ther. Drug Monit. 22 (2000) 271.
[16] E. Lacassie, J.M. Gaulier, P. Marquet, J.F. Rabatel, G. Lachatre, J. Chromatogr. B 742 (2000) 229.
[17] M.A. Martinez, C. Sanchez de la Torre, E.A. Almarza, J. Anal. Toxicol. 28 (2004) 174.
[18] S.M.R. Wille, K.E. Maudens, C.H. Van Peteghem, W.E.E. Lambert, J. Chromatogr. A 1098 (2005) 19.
[19] J.W. Kwon, K.L. Armbrust, J. Pharmaceut. Biomed. 37 (2005) 643.
[20] R. Deubner, U. Holzgrabe, Magn. Reson. Chem. 40 (2002) 762.
[21] P.C. Davis, J. Wong, O. Gefvert, J. Pharmaceut. Biomed. 20 (1999) 271.
[22] R.H. Pullen, K.M. Palermo, M.A. Curtis, J. Chromatogr. 573 (1992) 49.
[23] J. Hasselstrom, K. Linnet, J. Chromatogr. B 798 (2003) 9.
[24] R. Mandrioli, S. Fanali, A. Ferranti, M.A. Raggi, J. Pharmaceut. Biomed. 30 (2002) 969.
[25] S.-N. Lin, Y. Chang, D.E. Moody, R.L. Foltz, J. Anal. Toxicol. 28 (2004) 443.
[26] Z. Zhou, X. Li, K. Li, Z. Xie, Z. Cheng, W. Peng, F. Wang, R. Zhu, H. Li, J. Chromatogr. B 802 (2004) 257.
[27] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM). "Guidance for Industry–Bioanalytical Method Validation", May 2001. Available from http://www.fda.gov/cder/guidance/4252fnl.pdf (Accessed on May 31, 2006).
[28] United States Pharmacopeia, 28th ed. United States Pharmacopeial Convention, Rockville (MD), 2005, p. 2748.
[29] R. Mandrioli, M.A. Saracino, S. Ferrari, D. Berardi, E. Kenndler, M.A. Raggi, J. Chromatogr. B 836 (2006) 116.