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Fully automated on-line quantification of quetiapine in human serum by solid phase extraction and liquid chromatography

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Abstract

A quantitative method for determination of quetiapine (QTP) in human serum is presented. The method is fully automated and based on high performance liquid chromatography (HPLC) with on-line solid phase extraction (SPE). The extraction procedure is based on a C2 cartridge, which is eluted with methanol. The eluate is injected onto a silica column with a mobile phase consisting of methanol:20 mM NH_4CH_3COO, pH 5.0 (99:1). Quetiapine is quantified by ultra-violet (UV) absorbance at 257 nM with trifluoperazine as the internal standard (I.S.). The extraction recoveries for quetiapine and trifluoperazine were 69 and 57%, respectively. The total inter day coefficient of variation was 11.1, 3.8 and 3.1% at 20, 500 and 1000 nM, respectively. The detection limit was 10.3 nM quetiapine. The method has been used in our therapeutic drug monitoring (TDM) laboratory where co-administered drugs often are observed. In an investigation of analytical interference from co-administered drugs, demethyl-mianserine was the only drug which interfered with the internal standard. There was no interference with quetiapine itself. The method showed good agreement with mass spectrometric quantification of quetiapine.

Keywords: Quetiapine

1. Introduction

Quetiapine (QTP) is a new antipsychotic drug with a dibenzoethiazepine structure which makes the drug closely related to clozapine (Fig. 1). These antipsychotics have a low incidence of extrapyramidal side effects and tardive dyskinesias compared to older antipsychotics and are therefore classified as atypical antipsychotics. They interact with a wide range of neurotransmitter systems including dopamine D1 and D2, serotonergic $5HT_{1A}$ and $5HT_{2A}$, and α_1- and α_2-adrenergic receptor sites [1].

The atypical antipsychotics are gradually replacing the older typical antipsychotics due to the low incidence of side effects and effective reduction of schizophrenic symptoms. As a consequence, there is an increasing demand for therapeutic drug monitoring (TDM) and research on metabolism of these drugs. Many patients receive several antipsychotics and antidepressants at the same time and due to inter-individual pharmacokinetic variability, interactions, non-compliance, and toxic effects the number of TDM analyses in our laboratory has increased during the last decade. QTP is primarily metabolized by cytochrome P450 3A4 (CYP3A4) with a small contribution from CYP2D6 [2].

Our laboratory provides drug monitoring for a wide range of antipsychotics and antidepressants and most of the analyses are fully automated on a combined solid phase extraction (SPE) and high performance liquid chromatography (HPLC) system with ultra-violet (UV) detection [3,4]. Only a few articles have been published concerning quantification of QTP in human serum [5–7]. Here, we present an analytical method for quantification of QTP, which contrary to previous procedures is fully automated with SPE on-line extraction.

2. Experimental

2.1. Chemicals

QTP 2-[2-(4-dibenzo[*b*,*f*]-1,4-thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol was obtained as a gift from AstraZeneca, Copenhagen, Denmark, and the internal standard

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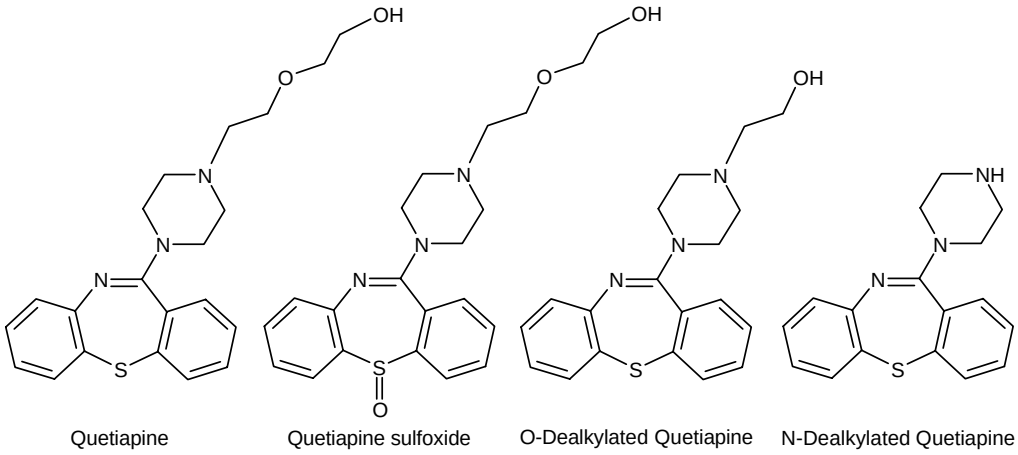

Fig. 1. Structure of quetiapine and three metabolites.

(I.S.), trifluoperazine 10-[3-(4-methyl-1-piperazinyl)-propyl]-2-(trifluoromethyl)-10H-phenotiazine (TFP), was purchased from Sigma. Stock solutions were prepared in ethanol and dilutions were done with methanol. All other chemicals and reagents were of analytical grade.

2.2. Apparatus

2.2.1. HPLC

The extraction procedure was carried out on a Gilson (Villiers le Belle, France) ASPEC XL apparatus with 735 Sampler Software version 3.10 using 1 ml cartridges containing 100 mg ethyl-bonded silica-gel from Isolute (International Sorbent Technology; Hengoed, Mid Glamorgan, United Kingdom).

The HPLC-system was a Gilson apparatus consisting of a 805 manometric module, a 306 isocratic pump, a 118 UV-Vis detector and Unipoint Software version 3.0. The analytical column was a Spherisorb S5W (150 mm × 4.6 mm i.d., 5 μm) from Waters (Taunton, MA, USA), and the guard column was a Phenomenex (Torrance, CA, USA) silica (4 mm × 3 mm i.d.).

2.3. Mass spectrometric method (LC-MS-MS)

The HPLC method was verified against a LC-MS-MS method. For this method the extraction procedure was carried out on a Gilson (Villiers le Belle, France) ASPEC XL4 apparatus with 735 Sampler Software version 3.10 without on-line injection. The HPLC-system was a HP1100 apparatus (Agilent Technologies Deutschland GmbH, Waldbrom, Germany) consisting of a solvent micro degasser (G1379A), a binary pump with seal wash and solvent selection (G1312A), a thermostated well plate autosampler (G1367A), a single wave UV-detector (G1314A) and a control module (G1323B). The flow was split to 250 μl/min to the mass spectrometer and 750 μl/min to waste. Mass spectrometric detection was performed on a Quattro Micro (Micromass UK Limited, Manchester, United Kingdom) instrument operating in positive electrospray ionization (ESI) mode. The optimal settings for multiple reaction monitoring (MRM) quantitation of the transitions QTP (m/z 384.15 → 253.15) and TFP (m/z 408.05 → 141.05) were: cone voltage, 35 V; collision energy, 23 eV (QTP) and 22 eV (TFP); source and desolvation temperature, 130 and 300 °C; desolvation gas flow, 800 l/h and collision gas pressure, 3.9×10^{-3} mbar. Nitrogen was produced by a Micro 90 N_2 generator (Parker, Etten-Leur, Netherlands) (purity: 99.0%) and used as nebulizer and desolvation gas. Argon (purity: >99.996%) served as collision gas. The dwell time was 0.3 s. The HP1100 and the Quattro Micro were both controlled by MassLynx software version 3.5.

2.4. Extraction procedure

The extraction steps were as follows: 750 μl serum was mixed with 2950 μl, 20 mM KH_2PO_4, pH 11.0 (adjusted with 10 M potassium hydroxide) and 50 μl, 8 μM TFP in 50% methanol used as internal standard. Initially, the cartridges were conditioned with 2000 μl methanol and subsequently with 1000 μl, 20 mM KH_2PO_4, pH 11.0. Of the serum mixture 2500 μl (corresponding to 500 μl serum) was loaded onto the cartridges at 1 ml/min. Subsequently, three washing steps were carried out: first 3000 μl, 20 mM KH_2PO_4, pH 11.0 and secondly 2 × 2000 μl 9% methanol. Finally, the analytes were eluted with 250 μl methanol, of which 100 μl was injected onto the HPLC apparatus. For the LC-MS-MS analysis the sample vials were transferred to the autosampler and then injected. The rinse solvent of the extraction apparatus consisted of 20% methanol in order to avoid contamination of analytes and precipitation of proteins in the tubes.

2.5. Chromatographic conditions

The final conditions for the chromatographic run were ambient column temperature, flow rate at 1.0 ml/min, and detection at 257 nm. The mobile phase consisted of

methanol:buffer (20 mM ammonium acetate, pH 5.0 (99:1). The pH was adjusted with 4 M acetic acid.

The run time of each HPLC-analysis was 35 min in order to elute most of the potential interferents. As the SPE method was 22 min the total analysis time was 35 min corresponding to a maximum throughput of 40 samples per 24 h. Quantification was done on basis of the area ratio between the QTP and TFP and multiplied by the factor obtained from the calibration standard.

2.6. Validation

Evaluation of linearity was performed on the basis of eight levels (50, 75, 150, 250, 500, 1000, 2500 and 5000 nM) of QTP spiked in serum. At each level three repetitions were made. Extraction recovery was calculated at two levels (50 and 1000 nM) by comparing area averages of two serum samples at each level spiked with QTP and TFP to area averages in two injection standards at each level.

Specificity was evaluated by analysing six serum blanks and looking for interfering peaks at the retention time of QTP and TFP. Selectivity was evaluated by running injections standards of selected compounds (Fig. 2), which patients might receive as co-medication.

Control samples were analysed to calculate the intra and inter day precision and accuracy. Two replicates at three levels were analysed along with two calibration standards on each of 9 days using the same instrument. Lower limit of detection (LOD) was calculated as three times the total standard deviation (s) of the lowest control sample plus the mean of blank. Lower limit of quantification (LOQ) was calculated by multiplication of the same total standard deviation (s) by five plus the mean of blank corresponding to a coefficient of variation of the LOQ less than 20% [8,9]. Carryover was calculated by running serum blanks after the highest control sample. All statistic calculations were made by CBstat software version 4.2.1 (www.cbstat.com).

2.7. Stock solutions, serum calibration standards and serum controls

Stock solutions of QTP and TFP were prepared in ethanol and further dilution was performed with methanol:water (1:1). Serum calibration standards and controls were made by spiking serum from healthy drug-free donors with the methanol:water solution of QTP (0.01 ml/ml serum). Stock solutions were stored at −18 °C, and serum calibration standards and serum controls were stored at −80 °C.

2.8. Stability of QTP in serum

Nine patient samples were divided into two and were stored at −20 °C. One part was thawed 48 h before analysis and stored at room temperature until analysis, whereas the other part was thawed just before analysis. All 18 samples were analysed the same day and data were assessed by a paired t-test.

2.9. Patient samples

The patients were referred to the routine TDM service laboratory at the Psychiatric Hospital in Risskov, Denmark. Blood samples for quantification of QTP were drawn in the morning approximately 12 h after the last drug intake. The samples were separated into serum and red blood cells by centrifugation. The serum samples were stored at −18 °C until analysis.

3. Results

3.1. Chromatography and recovery

A blank serum sample without internal standard and a representative sample from a patient treated with QTP are shown in Fig. 3. The patient sample shows several metabolites. Preliminary identification by LC-MS-MS has suggested the following metabolites: M1: *O*-dealkylated quetiapine; M2: quetiapine sulfoxide; M3: *N*-dealkylated quetiapine [10]. There are no interfering peaks from the matrix. The mobile phase was selected on basis of interference from other drugs, which made it necessary to have a chromatography time of 35 min. The average (50 and 1000 nM levels) absolute recoveries of QTP and TFP were determined to 69 and 57%, respectively.

3.2. Linearity, precision and accuracy

Linearity was evaluated by plotting the observed detector response as peak area ratio of QTP to TFP versus the concentration of QTP (range: 50–5000 nM). The squared correlation coefficient was 0.9948 following a linear equation, $y = 0.0009x$ (S.E.: 0.00001) − 0.0229 (S.E.: 0.02683). There was no significant deviation from linearity (F-test, $P = 0.96$). From the linearity evaluation, 500 nM QTP was selected as the level of the calibrator for one-point calibration in the validation studies.

Accuracy, and intra and inter day precision were determined by analysing blank serum samples spiked with three levels of QTP (Table 1). The accuracy was close to 100% (97.5–101.9%). The inter day variation was less than 11.1% at all levels and the intra day variation was less than 6.9% at all levels. The limit of detection and limit of quantification were determined to be 10 and 20 nM, respectively. Carryover was less than 1%.

3.3. Stability of QTP in serum

The mean of samples stored 48 h at room temperature (598 nM) was not significantly different from the mean of samples analysed just after thawing (589 nM) (paired t-test,

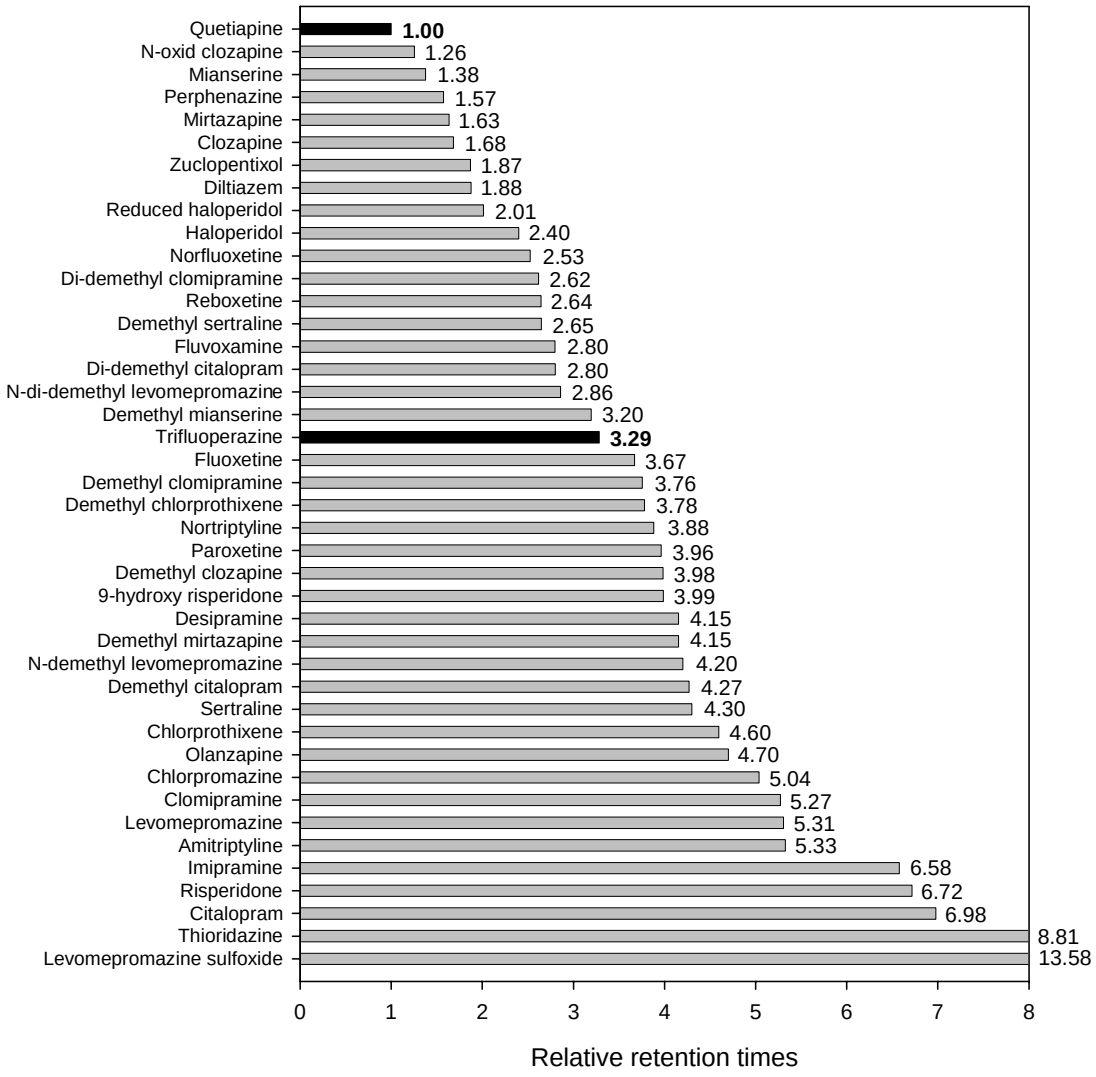

Fig. 2. Relative retention times of drugs and metabolites in relation to quetiapine.

$P > 0.05$). The experiment was undertaken to assure that mailed samples did not deteriorate during transport.

3.4. Interference

Patients in psychiatric treatment often receive several drugs simultaneously. These drugs and their metabolites can cause analytical interference with the I.S. or the drug of interest. Therefore, 40 drugs and metabolites were individually analysed for interference with either QTP or TFP.

Table 1
Precision and accuracy

QTP (nmol/l)	*n*	Intra day precision C.V. (%)	Inter day precision C.V. (%)	Accuracy (%)
20	10	6.9	11.1	97.5
250	9	2.9	3.8	99.5
1000	9	2.3	3.1	101.9

Fig. 2 shows that only demethyl-mianserine interfered with TFP. There was no interference with QTP. On basis of the retention time of late eluting potential interferents the run time was set to 35 min. Only thioridazine and levomepromazine sulfoxide eluted later.

3.5. Comparison with LC-MS-MS detection

The main purpose of comparison between UV and LC-MS-MS was to examine for matrix-effects and co-eluting metabolites of QTP as no metabolites were available as reference compounds. LC-MS-MS is not applied for routine measurements at the present time at our laboratory. Weighted Deming regression analysis applied on a set of paired patient sample measurements [11] showed no significant deviation of the slope from one and no significant deviation of the intercept from zero ($y = 1.0045x - 5.3414$), indicating a good agreement between the measurements (Fig. 4). Thus, the HPLC–UV method was capable of spe-

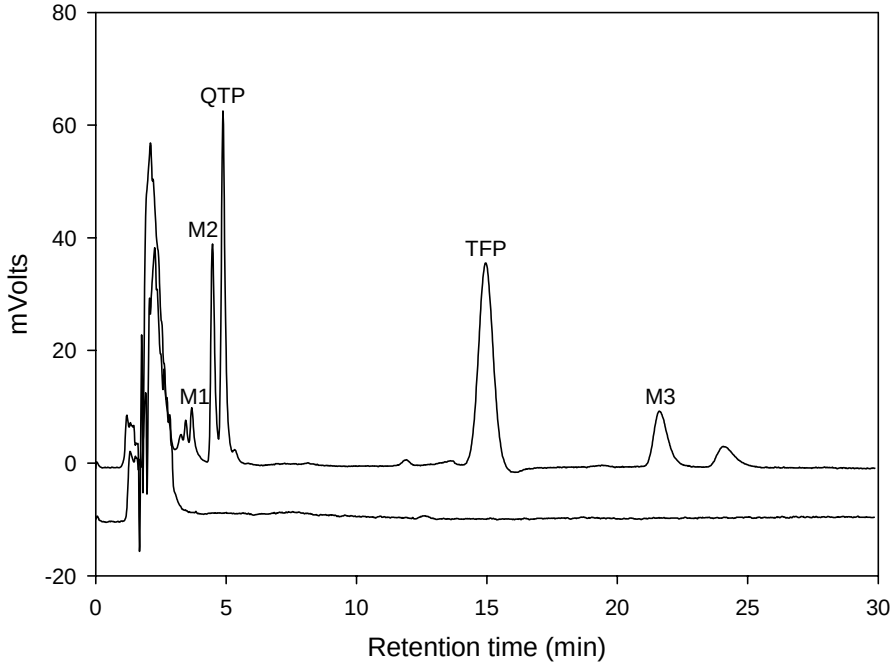

Fig. 3. Chromatograms of a patient sample (top, receiving 1200 mg/day) and serum blank sample (bottom) (QTP: quetiapine; TFP: trifluoperazine; M1: *O*-dealkylated quetiapine; M2: quetiapine sulfoxide and M3: *N*-dealkylated quetiapine).

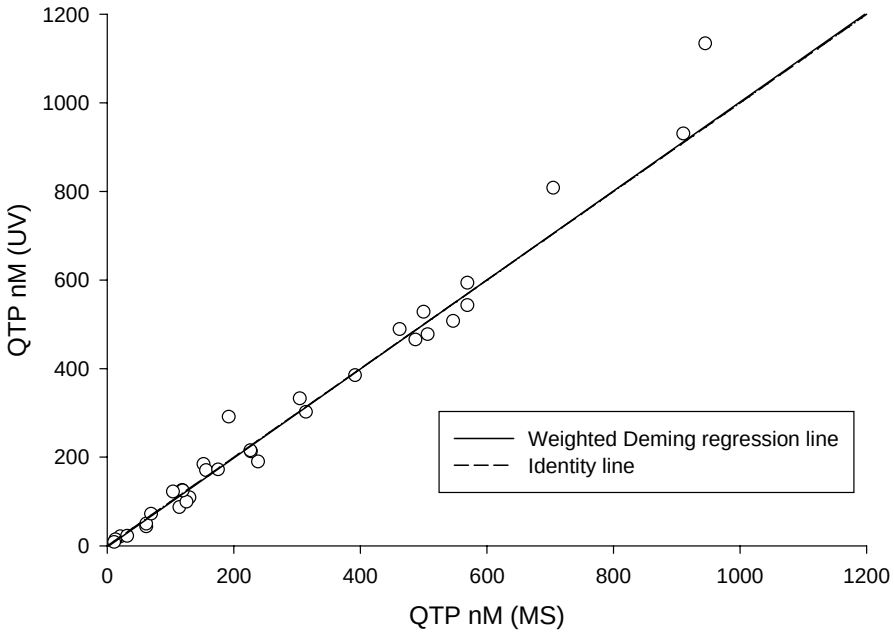

Fig. 4. Comparison of ultra-violet (UV) and mass spectrometric (MS) detection by weighted Deming regression for 33 patient samples.

cific measurement of QTP. Furthermore, the separation between QTP and the metabolites of QTP enables a qualitative detection of quetiapine sulfoxide (Fig. 1), which may be of value in assessing the metabolic rate of QTP. For example, measurement of a low QTP level in conjunction with relatively high metabolite level might suggest a rapid metabolic rate as opposed to non-compliance.

4. Discussion

Originally, we intended to analyse QTP by our existing method for clozapine and olanzapine [4]. However, although QTP has structural similarities to clozapine, the lipophilic properties differ significantly because of the ethoxy–ethanol group attached to the piperazine nitrogen. This minimizes

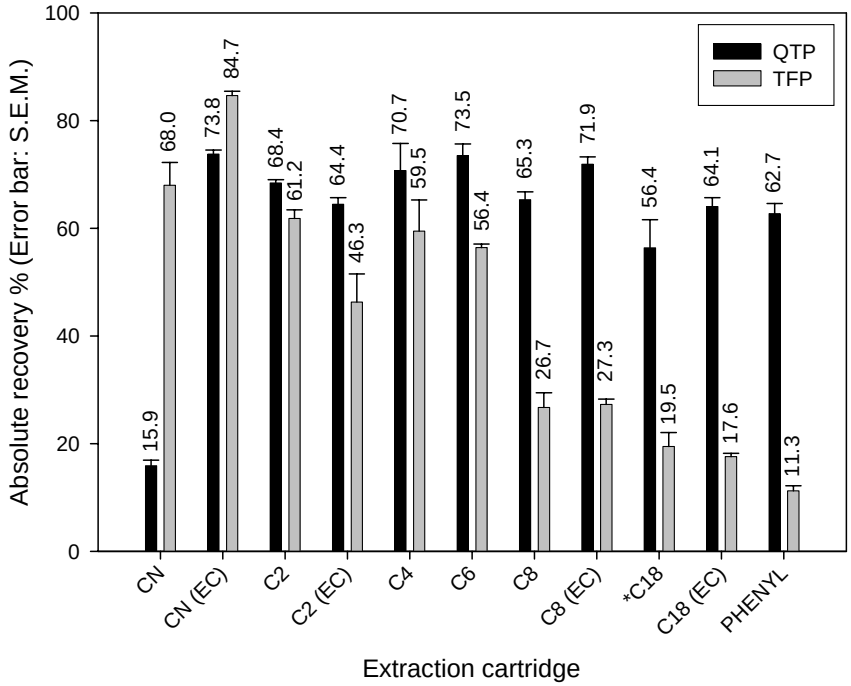

Fig. 5. Absolute recoveries of quetiapine and trifluoperazine at different extraction cartridges. S.E.M.: standard error mean. The experiment was carried out at calibrator level of quetiapine (500 nM) with three repetitions per extraction cartridge. Peak areas of the serum sample were compared to peak areas of a injection standard. The extraction was: 750 μl serum was mixed with 2950 μl, 20 mM KH_2PO_4, pH 11.0 and 50 μl, 8 μM trifluoperazine in 50% methanol. The extraction cartridge was the conditioned with 2000 μl methanol and 1000 μl, 20 mM KH_2PO_4, pH 11.0. Twenty-five hundred microliters of the serum mixture was then loaded on to the cartridge, which was washed subsequently with 3000 μl, 20 mM KH_2PO_4, pH 11.0 and two times 2000 μl, 9% methanol. Finally, the analytes were eluted with 250 μl methanol of which 100 μl was injected into the HPLC apparatus; asrerisks: two repetitions.

the polar effects of the protonized piperazine group, and it was clear that a thorough examination of both the extraction and the chromatography steps were necessary.

During the method development, several extraction cartridges were evaluated (CN, CN (EC), C2, C2 (EC), C4, C6, C8, C8 (EC), C18, C18 (EC) and phenyl). Extraction recoveries of the extraction cartridges were determined at one level (500 nM QTP) with three repetitions under the final conditions described in the sections of extraction procedure and chromatographic conditions. The results are shown in Fig. 5. Generally, the recovery of QTP increased slightly from C2 to C8 and significantly from CN to CN (EC), which was the cartridge where end-capping had the highest effect. Recovery of TFP decreased when the CN cartridge was exchanged with a non-polar C2–C18 and phenyl. The phenyl cartridge resulted in a broadened solvent front, which interfered with the QTP peak. In order to achieve an acceptable recovery of TFP, pH had to be above 10, while the recovery of QTP remained the same in the pH range 8–11. C2 was selected as a compromise of obtaining acceptable recovery of both QTP and TFP, and the achievement of no interference in blank serum samples (Fig. 3).

TFP was selected as I.S. on basis of experience in the laboratory of TFP stability and chromatographic behaviour. Although TFP and QTP share some structural similarities, their behaviour in the extraction process and chromatography differs. As an example, Fig. 6 shows the effect of solvent strength in the washing procedure during SPE. Applying increasing solvent strength in the washing procedure implies that the recovery of QTP decreases, while it remains the same for TFP. When washing with methanol, and subsequently eluting with methanol, the results indicate that TFP adsorbs stronger on the extraction cartridge than QTP.

In a previous publication [4] TFP was mixed with the solvent used to dilute the samples before extraction. This is done in the methods of clozapine and olanzapine at pH 8. When pH is raised to 11, TFP is no longer stable and will be subject to autooxidation [12]. In this case, the concentration of TFP was reduced by approximately 50% during 24 h at pH 11. This problem was avoided by reprogramming the XLi apparatus so that internal standard, diluting solvent and serum were mixed just before loading onto the cartridge.

The first step in developing the HPLC method was to establish a chromatography capable of measuring QTP and TFP without any analytical interference from the most common co-administered drugs. A silica column was chosen in order to avoid interference from commonly used psychiatric drugs as we have observed previously [4].

A recently published method on quantification of quetiapine in human serum applied SPE and HPLC with UV detection [7]. However, there was no direct injection of the eluate onto the HPLC column, which also was the case in Pullen et al. [5] and Davis et al. [6].

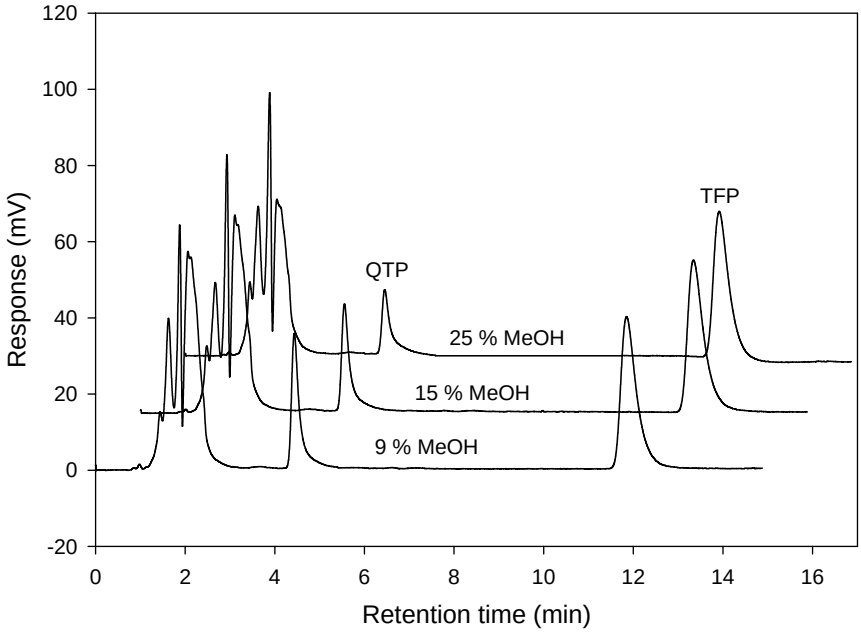

Fig. 6. Effect of increasing percentage of methanol (MeOH) in cartridge wash on recovery of quetiapine (QTP) and trifluoperazine (TFP) (method: solid phase extraction: 750 μl serum + 50 μl, 8 μM TFP + 2950 μl, 20 mM K_2HPO_4, pH 11.0 was mixed. Twenty-five hundred microliters mix was loaded on the C2 cartridge at 1 ml/min. The cartridge was then washed with 3000 μl, 20 mM K_2HPO_4, pH 11.0 and two times 1000 μl, 9, 15 or 25% MeOH. The analytes were eluted with 250 μl MeOH. HPLC: mobile phase: MeOH:20 mM NH_4CH_3COO, pH 5.0 (99:1), 257 nM UV-detection, flow: 1 ml/min, silica column).

The critical step in on-line SPE and HPLC is the compatibility of the eluent and the mobile phase. The optimal procedure is elution with the mobile phase which, however, often results in too low recovery, or with an eluent of weaker solvent strength than the mobile phase. Elution with solvents of different composition and pH than the mobile phase can give broadened peaks and unstable retention times during a run of a series of samples. Here, methanol gave the best result after the dilution solvent was changed. Initially, 100 mM ammonium acetate, pH 11, adjusted with ammonia was tried as dilution solvent in the extraction process. The relatively high concentration of ammonia resulted in a basic eluate, because ammonia adsorbs to the C2 cartridges and is highly soluble in methanol. As a result of the basic eluate, retention times of both QTP and the internal standard, TFP, decreased drastically during a series of five samples. With the exchange of ammonium acetate with potassium dihydrogen phosphate these problems were avoided. This effect can be caused by changes in the ionization of silanol groups on the HPLC column and lack of equilibration time. Increasing pH in the mobile phase results in decreasing retention times and the opposite for decreasing pH. Increasing the percentage of methanol and decreasing pH in the mobile phase resulted in increased retention times of especially TFP and optimal separation of the drugs and their metabolites.

The major issue in TDM is selective measurement of the drug of interest with no interference from other drugs. Comedication of psychiatric patients with 2–10 drugs is frequently observed in our TDM laboratory. Mandrioli et al. [7] give no detailed information about possible interfering compounds eluting later than 11 min, and they do not investigate the retention times of relevant metabolites. Nor do Pullen et al. [5] and Davis et al. [6] examine for relevant interfering compounds other than the metabolites of QTP. These issues can cause interference with the I.S. or the drug of interest. Searching for interfering compounds revealed no major interferences except for the active metabolite demethylmianserine and the late eluting thioridazine and levomepromazine sulfoxide, which can cause interference in the following run. Thioridazine is hardly used anymore in our area, and the typical antipsychotic levomepromazine is relatively seldom used.

The TDM laboratory has measured QTP in patients every week for 1 year with the described method. During that period it has exhibited stable performance and has turned out to be suitable for routine TDM of quetiapine. The drug measurements at the TDM laboratory represent the trough value (12 h). The concentration–time profile and $T_{(1/2)}$ (5.3 h) has been studied by Davis et al. [6] and Gefvert et al. [13].

Acknowledgements

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