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ORIGINAL ARTICLE

UHPLC-MS/MS assay using environment friendly organic solvents: A green approach for fast determination of quetiapine in rat plasma

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Abstract Reduction or elimination of traditional hazardous chemicals in chromatographic separation and sample preparation is comparatively safer and can be considered as a greener approach for the determination of analytes in biological matrices. A rapid and sensitive UHPLC-MS/MS assay for determination of quetiapine in rat plasma was developed and validated using environment friendly and low toxic organic solvents as organic modifier in mobile phase and sample preparation. Quetiapine and risperidone (internal standard) were separated on Acquity BEH™ C_{18} column (50 × 2.1 mm, i.d. 1.7 μm) using ethanol–water-formic acid (80:20:0.1, v/v/v) as mobile phase at a flow rate of 0.3 mL/min. Detection was performed on tandem mass spectrometer using positive electrospray ionization source by multiple reaction monitoring mode. The precursor to product ion transitions of m/z 384.13 > 253.00 for quetiapine and m/z 411.18 > 191.07 for IS was used to quantify them respectively. The method was found to be linear in the concentration range of 0.45–500 ng/mL. All validation parameters were within acceptable range, as defined by guidelines of bio-analytical method validation. In conclusion, bioanalytical assay using UHPLC-MS/MS together with environment friendly and low toxic organic solvents in mobile phase and sample preparation may be considered as greener approach for quantification of analytes in plasma samples.

1. Introduction

Solvents, commonly used in analytical methodologies, are mostly volatile organic compounds that are hazardous to our environments. They lead to air pollution, are flammable in nature and can cause toxicity and/or carcinogenicity (Salvador Garrigues and de la Guardia, 2010). Green analytical chemistry is a modern era technique that involves methods to reduce

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or eliminate the use of the hazardous solvents, reagents, preservative and other chemicals that are harmful to human health or environment without compromising the performance and quality of analysis (Keith et al., 2007; Namieśnik, 2001). In the recent years, rapid rise in development of green analytical methods has been observed that has results to extensive research and high rate of publications in this area (Agnieszka Gauszka and Namieśnik, 2013; Christopher J. Welch et al., 2010; Haq et al., in press; Armenta et al., 2008; Tache et al., 2013; Tobiszewski et al., 2010; Vaher and Kaljurand, 2012).

Recently, few attempts have also been explored in literature for the use of environment friendly approach in bioanalytical technique (Bojko and Pawliszyn, 2012; Fountain, 2012; Ghosh, 2012; Heudi, 2012; Kaljurand and Koel, 2012; Rainville et al., 2012; Tsai et al., 2012). Green approach in bioanalytical methods can be implemented during chromatographic separation and sample preparation. Conventional HPLC mobile phase techniques (which use acetonitrile and/or methanol) are replaced with environment friendly alternatives such as water, ethanol, isopropanol or propyl carbonate (Rainville et al., 2012; Smith, 2008; Tache et al., 2013). Similarly, use of carbon dioxide in supercritical fluid chromatography (Dispas et al., 2012), reduction in consumption of solvents or additives through reduced column internal diameters (narrow or microbore ranges) and dimensions of packing ($\leqslant$2 μm) (Sandra et al., 2010; Zotou, 2012) adapted as the environment friendly approach in chromatographic separation. Additionally, selection of less harmful organic substitutes (e.g. ethyl acetate, 2-butanol) in liquid–liquid extraction (LLE), practices of liquid and solid phase micro-extraction (Abdel-Rehim and Abdel-Rehim, 2013; Farhadi et al., 2012; Kataoka, 2011; Kataoka and Saito, 2011) and sample reduction using dried blood sampling (Heudi, 2012) are becoming more popular methods and favorable choice in sample preparation.

Among the available analytical techniques; ultra-high performance liquid chromatography (UHPLC) has gained a considerable attention as both chromatographic separation and assay selectivity have been significantly improved using this technology (Liu et al., 2009). Use of Aquity BEH column in UHPLC, which not only increase the separation throughput and efficiency but also reduces the retention time and volume of solvent required during separation (Kumar et al., 2012; Li et al., 2008; Novakova et al., 2006). Considering all these above mentioned advantages, UHPLC may be useful for greener approaches in bioanalytical assay (Cielecka-Piontek et al., 2013).

Quetiapine (QUE), a dibenzothiazepine derivative is an atypical antipsychotic, indicated for the treatment of schizophrenia, acute mania in bipolar disorders, and bipolar depression in adults. It has also been found to be effective as a monotherapy and as an adjunctive to a mood stabilizer (Altamura et al., 2011; Chiesa et al., 2012; Dispas et al., 2012; Patteet et al., 2012; Vieta et al., 2010). Therapeutic drug monitoring is a common and an important requirement in antipsychotic treatment to control the compliance and avoidance of extrapyramidal side effects (Hiemke et al., 2004; Musenga et al., 2009). It is important to perform accurate monitoring of QUE levels in blood in order to reduce the side effects and to optimize treatment. In view of this, to date, innumerable assays have been reported for quantification of QUE (alone and in combination with other drugs) in biological matrices (e.g. plasma, serum, tissue, oral fluids and microsomes). These techniques include HPLC with UV detection (Davis et al., 1999; Frahnert et al., 2003; Hasselstrom and Linnet, 2003; Mandrioli et al., 2002; Mercolini et al., 2007; Sachse et al., 2006; Saracino et al., 2006) gas chromatography mass spectroscopy (GC MS) (da Fonseca et al., 2013; Langman et al., 2004; Pullen et al., 1992) liquid chromatography mass spectroscopy (Zhou et al., 2004), liquid chromatography tandem mass spectroscopy (LC-MS/MS) (Barrett et al., 2007; Davis et al., 2010; Fisher et al., 2012a,b; Kirchherr and Kuhn-Velten, 2006; Li et al., 2004; Lin et al., 2004; Nirogi et al., 2008; Ostad Haji et al., 2013; Pan et al., 2012; Xiong et al., 2013) and UHPLC-MS/MS (Amundsen et al., 2013; Ansermot et al., 2013; Ji-Ying Tu et al., 2008; Li et al., 2007; Patteet et al., 2013). Most of the above mentioned techniques are low sensitive as well as time consuming. Although, the previously reported LC-MS/MS and UHPLC-MS/MS methods were also sensitive, but the solvents used in chromatographic separation and sample preparation falls under the category of hazardous wastes which causes serious environment, health, and safety (EHS) concerns, including human and eco-toxicity issues (Cielecka-Piontek et al., 2013; Rainville et al., 2012; Salvador Garrigues and de la Guardia, 2010).

Despite of available possibilities for green bioanalytical approach, very meager information exists regarding its use. To the best of our knowledge, only one validated method is available in literature, which used the greener approach for bioanalysis throughout the study (Cheregi et al., 2013).

Therefore, the present study aimed to develop a generic UHPLC-MS/MS method using environment friendly and low toxic solvents, a green approach, which can facilitate the rapid determination of QUE in plasma samples.

2. Experimental

2.1. Chemicals and reagents

Quetiapine (purity $\geqslant$ 98 %) and risperidone (internal standard; purity $\geqslant$ 98%) were used as reference substance in this study. QUE was received as a gratis from Ranbaxy Laboratories India and risperidone was purchased from Sigma–Aldrich (St. Louis, MO), USA. Ethanol, ethyl acetate and methanol were of HPLC grade and were obtained from VWR International Ltd. UK. Formic acid and sodium hydroxide were of analytical grade and obtained from Qualikems Fine Chem. Pvt. Ltd., Vadodara India. All aqueous solutions used in this study were prepared by using Milli-Q water purified method using Milli-QR Gradient A10R (Millipore, Moscheim Cedex, France) with pore size 0.22 μm. Blank plasma was prepared in laboratory from drug free healthy rats which were obtained from the Animal Care and Use Centre, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia and was stored at −80 °C until used.

2.2. Calibration standards and quality control samples

Standard stock solutions (100 μg/mL) of QUE and risperidone (IS) were prepared in methanol and kept in the refrigerator at 4 °C for use within 15 days of preparation. Working solutions

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of QUE for calibration standards were prepared from stock solution using methanol:water (50:50, v/v). A 20 μL aliquot of each working solutions was added to 200 μL of blank rat plasma to obtain the final calibration standard between 0.45 and 500 ng/mL. Quality control (QC) samples at four different concentrations: 0.48, 1.5, 60 and 400 ng/mL in blank rat plasma were also prepared in a similar manner and treated as lower limit of quantification for QC (LLOQ QC), low QC (LQC), mid QC (MQC) and high QC (HQC) respectively. Spiked plasma calibration standards and quality control samples were kept at −80 °C until used during validation and/or analysis. The IS working solution (200 ng/mL) for routine use was prepared by diluting the risperidone stock solution in methanol:water (50:50, v/v) and was kept in the refrigerator at 4 °C.

2.3. UHPLC separation and MS/MS parameters

The equipment consisted of ACQUITY™ UHPLC system coupled to triple–quadruple tandem mass spectrometer Micromass® Quattro micro™ (Waters Corp., Milford, MA, USA). The UHPLC system included quaternary solvent manager, a binary pump, degasser, autosampler with an injection loop of 10 μL and a column heater–cooler. The chromatographic separation was performed on Acquity BEH™ C_{18} column (50 × 2.1 mm, i.d., 1.7 μm, Waters, USA) maintained at temperature of 40 ± 2 °C. The mobile phase composed of ethanol–water-formic acid (80:20:0.1, v/v/v) at a flow rate of 0.3 mL/min. The injection volume was 10 μL in partial loop mode and the temperature of the autosampler was 15 ± 2 °C.

Triple–quadruple tandem mass spectrometer equipped with electrospray ionization (ESI) interface was used for analytical detection. The detection was performed in ESI positive mode using multiple reaction monitoring (MRM) by the ion transitions of m/z 384.13 > 253.00 for QUE and m/z 411.18 > 191.07 for IS, having dwell time of 0.161 s. Nitrogen was used as a desolvating gas at a flow rate of 650 L/h. The desolvating temperature was 350 °C whereas source temperature was 150 °C. The collision gas (argon) flow was 0.1 mL/min and capillary voltage was set at 3.5 kV. The compound parameters such as cone voltage and collision energy were set at 40 V and 24 eV for QUE and 42 V and 30 eV for IS respectively. The Mass Lynx software (Version 4.1, SCN 714) was used to control the UHPLC-MS/MS system. Data were collected and processed using TargetLynx™ program.

2.4. Sample preparation

Plasma samples stored at −80 °C were thawed at room temperature and vortexed for 30 s before sample preparation. In a clean 10 mL-glass tube, an aliquot of 200 μL of spiked calibration standards, QCs and unknown plasma samples were transferred and 10 μL of IS (200 ng/mL) was added in each tube except blank. The 50 μL of 0.1 M sodium hydroxide was added into each tube and vortexed for 30 s. Then, 2 mL of ethyl acetate was added into each tube and again vortexed gently for 3 min followed by centrifugation for 5 min at 4000*g* and 4 °C. After centrifugation, upper portion of the organic layer was transferred to a clean test tube, and evaporated to dryness under a stream of nitrogen at approximately 40 °C. The dried extracts were reconstituted in 200 μL of ethanol and transferred into HPLC vials for the analysis.

2.5. Method validation

The developed assay was fully validated in rat plasma according to guidelines of bioanalytical method validation set by the United States Food and Drug Administration and European Medicines Agency (EMA, 2011; FDA, 2001). The evaluated validation parameters included selectivity, linearity of response, accuracy, precision, matrix effects, recovery, dilution integrity and stability of analytes in both short-term sample processing and long-term storage.

Method selectivity was assessed by comparing the chromatograms of blank rat plasma samples with chromatograms of spiked plasma samples at LLOQ (0.45 ng/mL) and IS (20 ng/ml). The response of interferences in blank plasma was limited to ⩽20% of the response of analytes and ⩽5% of the IS. The linearity of the method was determined by plotting the plasma calibration curve in triplicate at eight different concentrations ranging from 0.45 to 500 ng/mL. The precision of the assay was expressed as percentage coefficient of variation (% CV) at four different concentrations whereas accuracy was expressed as a percentage of deviation from the respective nominal value. The intra-day and inter-day precision and accuracy were evaluated at four different QC concentration levels (LLOQ QC, LQC, MQC and HQC) in six replicate on the same day and on three different days, respectively. The recoveries of QUE at three QC levels were determined by comparing peak area ratios of plasma spiked with analyte prior to extraction with the peak area ratios of plasma spiked with analyte after the extraction. The matrix effects were determined by calculating the peak area ratio of extracted rat plasma postspiked with analyte to the peak area of the analyte in mobile phase in the same concentration. Deviation in analyte concentrations of a maximum of 15% was considered acceptable and in line with current EMEA guideline (EMA, 2011). The extraction recovery and matrix effects of IS were also evaluated using the same procedure.

The stability of QUE in rat plasma under different anticipated conditions was assessed by using six replicates of LQC and HQC. Bench-top stability was assessed after keeping the plasma samples to room temperature for ~6 h, the freeze–thaw stability was determined after three cycles of freeze–thaw. Autosampler storage stability was determined by storing the reconstituted QC samples for ~48 h under autosampler condition (maintained at 15 °C) before analysis. Long-term stability was assessed after storage of the test samples at around −80 °C for 60 days. The working solutions and stock solutions of QUE and the IS were also evaluated for their stability at room temperature for 12 h and at refrigerator temperature (below 10 °C) for 15 days. Dilution integrity exercise was carried out to ensure the integrity of analyte in those samples which were beyond upper limit of the standard curve and need to be diluted. A fresh stock of QUE was prepared and spiked in plasma to get a concentration level of 1.8 times of highest standard of the usual calibration standard, and then diluted 2 and 4 times with the same plasma. Six aliquots of both dilutions were processed along with freshly spiked calibration standards and analyzed by back calculation using regression equation obtained.

2.6. Application to pharmacokinetic interaction study in rats

To demonstrate the utility of this assay, method was applied to pharmacokinetic interaction study of QUE with green tea

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extracts (GTE) in rats. Twelve male wistar albino rats weighing from 180 to 220 g were randomly divided into two groups (6 in each). Experiments were carried out according to the guidelines of the animal care and use committee at King Saud University. Rats in treatment group received GTE (175 mg/kg, oral) for seven days, while control group received normal saline in same volume. Blood samples (approximately 0.5 mL) were collected from the retro-orbital plexus into heparinized microfuge tubes at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after administration of QUE (25 mg/kg, oral; suspended in 1% hydroxyl propyl methyl cellulose) in both groups. Plasma samples were harvested by centrifuging the blood at 5000*g* for 10 min and stored at −80 ± 10 °C until analysis.

3. Results

3.1. Method development

UHPLC-MS/MS operation parameters were carefully optimized before detection by tandem mass spectrometry using MRM of precursor–product ion transitions at m/z 384.13 > 253.0 for QUE and m/z 411.18 > 191.07 for IS. Initially the standard solution (200 ng/mL) of both QUE and IS was directly infused into the mass spectrometer for tuning in both positive and negative modes using ESI as the ionization source. It was observed that the signal intensity of positive ion (ESI^+) was much higher than that of negative ion for both analyte and IS. Parameters, such as capillary and cone voltage, desolvation temperature, ESI source temperature and flow rate of desolvation gas and cone gas were optimized to obtain the optimum intensity of protonated molecular ions $(M–H)^+$ of QUE and IS. Analyte produced the strongest product ion and daughter ion signals when cone voltage and collision energy were set at 40 V and 24 eV respectively. For IS, the optimized cone voltage and collision energy were 42 V and 30 eV respectively. The precursor and product ion spectra of QUE and IS are shown in Fig. 1.

Feasibility of different compositions of ethanol and isopropranol and water containing 0.1% formic acid as mobile phase were tried for separation of analyte and IS with altered flow-rates (in the range of 0.2–0.4 mL/min) on BEH™ C18 column (50 × 2.1 mm, i.d. 1.7 μm). The best peak resolution along with high sensitivity was achieved with an isocratic elution by a mobile phase comprising of ethanol–water-formic acid (80:20:0.1, v/v/v) at a flow-rate of 0.3 mL/min. The column oven temperature also optimized at 40 °C to adjust the back

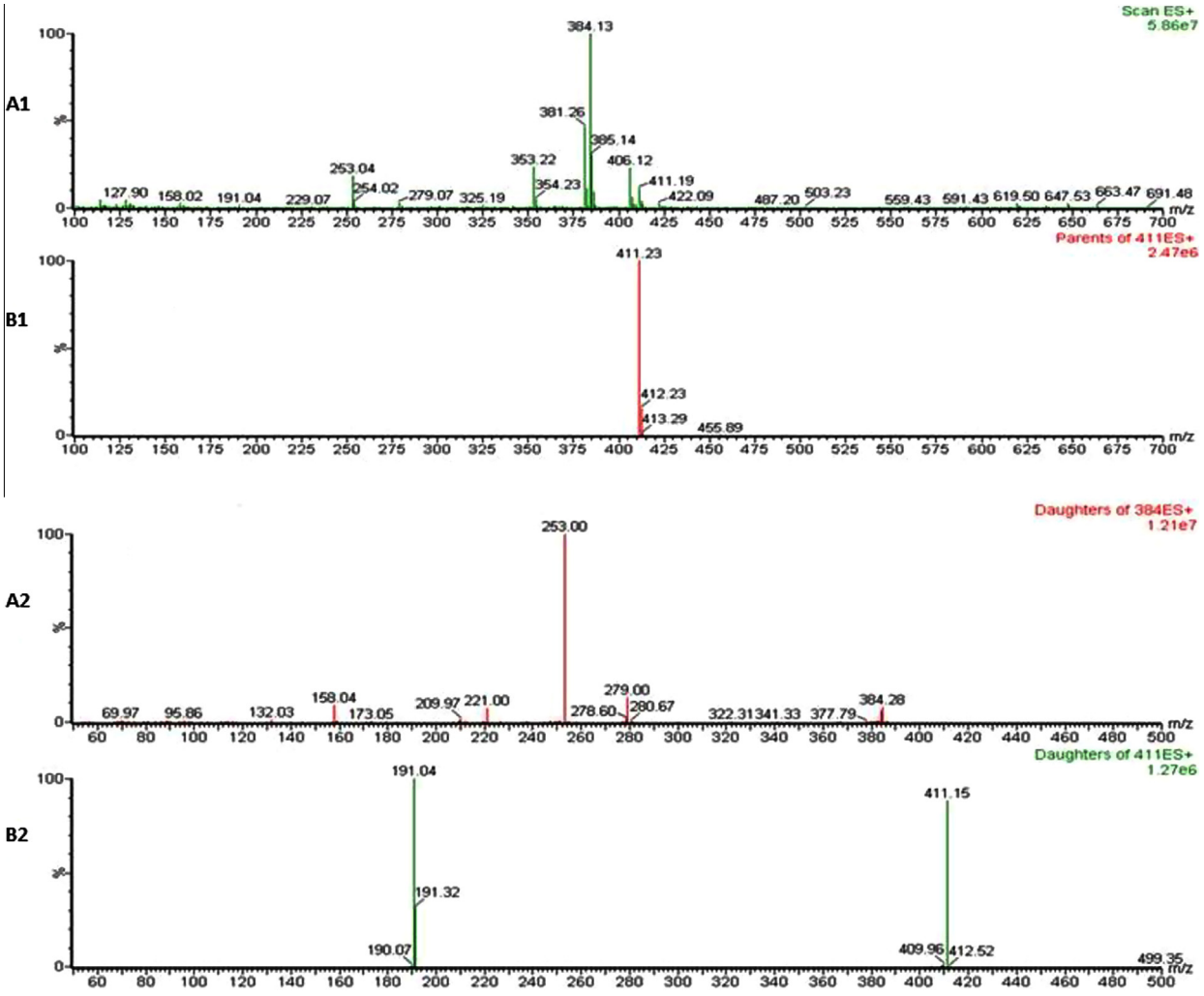

Figure 1 Precursor and product ion spectra for quetiapine (A1 and A2) and IS (B1 and B2), respectively.

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pressure which was finally set to approx. 8000 psi. The selected conditions were found to be suitable for the separation and determination of electrospray response for QUE and IS.

Clean samples are essential to minimize ion suppression and matrix effect in bioanalysis. Initially protein precipitation using ethanol and/or isopropranol was evaluated for sample preparation but the results were not satisfactory in terms of recovery and matrix effects. Finally, liquid-liquid extraction (LLE) using ethyl acetate, tertiary butyl ethyl ether and 2-butanol was tried as extracting solvents. Ethyl acetate produced better results compared to others. Extraction rate was also increased by alkalization of the compounds by adding 50 μL of 0.1 M sodium hydroxide before ethyl acetate. Effects of different extracting solvents (ethyl acetate, tertiary butyl ethyl ether and 2-butanol) on quetiapine recovery and matrix effects are shown in Fig. 2.

3.2. Method validation

3.2.1. Selectivity

No significant peaks in blank plasma samples were detected at the corresponding retention time of analyte and IS. Representative MRM chromatograms of analyte and IS for blank plasma (showing no significant interference at the retention time of analyte and IS) as well as plasma spiked at LLOQ level are shown in Fig. 3A and B, respectively.

3.2.2. Linearity and lower limit of quantification

The linearity of the method was determined by a weighted ($1/X^2$) least square regression analysis of standard plot associated with eight-point standard curve. The calibration curves were generated by plotting area ratio (analyte/IS) as a function of QUE concentration. The method was found to be linear in range between 0.45 and 500 ng/mL for QUE in rat plasma and LLOQ for this assay was 0.45 ng/mL. The determination coefficients (r^2) were found to be consistently $\geqslant$0.997 during the course of validation.

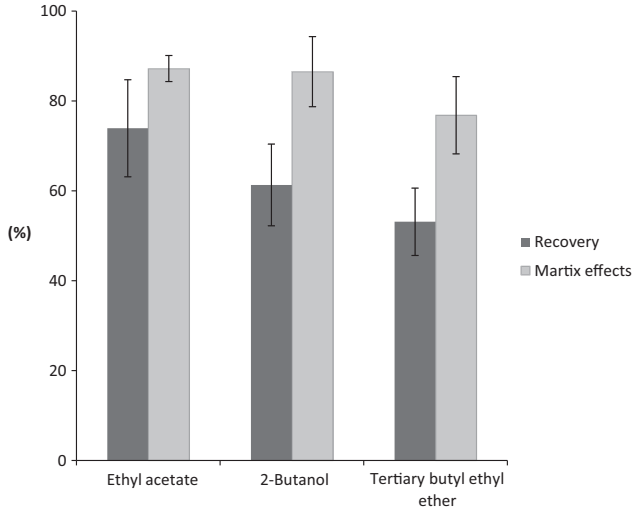

Figure 2 Extraction recovery and matrix effects (%Mean ± %CV) of different extracting solvents at MQC concentration of quetiapine in rat plasma (n = 3).

3.2.3. Precision and accuracy

The precision and accuracy data for QC samples (both inter-day and intra-day) are listed in Table 1. The intra-day and inter-day precision results were 3.8–11.3% and 4.5–9.1%, respectively; whereas the intra-day and inter-day accuracies were 91.1–112% and 94.5–112%, respectively. The accuracy was required to be limited to within ±15% (20% for LOQ QC) and precision was not to exceed ±15% (20% for LOQ QC.)

3.2.4. Recovery and matrix effects

The percentage recoveries (mean ± SD) of QUE obtained from plasma at three different QC concentrations level (1.50, 60 and 400 ng/mL) were 76.9%, 73.9% and 71.4% respectively. Mean recovery for the risperidone (IS) at the concentration employed was 67.3% (Table 2). These results indicate that the extraction efficiency for QUE using LLE method was satisfactory, consistent and concentration independent. The ion suppression effects were within $\leqslant$15% and its relative standard deviation (%CV) was within ±5% among all QC concentration, indicated no relative matrix effects (Table 2).

3.2.5. Stability and dilution integrity

The stability and dilution integrity results are summarized in Table 3. These indicate that QUE spiked in rat plasma was stable for at least 6.0 h at room temperature, 48 h in autosampler, 60 days at around −80 °C and during three cycle of freeze–thaw when stored at around −80 °C. The stock solutions and working standard of QUE and IS were also stable for 15 days at refrigerator temperature (below 10 °C) and for 12 h at room temperature. In dilution integrity study, the percentage accuracy of two and four times diluted samples was to 96.3% and 97.0% of the nominal concentration for QUE. These results indicated that the dilution of the concentrated plasma sample up to four times maintains legibility and integrity of QUE concentration.

3.3. Pharmacokinetic interaction study in rats

The applicability of this validated method was demonstrated successfully in a pharmacokinetic interaction study of QUE with GTE. Representative MRM chromatogram of analyte and IS after one hour administration of QUE alone in rat are shown in Fig. 3C. The mean plasma concentration verses time profile of QUE is shown in Fig. 4.

4. Discussion

Binary mixture of water and polar organic solvents like methanol and acetonitrile comprise the majority of mobile phase utilized for liquid chromatography. These agents are used due to their miscibility with water, low viscosity, ability to produce good chromatographic peak shape and compatibility with UV and MS detection. Unfortunately, these solvents produce substantial hazards to the analysts during handling as well as produce harmful effects on the environment. In this study, the primary focus on the selection of the mobile phase was the replacement of these traditional (methanol and acetonitrile) solvents with non-traditional, less hazardous, environment friendly benign organic solvents. Non-traditional,

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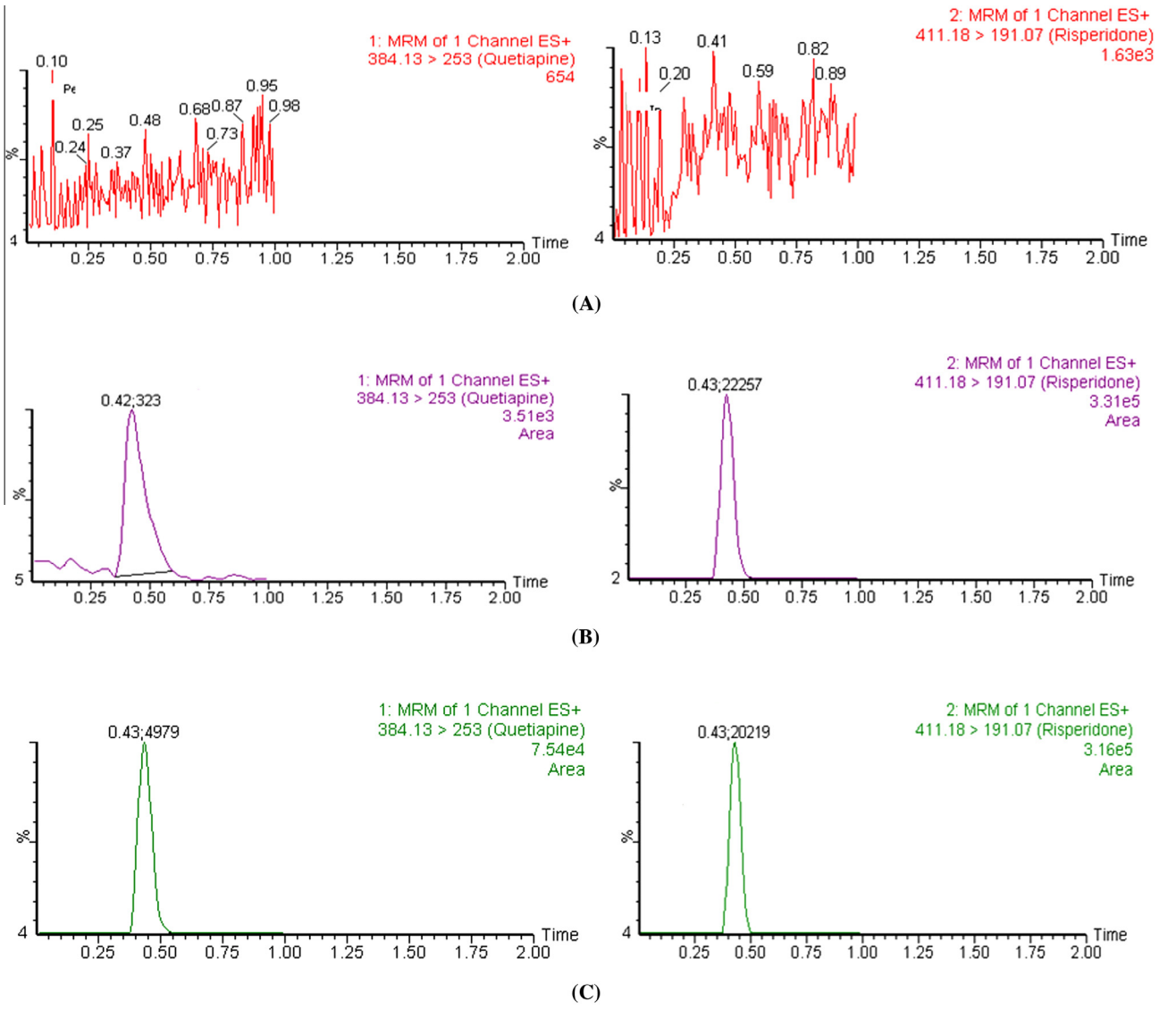

Figure 3 Representative MRM chromatograms of quetiapine and IS in (A) blank plasma; (B) plasma spiked at LLOQ level and (C) at one hour after oral administration of QUE 25 mg/kg in rat.

alcoholic mobile phase; like, ethanol and isopropranol revealed promising results in replacing the traditional use of acetonitrile and methanol (Rainville et al., 2012). However, high viscosity of these two non-traditional solvents was the main challenge which could result in high back pressure during the process of separation. UHPLC chromatographic system is designed in a special way to withstand high system back-pressures, and the separation on it can be easily performed under very high pressures (up to 1000 psi), without any negative impact on analytical column or other components of chromatographic system (Novakova et al., 2006). In this study, the use of ethanol with the adjusted back pressure (approx. 8000 psi) and the column oven temperature (40 °C) was found to be suitable for the separation and determination of electrospray response for QUE and IS. Finally, mixture of ethanol–water-formic acid (80:20:0.1) was selected for chromatographic separation of quetiapine. Chromatographic separation of previously reported green bio-analytical assay (Cheregi et al., 2013) was based on mixture of propylene carbonate/ethanol in 7:3 ratio.

Commonly used sample preparation technique such as protein precipitation, LLE and solid phase extraction (SPE) generally consume substantial amount of organic solvents. Phospholipids present in plasma are considered as one of the most significant matrixes interferences encounter in protein preparation methodologies (Phillips and Dodge, 1967). But, due to ease and compatibility with mobile phase, sample extraction by protein precipitation using acetonitrile and/or methanol is often preferred over other solvents. However, both of them have been reported to less effective in the removal of the phospholipids compared to ethanol and isopropranol (Rainville et al., 2012). During this study, protein precipitation using ethanol and/or isopropranol in the presence or absence of acetic acid/formic acid was evaluated for sample preparation but the efforts resulted poor recoveries and sensitivity. LLE is one of the preferred sample preparation techniques during assay development that provides cleaner extracts than protein precipitation processes in most cases. LLE is more cost effective than SPE, especially when only non-halogenate extraction solvents such as hexanes and ethyl acetate are used. Tertiary butyl methyl

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Table 1 Intra-day and inter-day precision and accuracy of QUE in rat plasma.

Nominal concentration (ng/mL)	Run	Mean ± SD (ng/mL)	Precision (CV%)	Accuracy (%)
Intra-day variation (six replicate at each concentration)				
0.48	1	0.54 ± 0.03	5.5	112
	2	0.53 ± 0.06	11.3	110
	3	0.53 ± 0.05	9.4	111
1.50	1	1.56 ± 0.11	7.0	104
	2	1.58 ± 0.13	8.2	105
	3	1.55 ± 0.06	3.8	103
60.0	1	57.3 ± 4.42	7.7	95.4
	2	61.7 ± 5.70	9.2	103
	3	62.8 ± 5.68	9.0	105
400	1	364 ± 20.3	5.5	91.1
	2	393 ± 37.0	9.4	98.1
	3	378 ± 16.2	4.3	94.4
Inter-day variation (18 replicate at each concentration)				
0.48		0.54 ± 0.04	7.4	112
1.50		1.57 ± 0.07	4.5	105
60.0		60.6 ± 5.56	9.1	101
400		378 ± 27.2	7.1	94.5

Table 2 Extraction recovery and matrix effect (three QC samples) in rat plasma (n = 6).

Compound	Nominal concentration (ng/mL)	Extraction recovery		Matrix effects	
		%Mean ± SD	CV%	%Mean ± SD	CV%
Quetiapine	1.50	76.9 ± 5.8	7.6	89.8 ± 3.7	4.1
	60.0	73.9 ± 8.0	10.8	87.2 ± 2.5	2.9
	400	71.4 ± 4.9	6.9	86.7 ± 3.8	4.4
	Mean	74.1 ± 2.7	3.7	87.9 ± 1.6	1.9
IS	20.0	67.3 ± 3.6	5.4	80.7 ± 3.2	4.0

Table 3 Stability and dilution integrity data of QUE in rat plasma.

Stability	Nominal concentration (ng/mL) (n = 6)	Mean ± SD (ng/mL)	Precision (CV %)	Accuracy (%)
Bench top (6 h)	1.50	1.60 ± 0.06	3.7	106
	400	374 ± 15.0	4.0	93.5
Freeze thaw (3 cycle)	1.50	1.55 ± 0.10	6.5	104
	400	372 ± 25.0	6.0	93.0
In injector (48 h)	1.50	1.63 ± 0.06	3.5	108
	400	428 ± 11.37	2.7	107
60 days at −80 °C	1.50	1.43 ± 0.13	8.8	95.0
	400	365 ± 29.12	7.9	91.2
Dilution integrity	225	218.2 ± 12.9	5.9	97.0
	450	433.0 ± 12.2	2.8	96.3

ether, diethyl ether, dichloromethane, ethyl acetate, chloroform and n-hexane are commonly used as extracting solvents for liquid–liquid extraction. But here considering environment, health, and safety concerns (EHS); ethyl acetate, tertiary butyl ethyl ether and 2-butanol were tried as extracting solvents. Finally, ethyl acetate produced better result compared to others and was used as extracting agent in sample preparation. Liquid–liquid extraction is also employed in sample preparation of previous green bio-analytical assay (Cheregi et al., 2013), but 1-octanol was used as an extracting agent there followed by direct injection of large volume of the organic layer for analysis.

The developed UHPLC-MS/MS method using environment friendly organic solvents allow a fast and reliable determination of QUE plasma concentrations using 200 µl of plasma with a calibration range of 0.45–500 ng/mL and LLOQ 0.45 ng/mL. The method was successfully validated according to the current effective official guidelines for bioanalytical method validation and was successfully applied in a pharmacokinetic interaction study of QUE in rats. Since this assay was developed and validated in rat plasma samples, so it cannot be directly used for application in clinical samples or therapeutic drug monitoring. At least, partial validation in terms of

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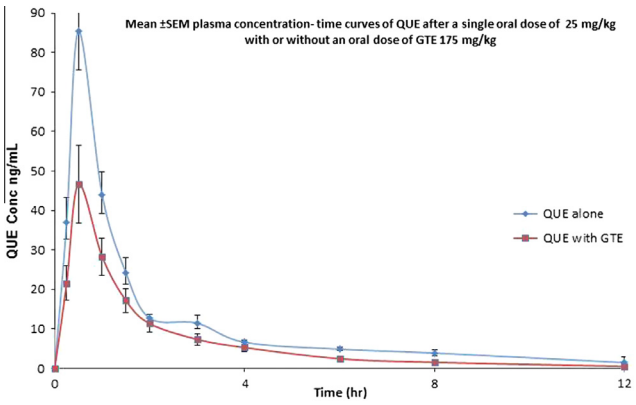

Figure 4 Mean ± SEM plasma concentration-time curves of QUE after a single oral dose of 25 mg/kg with or without an oral dose of GTE 175 mg/kg.

precision and accuracy is necessary before application in human samples.

5. Conclusions

A green bioanalytical UHPLC-MS/MS method was developed and validated for the rapid determination of QUE in rat plasma using environment friendly and low toxic organic solvents. The developed method was successfully applied in pharmacokinetic study of QUE with GTE in rats. Replacement of traditional organic modifiers such as methanol or acetonitrile with ethanol did not produce any impact on peak symmetry and elution of both analyte and IS. Capacity of withstanding high back pressure (can handle the high viscosity characteristic of ethanol) along with AQUITY BEH column, 50×2.1 mm, i.d. 1.7 μm (leads to reduction of solvent required for separation and shortening of analysis time) enable UHPLC-MS/MS, the suitable equipment for green bioanalytical assay. Therefore, the present study shows the advantage of using less harmful solvents which protect environment from hazardous and polluting chemicals. Moreover, laboratory staffs are less exposed to toxic and corrosive organic solvents.

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