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QUALITATIVE AND QUANTITATIVE EVALUATION OF DIFFERENT PHARMACEUTICAL PRODUCTS CONTAINING QUETIAPINE: BRAND VERSUS GENERIC

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ABSTRACT

Atypical antipsychotics are not only used for symptoms of schizophrenia, but also for the treatment of Behavioral Psychological Symptoms in Dementia. This work is focused on developing a selective, fast method for the quantitative and qualitative determination of quetiapine in Seroquel® 25mg and the generic Quetiapine 25 mg tablets. Analyses were conducted by dissolving tablets in a suitable solvent (water/ACN 50:50) and measuring the quetiapine amount using a UPLC-PDA instrument. Dissolution test, disintegration test, and thermal analysis were conducted with specific instrumentation according to the European Pharmacopea. Quantitative analysis showed that the difference between the two pharmaceutical products was about 0.055%, which is not statistically significant; qualitative analysis highlights a slight difference about the disintegration time (1 minute) and the dissolution, caused by different excipients. This is further confirmed by the thermal analysis (Differential Scanning Calorimetry). It is possible to conclude that no differences were identified among the reconstituted samples of the two different products containing quetiapine.

KEY WORDS: dementia, quetiapine, generics, UHPLC-PDA, disintegration, dissolution.

INTRODUCTION

Quetiapine, chemically {2-[4-(dibenzo[b,f] [1,4]thiazepin-11-yl)piperazin-1-yl]ethoxy}ethanol, is an atypical antipsychotic agent. The molecular formula of quetiapine fumarate (Seroquel®) is $(C_{21}H_{25}N_3O_2S)_2 \cdot C_4H_4O_4$ (Figure1) and its molecular weight is 883.1 g/mol. Its logP is 2.8 and the pKa is 3.3 , 6.8 and its melting point is 172-173 °C[1]. The dibenzothiazepine structure with two basic nitrogen atoms is responsible for its higher solubility under acidic conditions (HCl 0.1 M). At a pH above 4, the water solubility is poor; towards pH 2, an increase in solubility is noticeable. However, below pH 2, solubility is decreasing owing to the ion effect[2].

Its active human plasma metabolite, norquetiapine, interacts with a broad range of neurotransmitter

receptors. Quetiapine and norquetiapine exhibit affinity for brain serotonin (5HT2) and dopamine D1- and D2- receptors. It is this combination of receptor antagonism with a higher selectivity for 5HT2 relative to D2- receptors, which is believed to contribute to the clinical antipsychotic properties and low extrapyramidal side effect (EPS), liability of quetiapine compared to typical antipsychotics. Quetiapine is indicated for treatment of schizophrenia and bipolar disorder[3] but these characteristics make quetiapine well tolerated and effective in patients who are particularly susceptible to EPS effects, including the elderly, adolescents, and those with preexisting dopaminergic pathologies, such as Alzheimer's and Parkinson's disease[4].

Quetiapine is extensively metabolized by the hepatic cytochrome P 450 (CYP) system and primarily by the CYP3A4 isoenzyme[5].

The recommended quetiapine dosage for reducing positive symptoms of schizophrenia is in the 150-750 mg daily range and for reducing negative symptoms is of 300 mg daily[6]. For management of psychosis in the elderly, lower doses of quetiapine (50-150 mg daily) may be more appropriate[7,8]. Dosage adjustment is not necessary in patients with renal impairment but should be used with caution in patients with known hepatic impairment, especially during the initial dosing period[3].

The main drug's side effects are insomnia, somnolence, headache, dry mouth, constipation, asthenia, agitation, dizziness, postural hypotension, ALT increased, and dyspepsia[9]. Quetiapine is available as fumarate salt such Seroquel® and Quetiapine generic 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg tablets, in immediate- and extended-release formulations[9].

By definition, a generic product is considered equivalent with the innovator brand product and needs to demonstrate the same qualitative and quantitative composition in active substance, pharmaceutical form and bioequivalence with the reference product after a single dose[10]. Different excipients, color agents, flavors, and preservatives are allowed. Generics may also differ in characteristics such as color, size, shape, and release mechanism[11]. Anyway therapeutic equivalence between brand and generics is not necessarily guaranteed because no clinical efficacy data are required for generics[12], but only bioequivalence data are requested. Simple bioequivalence data may not suffice to ensure comparable clinical efficacy and safety, especially in psychiatric diseases because different excipients in generic formulations may affect absorption and bioavailability[13].

In our clinical setting there was the need for clinicians to highlight or exclude any quantitative difference between the two pharmaceutical products, Seroquel®25 mg tablets versus Quetiapine 25 mg generic tablets, because some patients and their caregivers reported changes in the symptoms with an apparent decrease of the therapeutic efficacy.

The purpose of the present study was to make a comparison between two different product containing quetiapine, Seroquel® 25 mg tablets versus Quetiapine 25 mg generic tablets, to exclude any significative differences about intrinsic characteristics of these medicinal products.
This work focused on developing a selective, fast method using UPLC-PDA advanced technique and to apply analytical instrumentation (dissolution test, disintegration test and Differential Scanning Calorimetry) to the quantitative and qualitative determination of quetiapine in these different pharmaceutical products.
To our knowledge in the literature there is no data about assay determination of this drug in pharmaceutical preparations as a comparison between branded drug and generics and few analytical methods are available. For example Pucci V. et al[14] determined quetiapine amount in tablets using a spectrophotometric method and a capillary zone electrophoretic (CZE) method, Bagade S.B et al[15] and Prasanth V.G. et al[16] developed and validated an UV-Spectrophotometric method for determination of quetiapine fumarate in different dose tablets. Also Krishna S.R. et al[17] described a stability indicating HPLC method for related substances of quetiapine fumarate and Belal F. et al[18] performed a similar method with the application to human plasma.

MATERIAL AND METHODS

Chemicals and Reagents

The pharmaceutical formulations Seroquel® 25 mg coated tablets [AstraZeneca-Milan, Italy] and Quetiapina gen. 25 mg coated tablets [TEVA-Milan, Italy] were purchased from respective pharmaceutical companies. Seroquel® tablets batches: N°22311, N°32308, N°45304 and Quetiapine TEVA tablets batches: N°0440713, N°0501013, N°0491013.
Inactive ingredients (excipients), in the tablet core are: povidone, calcium hydrogen phosphate dihydrate, microcrystalline cellulose, lactose monohydrate, sodium carboxymethyl-starch, magnesium stearate. In the coated tablet: hypromellose, macrogol, titanium dioxide. Same

excipients are present in the generic quetiapine tablets, except for macrogol. Colloidal anhydrous silica, triacetin and lactose monohydrate are presents only in the quetiapine generic tablets core.
Quetiapine powder (as reference material) was purchased from Sigma-Aldrich [Batch N° 033M4712V]. Acetonitrile UPLC grade was purchased from J.T. Baker (Deventer, Holland) and deionized water was produced using a Milli-DI system coupled with a Synergy 185 system by Millipore (Milan, Italy). Orthophosphoric acid and potassium dihydrogen phosphate were purchased from Sigma–Aldrich (Milan, Italy).

Sample preparation
Preliminary analyses were considered to find the suitable solvent to completely dissolve quetiapine tablets[19]. Various types of solvents were studied to optimize tablet dissolving: A= water/methanol (50:50 v/v), B= water/acetonitrile (50:50 v/v), C= methanol (100 %) and D acetonitrile (100 %).

Eight tablets, four of Seroquel® and four of Quetiapine gen., were crushed to fine powder.
A 2.5 mL volume of different solvent (A,B,C,D) was added, the mixture was agitated on a tumbler for 15 min, and then centrifuged at 4000 rpm for 10 min. The supernatant (1 mL), with a final concentration of 10 mg/mL of quetiapine, was used for preparing the working solutions, diluting (dil.1:1000) the stock mixture with two different diluents: water/acetonitrile 50:50, the same diluents used for standard solution, and acid phosphate buffer (KH_2PO_4 10 mM pH 1.3, ortophosphoric acid). These two different diluents were used to choose the most appropriate one to the valid method. The same procedure (dilution) was performed for every work session. Twelve solutions were prepared utilizing four tablets, two for every pharmaceutical product and for every batch. The solutions were visually inspected for precipitate, color change, and tested for pH. The pH value of each sample was measured at time 0, using a Orion model SA520 pH meter (Milan, Italy).

The solutions were assayed using an ultra-performance liquid chromatography (UPLC-PDA) method similar to previously adopted by Prasanth V.G. et al[16]. Samples were analyzed in double.

UPLC assay and chromatographic condition
The chromatographic separations were performed using an Aquity UPLC® H-Class System (Waters-Milan, Italy) composed by a Quaternary Solvent Manager (QSM), Sample Manager (SM) and a Photo-Diode Array (PDA) detector. System control, data collection, and data processing were accomplished using Waters Empower-2 chromatography data software.

The gradient run was performed as shown in Table 1. According to robustness criteria[20], the mobile phase, composed of Solvent A (KH_2PO_4 10 mM with *orto*-phosphoric acid, pH 3.2) and Solvent B (acetonitrile 100%), was prepared and pH was measured at the beginning of each chromatographic analysis. Autosampler temperature (Sample Manager) was set at 10°C. Column temperature was also studied and we found that 35°C temperature was appropriate, to perform a peak far from solvent front, with a good resolution and shape. The reversed-phase column was a BEH C_{18} ACQUITY UPLC 1.7 µm 2.1x50 mm with pre-column, and the flow rate was set at 0.4 mL/min. The PDA detector was set at 247 nm and the injection volume for each sample was 1 µL. Gradient run time was 3 minutes (Table 1). Each run was also monitored with a scan wavelength (range 200-600 nm) to identify possible degradation products.

Standard Solution and Calibration Curve
Standard solution was prepared by dissolving quetiapine pure powder with known purity (reference material), in diluent (water/acetonitrile 50:50) to obtain a final concentration of 10 mg/mL. Calibration curve, and the linearity assessment of the method, were evaluated at five concentrations (STD1: 0.625 mg/mL; STD2: 1.25 mg/mL; STD3: 2.5 mg/mL; STD4: 5.0 mg/mL; STD5: 10.0 mg/mL).

Linearity
Linearity was demonstrated using the five calibration levels described above, and the linear regression method was used for data evaluation. The peak area of the standard compound was plotted against quetiapine tablets concentration. Linearity was described by the linear correlation coefficient (R^2).

Precision
The precision of the system was determined on two replicate injections of every sample preparation, including standard preparation and tablets solutions. They were analyzed using the same proposed method. Samples were analyzed the same day to obtain the repeatability. Every sample was prepared twice.

Stability Studies
The International Conference on Harmonization (ICH) guideline[20] entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substances.

Forced degradation studies were performed to differentiate degradation products (related to drug products) from those that are generated from non-drug products[21] in the two formulations, Seroquel® and Quetiapine generic, and to understand the chemical properties of molecules supporting the suitability of the proposed analytical method.

Degradation of quetiapine in aqueous solution is influenced by pH, temperature, concentration, and involves degradation products by oxidation, dealkylation and dimer formation[22].

At first, samples were exposed to thermal degradation (100 °C for 45 min), acid hydrolysis: fuming HCl 37%, 2 µl (pH=1.3) and base hydrolysis: NaOH 5M, 2 µl (pH=12). All samples were then analyzed with the proposed method. This preliminary analysis showed that on acidic conditions quetiapine shows higher signal and concentration values than quetiapine standard solution. So, we hypothesized that heat allowed the evaporation of the drug solution and made it more concentrated. Therefore we decided to perform degradation studies without heating samples and use an HPLC-MS instrument to exclude degradation products, not visible with UPLC-PDA because in the same range wavelength of quetiapine.

Disintegration test

Tablets were tested according to Ph. Eur./USP with apparatus A at a temperature of 37±2 °C. Distilled water was the dissolution medium used (800 mL) because in the tablet's core there is lactose monohydrate, which is soluble in water. Disintegration test was performed using 6 tablets for every pharmaceutical products and time was noted[23].

Dissolution test

A dissolution test simulates the availability of an active substance and allows the prediction of the time for complete release of the material from the dosage form[24].

Dissolution was tested using the Ph. Eur./USP rotating basket apparatus (200 rpm) and distilled water (800 mL) at 37 °C, as dissolution medium. Quetiapine tablets, two for every pharmaceutical product, were analyzed *in-vitro* drug release study in water for 25 minutes. Samples of 5 mL volume were withdrawn at preselected time points: 5,10,15,20,25 min. The withdrawn volume was replaced immediately with water dissolution medium.

The amount of drug dissolved was determined using UPLC-PDA with the proposed method previously described.

Differential Scanning Calorimetry

Differential Scanning Calorimetry **(**DSC) is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. It was carried out using a Perkin Elmer DSC/7 (Perkin-Elmer, CT, USA) equipped with a TAC 7/DX instrument controller. The instrument was calibrated with indium for melting point and heat of fusion. A heating rate of 10°C/min was employed in the 25-200 °C temperature range. Analysis of the two pharmaceutical products was performed in triplicate under nitrogen purge.

Quantitative assay

Quantitative assay was performed by relating the areas of the chromatographic peaks of the samples analyzed (tablets) with those of points of the calibration curve (amount) at time 0. The analysis was conducted on 3 batches of the different pharmaceutical products.

Selection of Batches

This study was performed using 3 different batches, as reported above in Chemicals and Reagents chapter, for each medicinal product, as requested by the European guidelines on stability studies[20].

RESULTS AND DISCUSSION

Various types of solvent for the dilution (1:1000) of the samples were studied to optimize the method. We chose phosphate buffer KH_2PO_4 10 mM, pH 1.3/ACN 50:50 because quetiapine peak had a good resolution and a high chromatographic signal under acidic conditions, as shown in Table 2. The retention time of quetiapine was 0.65 minutes (Figure 2). Chromatographic separation between the drug peak and solvent front was allowed by choosing the appropriate gradient and the column temperature (35 °C).

The small volume of injection (1µL) was chosen to avoid saturation process of detector. Data from calibration curve and linearity response, as previously reported, showed the goodness of this decision. Moreover, manufacturer guide of autosampler (Waters), in particular in the Specifications of the Waters Sample Manager, indicates between 0.1 to 10.0 µL as standard for the injection volume range with a relative standard error <0.15% for six replicate injections. This data was supported by our continuous repeated injections results.

Linearity
Linearity was demonstrated using five calibration levels for quetiapine standard solution with referent material (pure powder), which performed a good confidence on analytical method with respect to linear range at the range 10-0.625 mg/mL . The response was linear for quetiapine standard concentration, and correlation coefficient (R^2) was also found greater than 0.998.

Precision
Assay precision showed that quetiapine solutions had a relative standard deviation of less than 1%.

Stability Studies
The assay to highlight quetiapine degradation products at room temperature is shown in Figure 3 and confirmed that heat allows evaporation of drug solution and makes it more concentrated. So this assay was performed with samples without heat. Assays of degraded quetiapine samples confirmed a net decrease of drug concentrations caused by acid and base hydrolysis. The percent of degradation was calculated from the peak area of degradation standard and degraded test solution. Under acidic pH, the decrease in drug concentration was up to 30% of initial concentration; under alkaline pH, the decrease was about 50%.

Disintegration test
Disintegration time for six tablets was found to be 5.28 minutes for quetiapine generic tablets and 6.30 min for Seroquel® tablets. Since this was less than 15 min, it indicates that disintegration time is within the specification limit[25]. The slight difference about disintegration times is due to the different excipients of the two pharmaceutical products, especially macrogol, which is present in the Seroquel tablets coat, and prolongs disintegration time.

Dissolution test
The dissolution profile of the different tablets is shown in Figure 4. At time intervals between 5 and 25 minutes, trend of drug concentration (mg/mL) is similar for the two products. Therefore, the amount of quetiapine released over time is similar and achieves a complete dissolution after 15 minutes. Data about kinetics of the dissolution process was in accordance with the disintegration time of the different tablets. This assay confirms that the presence of macrogol plays a role in the disintegration time of the tablets, as previously investigated by Radosław K. et al[26].

Differential Scanning Calorimetry
This analysis was performed to evaluate if different excipients of Seroquel® and Quetiapina gen. could provide different characteristics to the molecule, compared to the pure substance, i.e. whether there could be different interactions between the drug and the excipients in the different tablets. The quetiapine melting point, as reference material (pure substance), is 180 °C. The DSC thermograms demonstrated the same thermal behavior (Figure 5), however, the Seroquel melting point is shifted by about 10 degrees compared to that of the generic product and the pure substance. This difference was in agreement with the results of the two previous assays.

Quantitative assay
The UPLC-PDA method developed was sensitive and specific for the quantitative determination of quetiapine fumarate. This method was applied for the estimation of drug in different pharmaceutical products. Quetiapine fumarate tablets from two different manufacturers (brand and generic) were evaluated for the amount of the active ingredient.
An overview of the results (mean values observed) is shown in the Figures 6. Percentage deviation between quetiapine fumarate amount in Seroquel® and in Quetiapine generic was 0.055%, not statistically significant. One of three batches showed a variation between two pharmaceutical products of about 0.14%; for the other two batches there were no differences; however this variability between different batches of the same drug is allowed[27].
None of the tablets ingredients (excipients) interfered with the analytic peak. The spectrum of the drug extracted from the tablets corresponded with that of standard quetiapine fumarate (pure powder) showing the purity of quetiapine fumarate peak in different tablets.

CONCLUSION

A gradient UPLC-PDA method was successfully developed for the estimation of quetiapine in different pharmaceutical product: brand versus generic drug. The method validation results proved that it was selective, accurate, linear, and robust. The short run time (3.0 min) allowed a rapid determination of the drug. Moreover, this method was applied to establish *in-vitro* dissolution profile of Seroquel and generic tablets. The development and validation of a method for determination of quetiapine in pharmaceutical dosage form were previously investigated by K T.et al[22]. Furthermore, other authors investigated quetiapine fumarate content in tablets, for example Kiran B. V. et al[19] developed an HPLC with internal standard method.
To our knowledge, there isn't any study comparing brand and generic products for quetiapine; this is the novelty of this study.

Compared to previous methods, our chromatographic assay provides some technical and cost advantages, for example extraction simple dilution and injection in UPLC-PDA system.

The results of our study showed negligible differences (not statistically significant) between Seroquel® and Quetiapine generic tablets. Considering the UPLC data, disintegration test, dissolution test, and DSC it is possible to conclude that there are no differences between the reconstituted samples of the two different products containing quetiapine. The real equivalence between brand and generic quetiapine tablets 25 mg, in term of efficacy and efficiency, needs to be verified in clinical studies.

The entry of generic medicines in the market is certainly an opportunity for saving in healthcare management but, in some cases, it may compromise its clinical efficacy compared to the brand drug. Market authorization of generic equivalents only requires the documentation of bioequivalence with branded counterparts in healthy subjects, using one lot of branded product without considering country differences[28,29]. A small bioequivalence study of a generic quetiapine in healthy male volunteers was performed by Mahatthanatrakul W. et al[30]. This study highlighted that generic product was bioequivalent to Seroquel in terms of both rate and extent of absorption.

Since issues regarding drug-drug interactions are not addressed by bioequivalence studies, potential risks cannot be excluded. Unpredictable blood concentrations also expose patients to a higher risk of concentration-dependent drug-drug interactions[13]. In addition, different excipients and impurities may cause allergic reactions or even intolerance[31][32]. As reported by Das Arun K. et al[23], different excipients in quetiapine tablets in the immediate release formulation, may affect the release of the active ingredient. In this context, therapeutic drug monitoring should be a valid instrument to monitor quetiapine plasmatic concentrations to personalized therapy, to minimize drug-drug interactions, and to verify therapeutic adherence, especially for generic products.

In conclusion, these results demonstrate areas of concern in the pharmaceutical quality of generic products, such as the content of active substances (quantitative evaluation) and disintegration times, dissolution profile, and thermal behavior (DSC) (qualitative evaluation).

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Table 1: Gradients program for elution

Time	Flow rate (mL/min)	% Solvent A	% Solvent B
Initial	0.4	70	30
1.0	0.4	20	80
2.0	0.4	20	80
3.0	0.4	70	30

Solvent A= KH_2PO_4 10 mM, pH 3.2 (ortophosphoric acid)
Solvent B= Acetonitrile 100%

Table 2: Summary of solvent used for the samples dilution (Seroquel® and Quetiapine gen. tablets) to optimize the method

Solvent for dilution (1:1000)	Observation/Remarks
KH_2PO_4 pH= 3.2/ACN 50:50	Good peak resolution, very low signal (0.070)
KH_2PO_4 pH= 2.2/ACN 50:50	Good peak resolution, low signal (0.075)
KH_2PO_4 pH= 1.3/ACN 50:50	**Good peak resolution, high signal (0.100)**

ACN: Acetonitrile

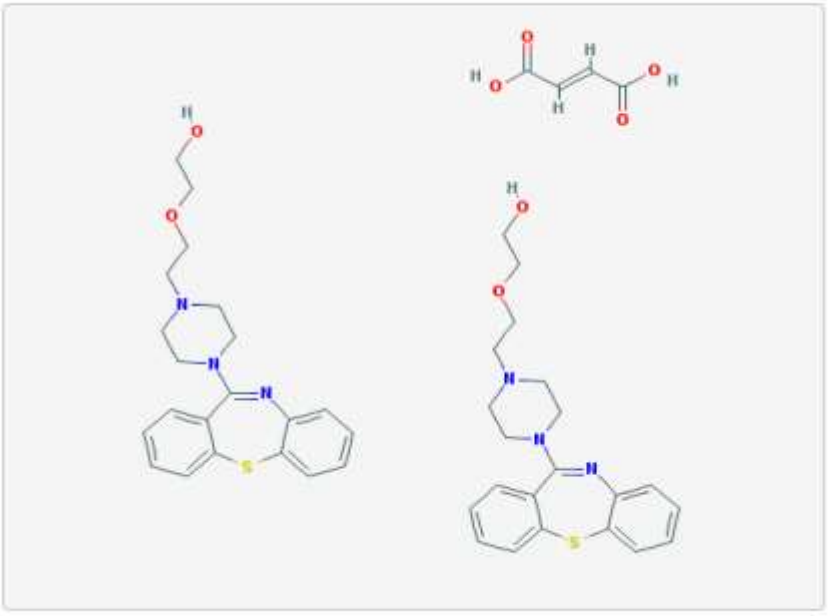

Figure 1. Molecular structure of quetiapine/quetiapine fumarate

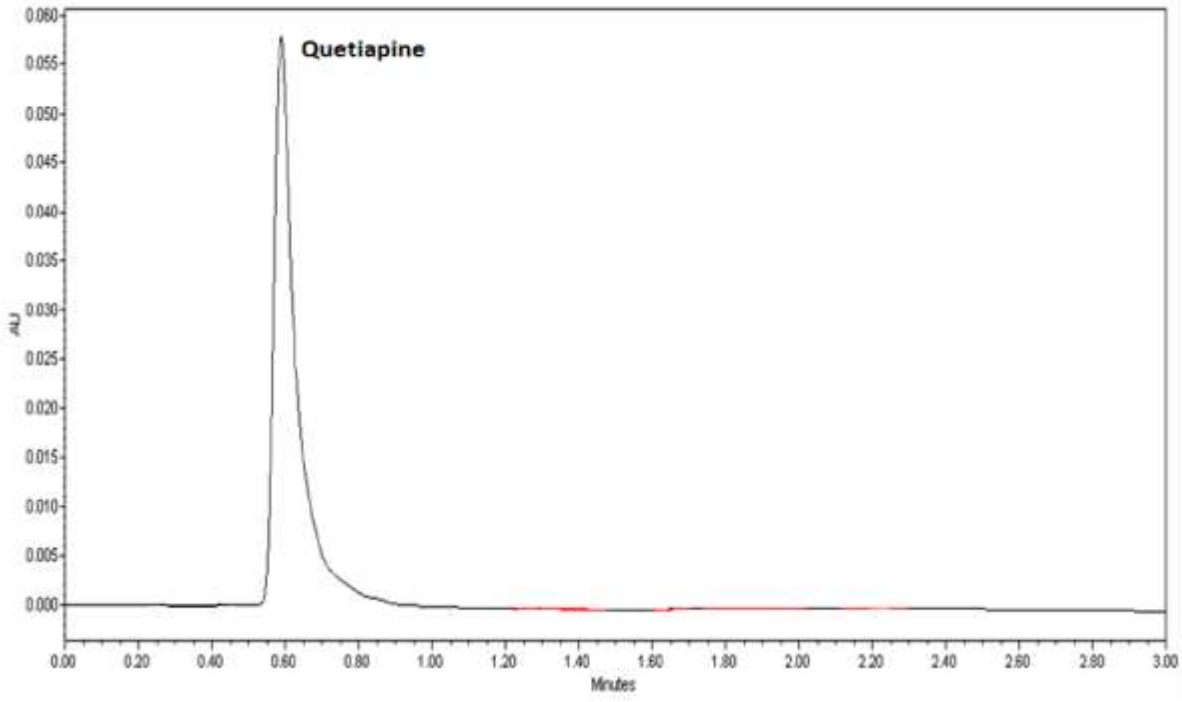

Figure 2. Quetiapine chromatogram (T_R= 0.65 min., 200-247 nm)

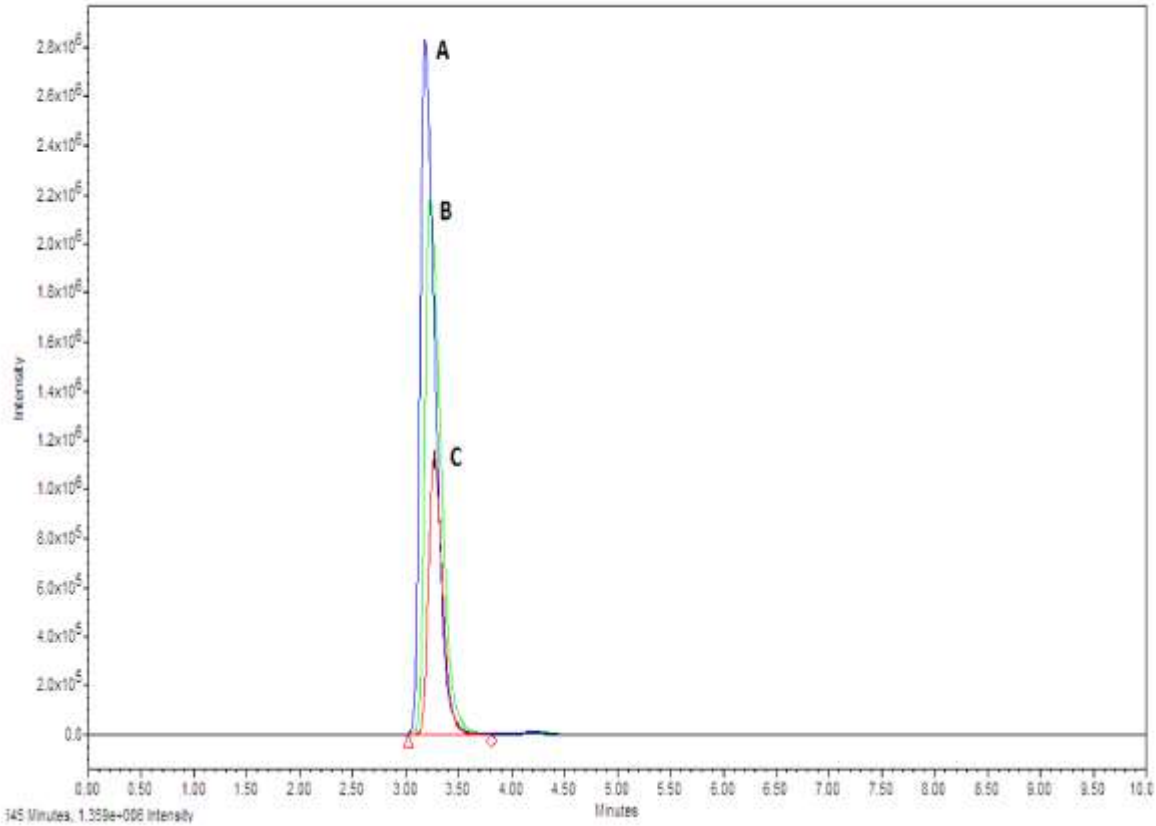

Figure 3. Quetiapine Stress Test: A. fresh stability sample, B. stress degradation sample: fuming HCl 37% and C. stress degradation sample: NaOH 5M.

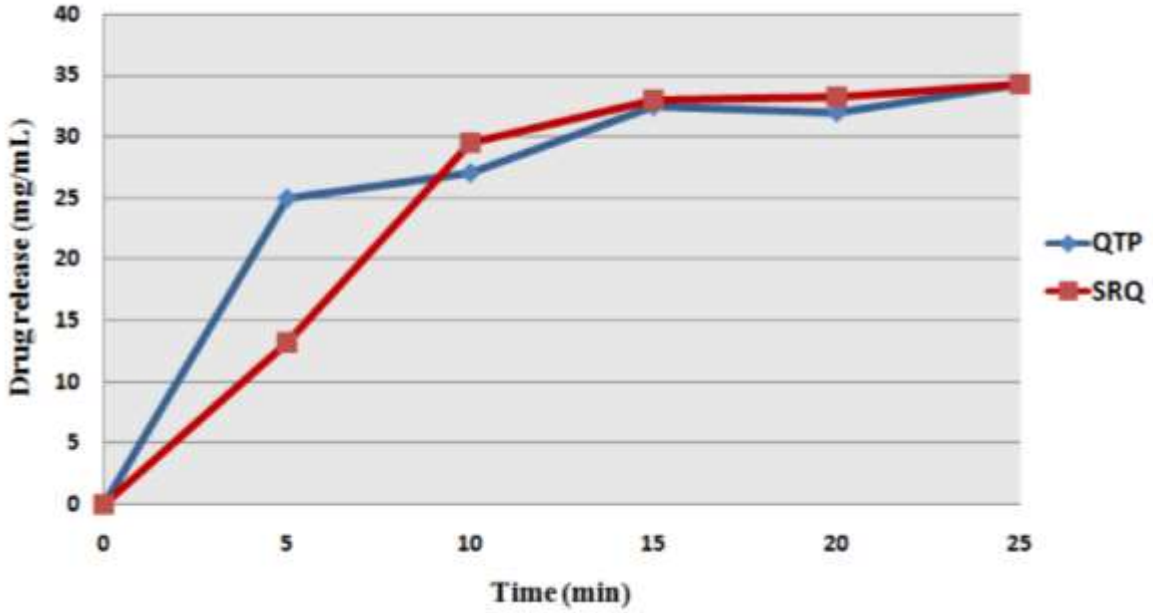

Figure 4. Dissolution Tests results: Seroquel® vs Quetiapine gen.

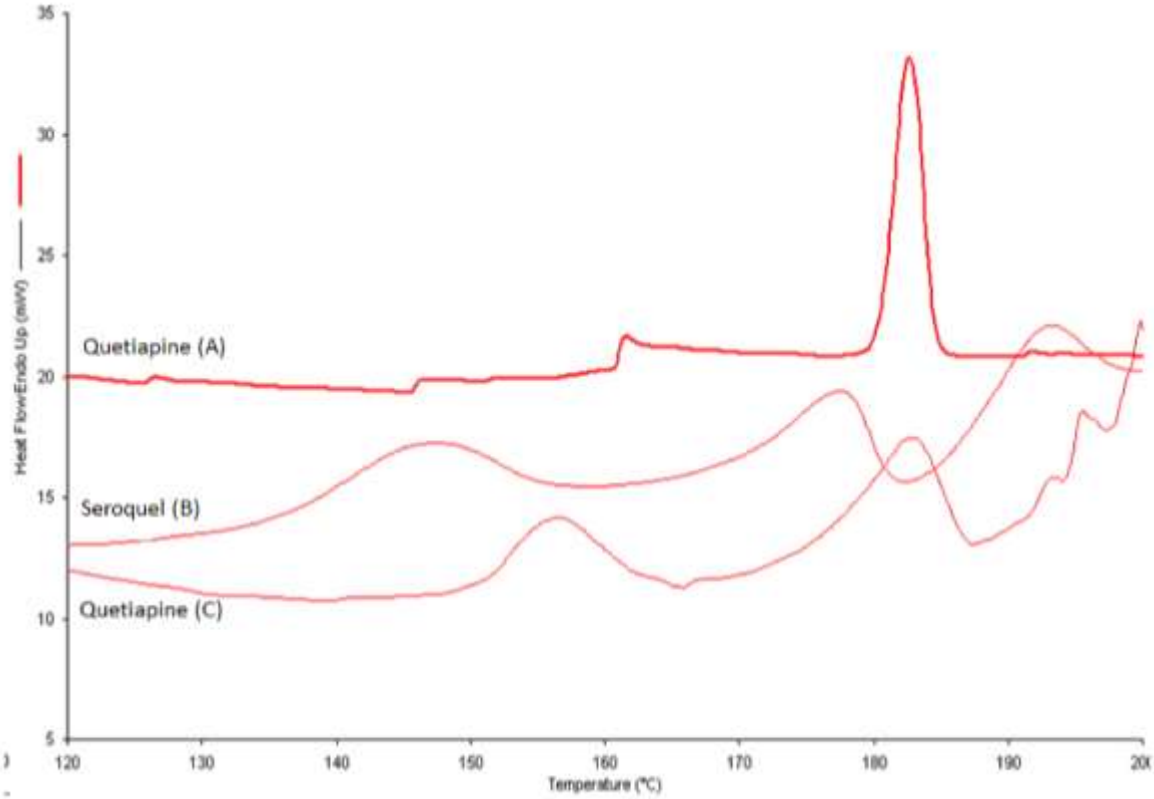

Figure 5. Differential Scanning Calorimetry (DSC): Quetiapine pure powder (A), Seroquel® (B) and the Quetiapine generic tablets (C).

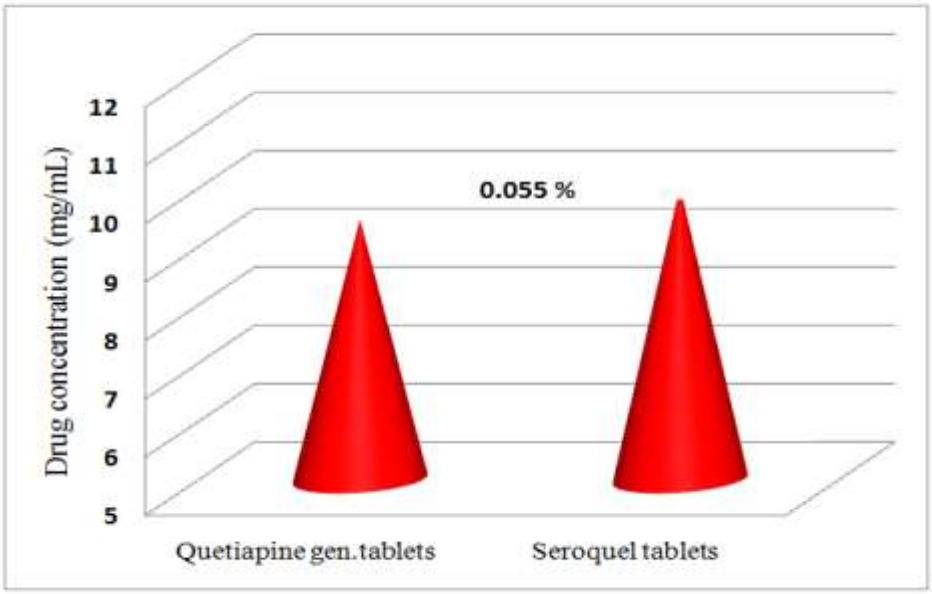

Figure 6. Quantitative analysis (mean values observed): percentage deviation between quetiapine fumarate amount in Seroquel® and in Quetiapine generic was 0.055%, not statistically significant.

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