Validated HPLC and Thin Layer-Densitometric Methods for Determination of Quetiapine Fumarate in Presence of its Related Compounds

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Abstract

Two chromatographic methods were developed for determination of quetiapine fumarate in presence of three related compounds; namely quetiapine N-oxide, des-ethanol quetiapine and quetiapine lactam, in pure form and pharmaceutical preparation. The first method depended on densitometric thin layer chromatography where the separation was achieved using silica gel 60F254 plates as stationary phase and toluene:1,4-dioxane:dimethylamine (5:8:2, v/v/v) as a mobile phase. The second method utilized the reverse phase high performance liquid chromatographic technique, using C18 column and methanol: acetonitrile: phosphate buffer (pH 5.3) in a ratio (19:40:41, v/v/v) as a mobile phase. The flow rate was 1 mL min⁻¹ and UV-detection was at wavelength 220 nm. The validation parameters of the developed methods were calculated and the results obtained were statistically compared with those of the HPLC manufacturer method.

Keywords: HPLC; Quetiapine fumarate related compounds; Thin layer-densitometry

Introduction

Quetiapine Fumarate (QTF) is a psychotropic agent belonging to a chemical class of dibenzothiazepine derivatives. The chemical designation is 2-[(4-dibenzo[b,f](1,4)thiazepin-11-yl-1-piperazinyl)ethoxy]-ethanol fumarate (2:1) (salt). It is present in tablets as the fumarate salt. It is a white or almost white powder, moderately soluble in water and soluble in methanol and 0.1N HCl. It is used to treat psychosis associated with Parkinson’s disease and chronic schizophrenia [1], synthesized originally by Warawa and Migler [2] and can be used alone or in combination with other medications to treat schizophrenia and bipolar disorder [3,4]. Several methods have been reported for the determination of QTF in bulk powder, pharmaceutical preparations and biological samples. These included UV-Visible spectrophotometric methods [5-9], HPTLC methods [10-12], capillary zone electrophoretic method [13], voltammetry [14] and HPLC methods [15-17].

The evaluation of QTF related compounds has been an important issue that was recommended by regulatory agencies. Few papers were published for estimation of QTF in presence of its related compounds including; a GC method for determination of QTF in presence of 2-(2-chloroethoxy)ethanol and n-methyl-2-pyrrolidinone [18] and four HPLC methods, where it was determined in presence of piperazine (PI), quetiapine lactam (QL) and dibenzothiazepine piperazinyl ethanol hemifumarate compounds in the first method [19], and in presence of two related compounds quetiapine N-oxide (QO) and (QL) in the second one [20], also in presence of (QL),(PI) and Des-ethanol quetiapine (DQ) in the third one with long run time (18 mins) [21], finally with (PI), (DQ) and quetiapine dimer using binary gradient mode in the fourth one [22]. And a RP-UPLC method for determination of QTF in presence of (QO), (PI), (DQ), S-oxide and quetiapine dimer using complicated binary gradient elution with linearity range (62.5-187.5 µg mL⁻¹) [23]. No method of the mentioned HPLC methods determined QTF in presence of this combination of related compounds: QO, DQ and QL in a simple isocratic isocratic elution mode for the mobile phase with good resolution between the four proposed components compared.

So far to our knowledge, only one stability-indicating HPTLC method for the determination of QTF in presence of its degradation products has been reported [24] but no TLC-densitometric method was reported for the determination of QTF in the presence of its related compounds.

The aim of our work was to develop more sensitive HPLC method with higher throughput using a mobile phase in a simple isocratic mode of elution and also to develop a selective, accurate, reproducible HPLC-densitometric method which has the advantage of being of low cost and a faster technique when compared to HPLC method for determination of QTF in presence of the three related compounds; QO, DQ and QL (Figure 1) in raw material and in dosage form.

Materials and Methods

Instruments

Camag TLC scanner (Camag, Muttenz, Switzerland) operated with winCATS software version 3.15, Linomat IV autosampler (Camag, Muttenz, Switzerland). 100-µL Camag microsyringe (Hamilton, Bonaduz, Switzerland), Precoated silica gel aluminium Plates 60 F254 (20 cm × 20 cm) 250 μm thicknesses (E. Merck, Darmstadt, Germany).

Agilent 1200 series chromatographic system equipped with quaternary pump, microvacuum degasser, thermostatic column compartment and variable wavelength UV–VIS detector was used. Sample injections were made through an Agilent 1200 series
by ultrasound sonicator for 50 minutes just prior to use. The analysis and filtered by vacuum filtration through 0.45 μm filter and degassed in a ratio of (19:40:41, v/v/v). The mobile phase was freshly prepared on TLC-plate was 180 mm.

saturated with the mobile phase for 15 min and the developing distance 2 cm from the bottom edge. The chromatographic chamber was pre-
microsyringe. Spots were applied 1.5 cm apart from each other and plates using Camag Linomat ΙV applicator along with 100 μL Camag sheets (E. Merck, Darmstadt, Germany). Samples were applied to the precoated 20 × 20 cm silica gel 60 F
Chromatographic conditions

HPTLC-densitometry: The mobile phase was selected as mixture of toluene, 1,4-dioxane and dimethylamine in a ratio of (5:8:2, v/v/v). The spots were scanned at 225 nm. The average peak area was calculated for each concentration of QTF and was plotted versus their concentration to obtain the calibration graph and the regression equation was then computed.

HPLC: Aliquots (0.1-3.0 mL) from QTF standard working solution were transferred into a series of 10 mL volumetric flasks and then diluted with methanol to obtain a concentration range of (1.0-30.0 μg mL⁻¹). Twenty microliters were injected for each concentration in triplicate and chromatographed using the HPLC conditions described above. The average peak area was calculated for each concentration of QTF and was plotted versus their concentration to obtain the calibration graph and the regression equation was then computed.

Application to pharmaceutical preparation (Seroquel tablet)

Seroquel tablet was individually weighed to get the average weight of the tablet. A sample of the powdered tablets, claimed to contain 100.0 mg of drug was transferred separately to 100 mL volumetric flask, sonicated for 20 minutes with 50 mL methanol, then the volume was brought to 100 mL with the same solvent and filtered to prepare stock solution, having a concentration of (1.0 mg mL⁻¹). A working standard solution (0.1 mg mL⁻¹) was prepared by further dilution of the stock solution with methanol.

In HPTLC-densitometric method, aliquot of 5.0 mL was transferred from the prepared stock solution into 10 mL volumetric flask, completed to the volume with methanol. Then, 10 μL were applied onto HPTLC plates (n=5). While, in HPLC method, aliquot of 1.0 mL was transferred from the prepared working solution into10-mL volumetric flask and the volume was completed with methanol. Then, 20 μL from this dilution were injected (n=5).

The general procedure described above for each method was followed. Then, the concentration of QTF in its pharmaceutical preparation was calculated.

Application of standard addition technique

To check the validity of the proposed chromatographic methods, standard addition technique was applied by adding known amounts of the pure drug to the previously analyzed tablets. In HPTLC-
HPTLC chromatogram of QO, DQ, QTF and QL mixture of
toluene:1,4-dioxane:dimethylamine in a ratio of (5:8:2, v/v/v). The optimum mobile phase used was a mixture of methanol, acetonitrile and phosphate buffer (pH adjusted to 6.4) in a ratio of (20:50:30, v/v/v) was used as a mobile. The peaks of QTF and QL overlapped. Increasing the ratio of buffer to (20:40:40, v/v/v) resulted in increasing the retention time of QTF and QL peaks to more than 10 minutes and there was still some overlapping between QTF and QL peaks. By decreasing pH to 5.3, the separation between QTF and QL overlapped peaks was resolved. The optimum mobile phase used for the simultaneous determination of QTF and its related compounds was a mixture of methanol, acetonitrile and phosphate buffer (pH adjusted to 5.3) in a ratio of (19:40:41, v/v/v). The average retention time (R_t)± SD, for 6 replicate injections for QTF, QO, DQ and QL were found to be 3.61 ± 0.04, 2.53 ± 0.01, 3.20 ± 0.02 and 4.24 ± 0.04, respectively. A typical chromatogram of bank injection and QTF and its related compounds are shown in Figures 3 and 4, respectively.

Calibration graph was obtained by plotting the average peak area against concentration of QTF (µg mL⁻¹). Linearity range was found to be 1.0-30.0 µg mL⁻¹ using the following regression equation:

\[ A = 684.53C - 278.13 \]
\[ r=0.9997 \]

Where A represents the average peak area, C is the concentration in µg mL⁻¹ and r is the correlation coefficient.

Method validation

International conference on Harmonization (ICH) guidelines [29] for method validation was followed for validation of the suggested methods.

Linearly: The linearity of the proposed methods for determination of QTF was evaluated by analyzing a series of different concentrations of the drug in the range of 1.0-11.0 µg spot⁻¹ for HPTLC-densitometric method and 1.0-30.0 µg mL⁻¹ for HPLC method. Each concentration was repeated three times, in order to provide information on the variation in peak areas values among samples of the same concentration. Linear relationships were obtained by plotting the drug concentrations against the average peak area obtained for each concentration of QTF. The validation parameters for the regression equation of the adopted

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chromatographic methods are given in Table 1.

Accuracy: The accuracy of the proposed method was checked by the analysis of five different concentrations of authentic samples in triplicate. The concentrations of QTF were calculated using the regression equation for each of the two proposed chromatographic methods and then the mean percentage recovery and the relative standard deviation (RSD%) were calculated, as shown in Table 1 indicating the satisfactory accuracy of the proposed methods.

Precision: Three replicates of each concentration were analyzed on the same day to determine the intra-day precision of the methods. To confirm the inter-day precision, three replicates of each concentration were analyzed at three separate days using the developed chromatographic methods and calculating RSD%. Results in Table 1 indicate satisfactory precision of the proposed methods.

Detection and quantitation limits: According to (ICH) recommendation [29] the limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ=k SD/slope of the response, where k=3.3 for LOD and 10 for LOQ and QL (10.0 μg mL\(^{-1}\)) mixture, using methanol:acetonitrile:phosphate buffer (pH=5.3) (19:40:41, v/v/v) at 220 nm.

Accuracy (Recovery% ± RSD)

Precision (RSD%, n=9)

| Validation parameters | HPTLC-densitometry (LOQ) | HPLC (LOQ) |
|-----------------------|--------------------------|------------|
| Linearity range       | 1.0-11.0 μg spot\(^{-1}\) | 1.0-30.0 μg ml\(^{-1}\) |
| Correlation coefficient (r) | 0.9997 | 0.9998 |
| Slope                 | 684.53                  | 68.61 |
| Intercept             | -278.13                 | -2.24 |
| LOD                   | 0.29 μg spot\(^{-1}\)   | 0.20 μg ml\(^{-1}\) |
| LOQ                   | 0.90 μg spot\(^{-1}\)   | 0.56 μg ml\(^{-1}\) |
| Accuracy (Recovery% ± RSD) | 100.36 ± 0.41       |        |
| Precision (RSD%, n=9) | Intra-day: 1.65          | 0.36 |
|                      | Inter-day: 1.93          | 1.93 |

Table 1: Characteristic parameters for the regression equations of the proposed methods, for determination of QTF.
Table 2: Results obtained for the analysis of laboratory prepared mixtures containing different ratios of the intact QTF with its related compounds, by the proposed chromatographic methods.

| Ratio(QTF:QO:DQ:QL)       | HPTLC (%Recovery* of QTF) | HPLC (%Recovery* of QTF) |
|---------------------------|---------------------------|--------------------------|
| 10:0.01:0.01:0.01         | 99.76                     | 100.16                   |
| 10:0.02:0.02:0.02         | 99.34                     | 100.18                   |
| 10:0.03:0.03:0.03         | 101.20                    | 101.22                   |
| 10:0.06:0.06:0.06         | 100.65                    | 100.17                   |
| 10:0.1:0.1:0.1            | 101.12                    | 100.79                   |
| 10:0.2:0.2:0.2            | 101.75                    | 101.15                   |
| Mean % ± SD               | 100.63 ± 0.91             | 100.61 ± 0.50            |

*Average of three determinations.

Table 3: System suitability test results of the developed chromatographic methods for determination of QTF.

| Parameters                              | HPTLC-densitometry | HPLC | Reference values |
|-----------------------------------------|---------------------|------|------------------|
|                                         | QO      | DQ    | QTF  | QL  | QO      | DQ   | QTF  | QL  |
| Le., min (HPLC)/R2 (HPTLC)              | 0.01    | 0.08  | 0.70 | 0.81| 2.53    | 3.20 | 3.61 | 4.24|
| Tailing factor (T)                      | 0.90    | 0.76  | 0.93 | 1.02| 0.73    | 0.96 | 1.01 | 1.18|
| Plates number (N)                       | 3040    | 4608  | 4688 | 3991| N>2000  |      |      |     |
| Height equivalent to theoretical plate (HETP; cm plate⁻¹) | 0.049   | 0.032 | 0.031| 0.037|        |      |      |     |
| Resolution (R2)                         | 16      | 10.47 | -    | 1.6 | 2.37    | 2.03 | -    | 2.64|

The smaller the value, the higher the column efficiency

R2 ≥ 2
**Conclusion**

The quality of pharmaceutical products is of vital importance for patient safety. The presence of related compounds or impurities may affect the efficacy and safety of pharmaceuticals. In this work, sensitive, accurate, precise and reproducible HPTLC-densitometric and HPLC methods were developed for the determination of QTF in the presence of three related compounds (QO, DQ and QL) which may be present in the pharmaceutical products in bulk powder and pharmaceutical formulation. The HPTLC-densitometric method has the advantage of being of low cost and is a faster technique when compared to HPLC. The proposed HPLC method offers high sensitivity, short run time and the use of isocratic elution mode for the mobile phase with good resolution between the four proposed components compared with the reported HPLC methods. Thus both methods can be used for routine analysis and quality control labs.

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