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Different stability-indicating methods for the determination of quetiapine fumarate

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ABSTRACT

Five accurate, precise and sensitive methods were developed for the determination of Quetiapine fumarate (QF) in presence of its degradation product. Method (A) was based on second derivative spectrophotometry ^{2}D, then measuring the amplitude at 266 nm. Method (B) was depended on measuring the peak amplitudes of the first derivative of the ratio spectra ^{1}DD at 244, 285 and 344 nm. Method (C) was based on the separation of the drug from its oxidized degradation product followed by densitometric measurement of the intact drug band at 302 nm. The separation was carried out on Fluka TLC plates of silica gel 60 F_{254} using ethyl acetate/ methanol /10% ammonium hydroxide (8.5:1:0.5 by volume) as a mobile phase. Method (D) was high performance liquid chromatographic one, separation by HPLC was achieved using an Eclipse XDB C18RP-column and methanol / water in a ratio of 80:20 (v/v) as a mobile phase. The flow rate was 1ml/min. Method (E) was based on the reaction of QF with P-Chloranilic acid (P-CA) in presence of its degradation product. Linearities were obtained in concentration range 10 – 60 μg/ml in case of methods (A) and (B). While in case of methods (C) and (D), linearities were obtained in concentration range of 4-20 μg/band and 1-20 μg/ml respectively. While in method (E), the linearity was achieved in the range of 40-400 μg/ml. In method (A), the mean percentage recovery was 99.9 ± 0.6%. In method (B) the mean percentage recoveries were 99.9 ± 0.4%, 99.2 ± 0.8% and 99.4 ± 0.8% at 244, 285 and 344 m respectively. Method (C) showed percentage mean recovery of 99.9 ± 0.7%, while in methods (D) and (E) were 99.8 ± 0.7% and 99.9 ± 0.4% respectively. The degradation product was obtained in oxidative stress condition, separated, and identified by IR and MS spectral analysis, from which the degradation product was confirmed, and the degradation pathway was suggested. The five methods were found to be specific for QF in presence of different concentration % of its degradation product. The proposed methods were validated according to ICH guidelines Q2 (R1). The five proposed methods were successfully applied for the determination of QF in Seroquel tablets. Statistical comparison between the results obtained by these methods and that obtained by the manufacturer method for the determination of the drug was done, and it was found that there was no significant differences between them.

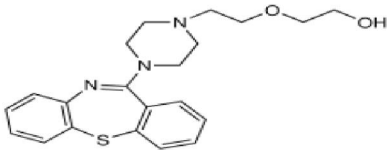
KEYWORDS

Second derivative;
Derivative ratio;
TLC;
HPLC;
P-chroranilic acid;
Quetiapine fumarate;
Degradation product;
Stability indicating methods.

INTRODUCTION

Quetiapine fumarate (QF) is 2-[2-(4-Dibenzo [*b*, *f*] [1, 4] thiazepin-11-yl-1-piperazinyl) ethoxy] ethanol fumarate (2:1)[1].

Quetiapine fumarate is a dibenzothiazepine a typical antipsychotic drug[1]. It is reported to have affinity for serotonin (5-HT_2), histamine (H_1), and adrenergic (α_1 and α_2) receptors as well as dopamine D_2 receptors[2]. Quetiapine is used in the treatment of schizophrenia and of mania associated with bipolar disorder and tried as an antipsychotic in patients with Parkinsonism[1].



Structure of quetiapine[1] molecular formula: ($C_{21}H_{25}N_3O_2S)_2$, $C_4H_4O_4$; Mol. weight 883.1

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No stability indicating methods have been cited in the literature for determination of QF in presence of its degradation product. Few reported methods were concerned with determination of QF alone involving titrimetric method[3] and HPLC methods[4-6]. Therefore this work presented a method for preparation of degradation of QF that was produced under the stress conditions mentioned in the International Conference on Harmonization (ICH) guidelines Q2 (R1)[7] followed by five stability indicating methods for its determination.

EXPERIMENTAL

Instrumentation

UV-Visible spectrophotometer (Unicam UV 300, Kyoto, Japan). Infrared (IR) spectrophotometer - Vector 22 (Bruker Optics, Ettlingen, Germany). MS spectrometer instrument-GC-MS-QPI000EX quadruple spectrophotometer equipped with electron multiplier detector (Shimadzu-Japan), Shimadzu CS-9301 PC dual wavelength flying spot scanning densitometer (Shimadzu, Tokyo, Japan), thin layer chromatographic plates precoated with silica gel 60 F_{254} 10×20 cm were obtained from Fluka (Switzerland, Germany) and HPLC, (Agilent 1200, Waldbronn, Germany) consists of Agilent quaternary pump, equipped with variable wavelength detector, 20 µl volume injection loop and Eclipse XDB C18RP-column (150×4.6 mm i.d.).

Reference sample

Quetiapine fumarate (QF)- Pure sample was kindly supplied by AstraZeneca pharmaceuticals, batch number 4009430021, (Cairo, Egypt). Its purity was found to be 99.3 ± 0.5% according to manufacturer method[3].

Pharmaceutical formulation

Seroquel tablets - Manufactured by AstraZeneca (Cairo, Egypt). batch number 100120 labeled to contain 25 mg of QF in each tablet.

Degraded sample (QF deg)

QF (400 mg) was dissolved in 30 ml methanol. Then 20 ml aqueous solution containing 2 gm potassium permanganate and 82.4 mg disodium hydrogen phosphate was added to the QF solution. The solution was sonicated for 30 minutes, and then extracted with chloroform (3x20ml). The aqueous layer was acidified with about 40 ml dilute sulphuric acid and 4 ml methanol and warmed to decompose excess potassium permanganate and to dissolve the formed manganese dioxide, The aqueous layer was further extracted with chloroform (2x20ml) and rendered just alkaline (pH~7.5) using aqueous dilute sodium hydroxide, then re-extracted with 20 ml chloroform. The organic extracts were combined together and evaporated under vacuum till dryness. The residue was quantitatively transferred into a 100-ml volumetric flask then dissolved in methanol and the volume was completed to the mark with the same solvent and filtered[8,9]. The degraded solution was tested for complete degradation by TLC using ethyl acetate/ methanol /10% ammonium hydroxide (8.5:1:0.5 by volume) as a mobile phase. Only one spot was observed not corresponding to QF. The degraded solution was used to suggest the methods.

From QF degraded solution, 20 ml was taken, evaporated to dryness and the residue was dissolved in 10 ml methanol. The solution was applied as separate compact bands 20 mm from the bottom of TLC plates using 25 µl Hamilton micro syringe. The plates were developed using the previously mentioned mobile phase. The degradate was extracted with methanol. The or-

ganic solution was evaporated and subjected to IR and MS spectral scans.

Reagents

Methanol, ethyl acetate, methanol and ammonium hydroxide 10% and acetonitrile,; (Merck, Munich, Germany). P-chloranilic acid (P-CA) ($2.5Mx10^{-3}$), potassium dihydrogen phosphate and potassium permanganre; (Sigma, Aldrich, USA). Methanol HPLC grade; (Prolabo, VWR International, West Chester, PA).

Standard solutions

(a) QF stock standard solutions

- (1mg/ml) in methanol for methods A,B,C and D, prepared by dissolving 0.1 gm of QF in a 100- ml volumetric flask then the volume was completed to the mark with methanol.
- (4 mg/ml) in methanol for method E, prepared by dissolving 0.4 gm of QF in a 100-ml volumetric flask then the volume was completed to the mark with methanol.

(b) QF working standard solutions

- (0.2 mg/ml) in methanol for methods A and B, prepared by transferring 20 ml of QF stock standard solution (1 mg/ml) in a 100- ml volumetric flask then the volume was completed to the mark with methanol.
- (0.02 mg /ml) in the mobile phase for method D, prepared by transferring 2 ml of QF stock standard solution (1 mg/ml) in a 100-ml volumetric flask then the volume was completed to the mark with mobile phase.
- (2 mg/ml) in acetonitrile for method E, prepared by transferring 50 ml of QF stock standard solution (4mg/ml) in a 100-ml volumetric flask then the volume was completed to the mark with acetonitrile.

Degradation solutions

(a) QF stock degraded solutions (QF deg)

- (1mg/ml) in methanol for methods A, B, C and D, prepared as mentioned under degraded sample.
- (4mg/ml) in methanol for method E, prepared as mentioned under degraded sample.

(b) QF working degraded solutions (QF deg)

- (0.2 mg/ml) in methanol for methods A and B, prepared by transferring 20 ml of QF stock degraded solution (1 mg/ml) in a 100- ml volumetric flask then the volume was completed to the mark with methanol.
- (0.02 mg /ml) in the mobile phase for method D, prepared by transferring 2 ml of QF stock degraded solution (1 mg/ml) in a 100-ml volumetric flask then the volume was completed to the mark with mobile phase.
- (2 mg/ml) in acetonitrile for method E, prepared by transferring 50 ml of QF stock degraded solution (4 mg/ml) in a 100-ml volumetric flask then the volume was completed to the mark with acetonitrile.

Laboratory prepared mixtures containing different ratios of QF and its degradation product

(a) Methods (A) and (B)

Aliquots (2.7 - 0.3 ml) of QF working standard solution (0.2 mg/ml) equivalent to (540 – 60 µg) were accurately transferred into a series of 10-ml volumetric flasks. Aliquots (0.3 – 2.7 ml) of QF deg working solution (0.2 mg/ml) equivalent to (60 – 540 µg) were added, the volume was completed with methanol to prepare mixtures containing 10 - 90 % of the degradation product.

(b) Method (C)

Aliquots (4.5 - 0.5 ml) of QF stock standard solution (1 mg/ml) equivalent to (4.5 – 0.5 mg) were accurately transferred into a series of 5-ml volumetric flasks. Aliquots (0.5 – 4.5 ml) of QF deg stock solution (1 mg/ml) equivalent to (0.5 – 4.5 mg) were added to prepare mixtures containing 10 - 90 % of the degradation product.

(c) Method (D)

Aliquots (9 - 1 ml) of QF working standard solution (0.02 mg/ml) equivalent to (180 – 20 µg) were accurately transferred into a series of 10-ml volumetric flasks. Aliquots (1 – 9 ml) of QF deg working solution (0.02 mg/ml) equivalent to (20 – 180 µg) were added to prepare mixtures containing 10 - 90 % of the degradation product.

(d) Method (E)

Aliquots (1.8 – 0.2 ml) of QF working standard solution (2 mg/ml) equivalent to (3.6 – 0.4 mg) were accurately transferred into a series of 10-ml volumetric flasks. Aliquots (0.2 – 1.8 ml) of QF deg working solution (2 mg/ml) equivalent to (0.4 – 3.6 mg) were added, the

volume was completed with acetonitrile to prepare mixtures containing 10 - 90 % of the degradation product.

Construction of the calibration graphs for method (A) [^{2}D]

Aliquots (0.5, 1, ……..3.0 ml) of QF working standard solution (0.2 mg/ml) were transferred into a series of 10-ml volumetric flasks. The volume was then completed to mark with methanol.

The zero-order spectra were recorded then ^{2}D spectra of each solution were obtained with $\Delta\lambda = 4$ nm and scaling factor 10. The peak amplitudes at 266 nm were measured. Linear calibration curve was constructed relating the peak amplitudes at 266 nm to the corresponding concentrations of QF and the corresponding regression equation was computed.

Construction of the calibration graph for method (B) [^{1}DD]

Alternatively, the zero-order spectra of the prepared solutions for method (A) were divided by the spectrum of 10 µg/ml of its degradation product and the ratio spectra were obtained. Then the first derivative of the ratio spectra ^{1}DD with $\Delta\lambda = 4$ and scaling factor 10 were obtained. The peak amplitudes of the first derivative of the ratio spectra at 244, 285 and 344 nm ($^1DD_{244,}$ $^1DD_{285,}$ $^1DD_{344}$) were measured. Linear calibration curves were constructed relating the peak amplitudes of the first derivative of the ratio spectra at 244, 285 and 344 nm to the corresponding concentrations of QF and the regression equations were computed.

Construction of the calibration graph for method (C) [TLC]

Aliquots (1, 2 ….., 5 ml) of stock standard solution (1mg/ml) were transferred into a series of 5-ml volumetric flasks. The volume was then completed to mark with methanol. Twenty µl of each solution were spotted on TLC plates, using Camag Linomat autosampler with micro syringe (25 µl), the following mentioned chromatographic conditions were adopted. The scanning profile for QF was obtained. The calibration curve relating the integrated area under the peak to the corresponding concentration was constructed and the regression equation was computed.

- **Chromatographic conditions**

The plates were first washed and developed with the mobile phase by mixing ethyl acetate/methanol/ammonium hydroxide10% (8.5:1.0:0.5 by volume), then activated for 15 minutes by placing in an oven at 100^{o}C before use. For detection and quantitation, 20 µl of prepared standard solutions were applied as separate compact bands 20 mm apart and 15 mm from the bottom of the plates. The chromatographic tank was saturated with the mobile phase for 15 minutes. The plates were developed (over a distance of 15 cm) in an ascending manner, air-dried, and the plates were scanned under the following conditions:

- Source of radiation: deuterium lamp
- Scan mode: zigzag mode
- Slit dimension: 3 mm x 0.45 mm
- Scanning speed: 20 mm/s
- Output: chromatogram and integrated peak area
- Wave length: 302 nm
- Photo mode: Reflection
- Swing width: 10 mm

Construction of the calibration graph for method (D) [HPLC]

Aliquots (0.5, 2, 4, 6, 8, 10 ml) of working standard (0.02 mg/ml) were transferred into a series of 10-ml volumetric flasks The volume was then completed to mark with the mobile phase. Using 25 µl syringe, 20 µl volume of each solution was injected in triplicates into the liquid chromatograph under the following chromatographic conditions:

- Column: Eclipse XDB C18 RP (150 mm x 4.6 mm I.D)
- Mobile phase: Methanol / Water (80:20 v/v), it was filtered using 0.47 µm Teflon membrane filter and degassed. The samples were also filtered using 0.47 µm Teflon filters.
- Flow rate: 1ml/ min
- Wavelength: 302 nm
- Column temperature: room temperature

The relative peak area (using external standard of 20 µg/ml of QF) was plotted against the corresponding concentration of QF and the regression equation was computed.

Construction of the calibration graph for method (E) [P-CA]

Aliquots (0.2, 0.4, 0.8, 1.2, 1.6, 2 ml) of working standard (2 mg/ml) were transferred into a series of 10-ml volumetric flasks then 2 ml of P-CA (2.5 M x

10^{-3}) was added and the volume was completed to mark with acetonitrile. The reaction was allowed to proceed at room temperature. The absorbance of the purple color was measured at 525 nm and the calibration curve was plotted relating the absorbance intensity to the corresponding concentration of QF and the regression equation was computed.

Application of the proposed methods to the analysis of laboratory prepared mixtures of intact drug and its degradation product

(a) Methods (A) and (B)

The absorption spectra of the laboratory prepared mixtures were recorded. Then the procedures were completed as described in subsection of *Construction of the calibration graphs*. The concentrations of QF were calculated by substituting in the corresponding regression equations.

(b) Method (C)

Aliquots equivalent to twenty µl from the prepared mixtures in of the laboratory prepared mixtures were spotted on TLC plates and the procedure was completed as described in subsection of *Construction of the calibration graph* and the concentrations of QF were calculated from the corresponding regression equation.

(c) Method (D)

Aliquots equivalent to twenty µl from the prepared mixtures in of the laboratory prepared mixtures were injected into the liquid chromatograph and the procedure was completed as described in subsection of *Construction of the calibration graph* and the concentrations of QF were calculated from the corresponding regression equation.

(d) Method (E)

The absorption intensities of the laboratory prepared mixtures were recorded. Then the procedure was completed as described in subsection of *Construction of the calibration graphs*. The concentrations of QF were calculated by substituting in the corresponding regression equation.

Application of the proposed methods to the analysis of QF in pharmaceutical preparation

(a) Methods (A) and (B)

Five tablets were weighed accurately and powdered. An amount of powder equivalent to 25 mg of QF was accurately weighed into a 25-ml measuring flask and extracted with 15-ml methanol in an ultrasonic bath for 30 minutes, diluted to volume with the same solvent and filtered. Suitable dilutions were made using methanol to prepare tablet solution containing 0.2 mg/ml. One ml of the solution was accurately transferred to a 10-ml volumetric flask and was diluted to volume with methanol. Then the procedures were completed as described under *Construction of the calibration graphs*. The concentrations of QF were calculated by substituting in the corresponding regression equations.

(b) Method (C)

An amount of powder equivalent to 25 mg of QF was accurately weighed into a 25-ml measuring flask and extracted with 15-ml methanol in an ultrasonic bath for 30 minutes, diluted to volume with the same solvent and filtered. Two ml of the solution was accurately transferred to a 5-ml volumetric flask and was diluted to volume with methanol. Then the procedure was completed as described under *Construction of the calibration graphs*. The concentrations of QF were calculated by substituting in the corresponding regression equation.

(c) Method (D)

The dosage form was prepared as mentioned above. Then suitable dilution was made using mobile phase to prepare tablet solution containing 0.02mg/ml. Three ml of the solution was accurately transferred to a 10-ml volumetric flask and was diluted to volume with the mobile phase. Then the procedure was completed as described under *Construction of the calibration graphs*. The concentrations of QF were calculated by substituting in the corresponding regression equation.

System suitability tests

Capacity factor (K'), Selectivity factor (α),Resolution (Rs), Tailing factor (T) and Column efficiency were calculated.

(d) Method (E)

An amount of powder equivalent to 100 mg of QF was accurately weighed into a 25-ml measuring flask and extracted with 15-ml methanol in an ultrasonic bath for 30 minutes, diluted to volume with the same solvent and filtered. Suitable dilution with acetonitrile was made to prepare solution of concentration 2mg/ml. 0.4 ml of

the solution was accurately transferred to a 10-ml volumetric flask and was diluted to volume with acetonitrile. Then the procedure was completed as described under *Construction of the calibration graphs*. The concentrations of QF were calculated by substituting in the corresponding regression equation.

RESULTS AND DISCUSSION

The stability of QF was studied according to ICH guidelines Q2 (R1)[7] for:

(a) Stress Acid and Alkaline: 1 M HCl/1 M NaOH for 16 hrs, 2 M HCl/2 M NaOH for 16 hrs and 5 M HCl/5M NaOH for 4 hrs and for 5 hrs.
(b) Oxidative Condition: b_1: 3% H_2O_2 for 2 hrs, 4 hrs, 6 hrs and for 10 hrs. b_2: 10% $KMnO_4$
(c) Thermal Degradation: At 100°C in an oven for 2 hrs, 4 hrs, and fo r 6 hrs.
(d) Photo Degradation: UV lamp producing UVB radiation for 6 hrs, 8hrs, and for 10 hrs.

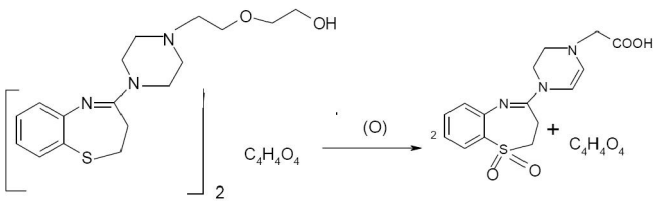
QF shows highly stability to acidic, alkaline, photo, and thermal conditions. The compound undergoes oxidative degradation into sulphoxide derivative. So the interest was focused on the oxidation of the cited drug using $KMnO_4$. The degradation process under the previously mentioned conditions was followed using TLC. It is a single component which confirmed by TLC as indicated by the appearance of one spot after complete degradation.

Since this work was concerned with the development of stability-indicating methods for the determination of QF, the degradation product was prepared in laboratory as mentioned in the section of *degraded sample*. The structure of the isolated acid degradation product was confirmed using IR and MS spectroscopy, (Figures 1-3).

Figure (1) showed the IR spectrum of intact QF, which is characterized by sharp peak at 3316 cm^{-1} of OH alcoholic, and absence of bands at 1650-1700 cm^{-1} of C=O carboxylic. On the other hand the IR spectrum of QF deg (Figure 2) revealed broad band at 3600-2800 cm^{-1} of O-H carboxylic, band at 1734 cm^{-1} of C=O carboxylic and band at 1656 cm^{-1} of C=N and bands at 1226 and 1155 cm^{-1} of sulfone. Mass spectrum of QF deg (Figure 3) showed the Mass of $C_{19}H_{17}N_3O_4S$ at M/Z 383. This finding suggested the following degradation pathway indicated that degradation product of QF has the following structure:

Methods (A) and (B)

Derivative spectrophotometry has been first suggested during the last decades and soon become a well established technique for the assay of drugs in mixtures and in pharmaceutical dosage forms. The first and second derivative methods can be applied for the determination of pure drug in presence of its degradation products by selecting a wavelength where contribution of



the degradation products is zero (or almost zero) while the drug to be determined has a reasonable value[10-12].

The zero-order spectra of QF and its degradation product show an overlap, (Figure 4), that prevents the use of direct spectrophotometric analysis of the drug in the presence of its degradation product. In an attempt to resolve this overlap, derivative method was applied. First-derivative failed to resolve this overlap (Figure 5). Upon examining the second-derivative spectra of the drug and its degradation (Figure 6), it is noticed that QF can be determined at 266 nm, where the degradation product has no contribution and shows zero reading.

Linearity of the peak amplitudes of the relationship of the second derivative curves to the corresponding concentrations of the drug was examined at the selected wavelength. The proposed method was found to be valid in the range of 10 – 60 µg/ml, and the regression equation was computed and found to be:

$$^2D_{266} = 0.003C + 0.001 \qquad r = 0.9997$$

Where $^2D_{266}$ is the peak amplitude at 266 nm respectively, C is the concentration of QF in µg/ml and r is the correlation coefficient.

The proposed method, was successfully applied for the determination of the drug in pure powder form with mean percentages recovery of 99.9 ± 0.6%,

(TABLE 1).

Alternatively derivative ratio spectra method is a successful and widely used application of derivative spectrophotometric technique for the resolution of some binary or ternary pharmaceutical mixtures[13-15].

Therefore derivative ratio spectrophotometry can also be applied. The zero-order absorption spectra of QF were divided by the spectrum of 10 µg/ml of its degradation product. This gave the best compromise in terms of sensitivity, repeatability, and signal-to noise ratio. The first derivative of the ratio spectra at 244, 285 and 344 nm with $\Delta\lambda = 4$ and scaling factor 10 ($^1DD_{244,}$ $^1DD_{285,}$ $^1DD_{344}$) were obtained. The peak amplitudes were measured at 244, 285 and 244 nm, (Figures 7,8).

The linearity between the concentrations of the drug and peak amplitudes at 244, 284 and 344 nm were studied. Linear relationships were obtained in the range of (10 – 60 µg/ml) of QF, and the regression equations were computed and found to be:

$^1DD_{244}$ = 0.0718C – 0.218 r = 0.9994

$^1DD_{285}$ = 0.1136 C – 0.314 r = 0.9996

$^1DD_{344}$ = 0.0738C – 0.216 r = 0.9991

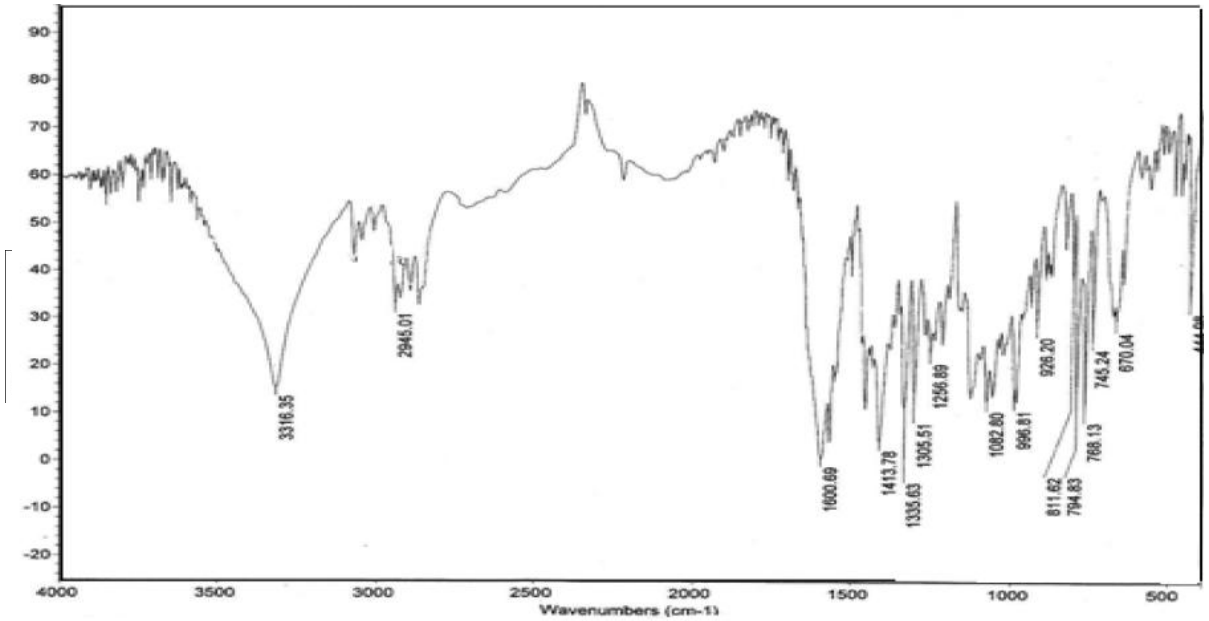


Figure 1 : IR spectrum of intact QF

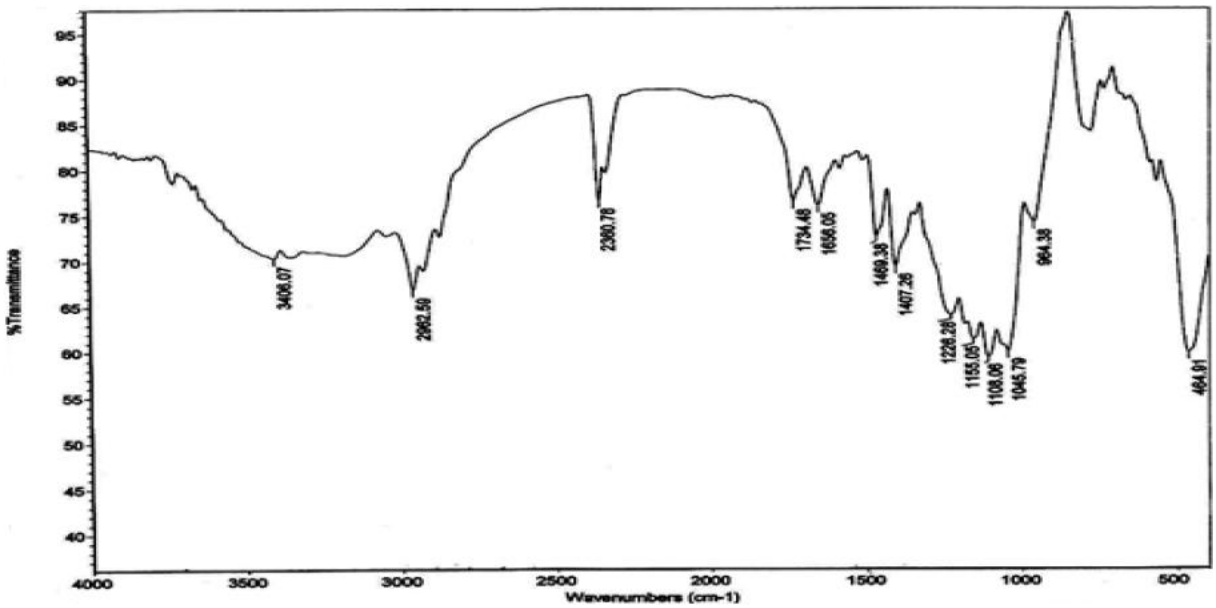


Figure 2 : IR spectrum of QF degraded

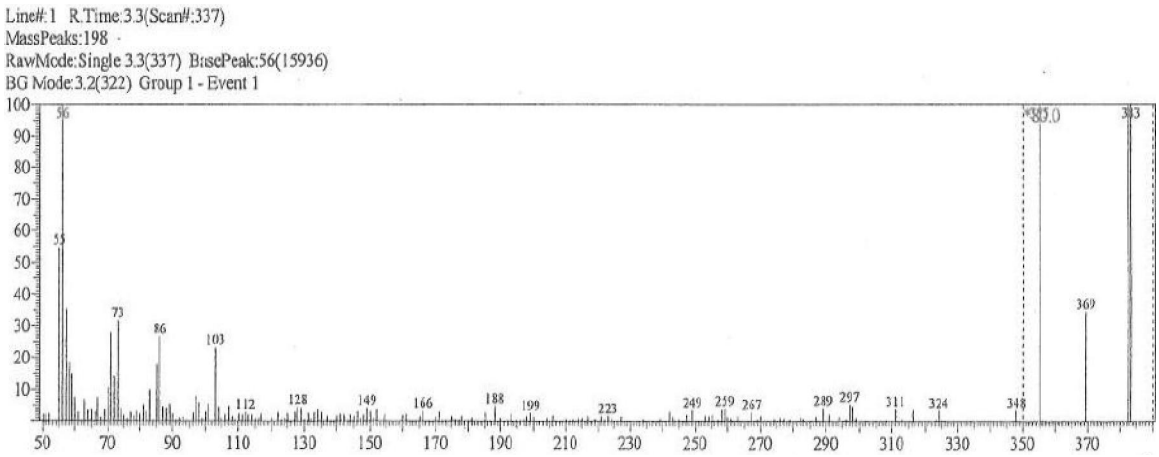


Figure 3 : Mass-spectrum of QF degraded

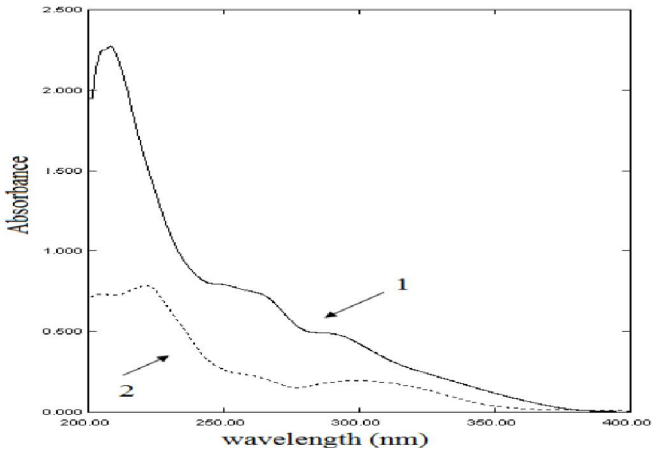


Figure 4 : Zero order absorption spectra of QF (——) and QF degraded (……) in methanol, concentration of each was 30 µg/ml

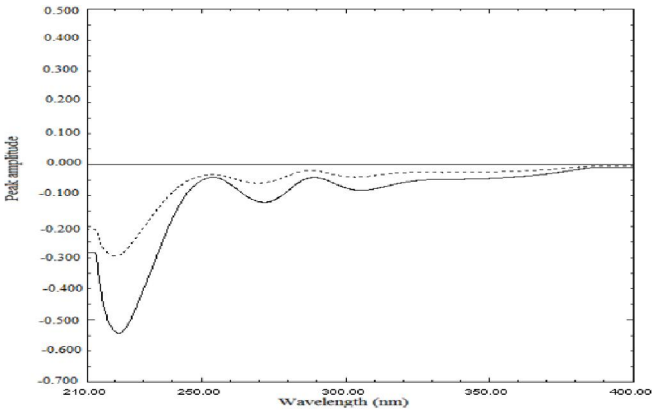


Figure 5 : First derivative absorption spectra of 30 µg/ml QF (_) and 30 µg/ml of QF deg (- - -) in methanol

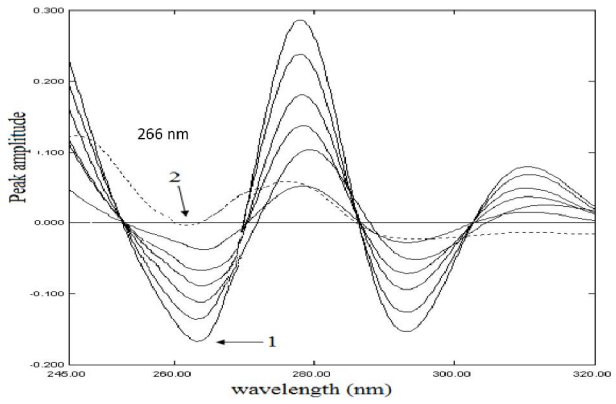


Figure 6 : Second derivative absorption spectra of 10– 60 µg/ml of QF (—) and 10 µg/ml of QF degraded (…..) in methanol

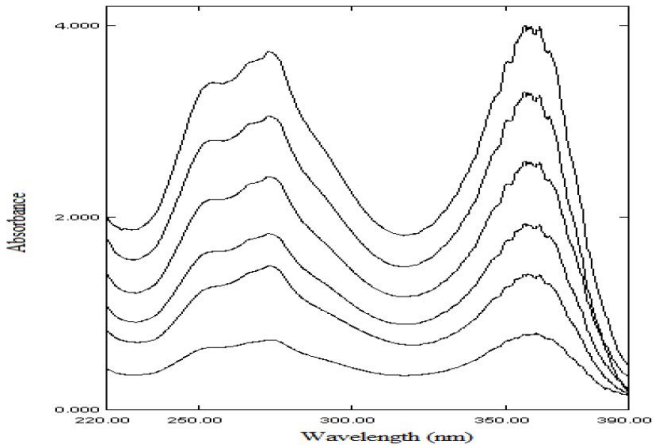


Figure 7 : Zero order absorption spectra of ratio spectra of QF (10-60µg/ml) in methanol using 10µg /ml of QF degraded as a divisor

Where [1]DD is the peak amplitude, C is the concentration of QF in µg/ml and r is the correlation coefficient.

The proposed method was successfully applied for the determination of the drug in pure powder form with mean percentages recoveries of 99.9 ± 0.4%, 99.2 ± 0.8%, and 99.4 ± 0.8% at 244, 285 and 344 nm respectively, (TABLE 1).

To study the two methods ruggedness, three different concentrations (20, 30, 50 µg/ml) solution of QF were analyzed using Unicam UV30 spectrophotom-

Review

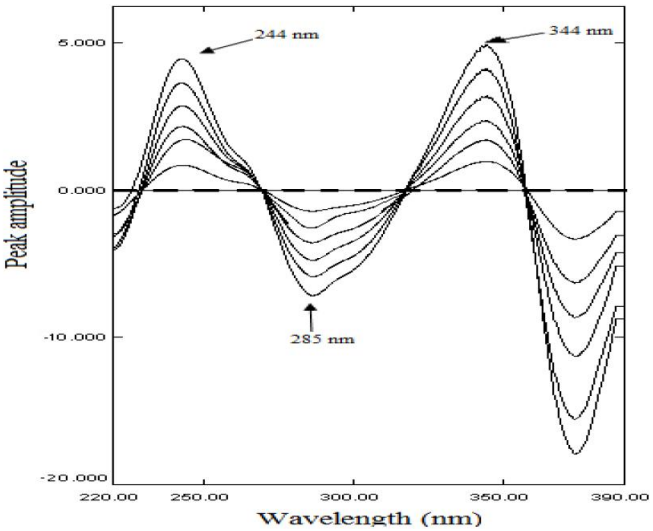


Figure 8 : First derivative of ratio spectra (^{1}DD) of QF (10–60 µg/ml) and 10 µg/ml of QF degraded in methanol using 10µg/ml of QF degraded as a divisor

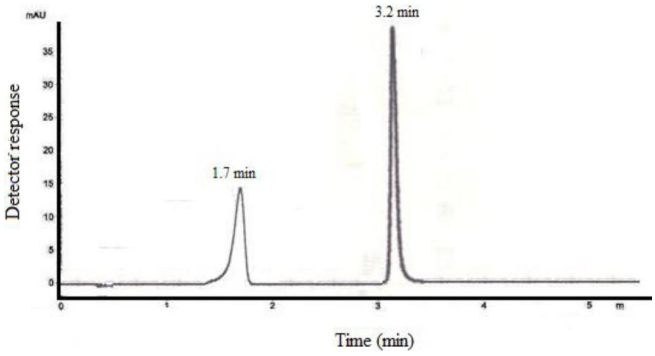


Figure 9 : HPLC chromatogram of resolved mixture of QF (R_t=3.2 min) and QF degraded (R_t= 1.7), (each of concentration 20µg/ml) using methanol/water (80:20v/v) as a mobile phase

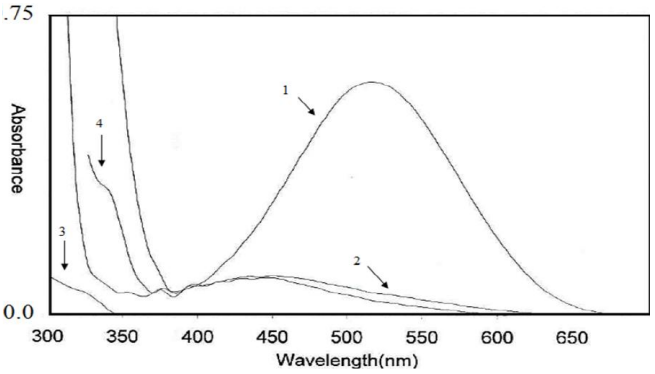


Figure 10 : Zero order absorption spectra of: (1) QF – P-CA complex, 200 µg/ml; (2) QF degraded– P-CA complex, 200 µg/ml; (3) QF in acetonitrile, 20 µg/ml; (4) Reagent blank

eter instead of Shimadzu UV-Visible spectrophotometer. The result in TABLE (1) proved the stability of the methods upon change of the instrument.

As for the robustness, determining (20, 30, 50 µg/ml) solution of QF in ethanol as solvent instead of methanol was studied, the methods demonstrated sufficient stability, (TABLE 1).

Method (C)

Densitometry is a simple method of quantitation directly on TLC plate by measuring the optical density of the separated spots [16,17].

The proposed method is based on the difference in the R_f values between the intact drug and its degradation product. The present work describes a sensitive, accurate and precise densitometric TLC method for the quantitative determination of QF in bulk powder, in dosage form and in the presence of its degradation product.

The suitable mobile phase has been selected to achieve the best separation of the drug from its oxidative degradation product; other necessary conditions have been established.

Different solvent systems with different ratios were tried; separation of QF from its degradation product was achieved upon using ethyl acetate/methanol/ammonium hydroxide10% (8.5:1.0:0.5 by volume). The instrumental conditions for densitometric measurement such as scan mode and wavelength detection were optimized. The scan mode chosen was zigzag mode, the scanning wavelength was 302 nm. QF was completely resolved from its degradation product and its R_f value was 0.54. On the other hand, the spot obtained from acid degradation had R_f value of 0.84. This would permit quantitative determination of QF in presence of its oxidative degradation product.

The relation between the concentration of QF and the integrated peak area of the bands was investigated. The linear relation was tested, resulting in a correlation coefficient (r) of 0.9996 for the concentration range of 4-20 µg/band with mean percentages recovery of 99.9 ± 0.7%, (TABLE 1).

To study the method ruggedness, three different concentrations (8, 12, 16 µg/ band) solution of QF were performed in two different laboratories using two different instrument. The result in TABLE (1) proved the stability of the method upon change of the instrument.

While for the robustness, determining (8, 12, 16 µg/band) solution of QF in ethyl acetate: methanol: ammonium hydroxide 10% (8.5:0.9:0.6 by volume) instead of

ethyl acetate: methanol: ammonium hydroxide 10% (8.5:1:0.5 by volume) as a mobile phase was studied, the method demonstrated sufficient stability, (TABLE 1).

Method (D)

High performance liquid chromatography (HPLC) is probably the most powerful and versatile tool for quantitative determination of many individual components in a mixture in one single procedure [18-20].

The proposed method is based on the difference in the R_t values between the intact drug and its degradation product. The present work describes a sensitive, accurate and precise HPLC method for separation and quantitative determination of QF in bulk powder, in dosage form and in the presence of its degradation product.

TABLE 1 : Results of validation parameters of the responses and the regression equations obtained by the proposed methods

Parameters	Method (A)	Method (B)			Method (C)	Method (D)	Method (E)
	^{2}D at	^{1}DD at					
	266 nm	244 nm	285nm	344 nm			
Slope[a]	0.003	0.0718	0.1136	0.0738	0.0739	0.0495	0.0027
Intercept[a]	0.001	0.218	0.3147	0.216	0.0356	0.0065	0.0072
Correlation coefficients	0.9997	0.9994	0.9996	0.9991	0.9996	0.9996	0.9995
Concentration range	10-60 µg/ml	10-60 µg/ml	10-60 µg/ml	10-60 µg/ml	4-20 µg/band	1-20 µg/ml	40-400 µg/ml
QL	1.5	6.15	5.5	4.98	0.2	0.3	10.7
DL	0.5	2.03	1.8	1.51	0.1	0.1	3.5
Average accuracy[a] (%)	99.9	99.9	99.2	99.4	99.9	99.8	99.9
Relative standard Deviation (precision %)	0.6	0.4	0.8	0.8	0.7	0.7	0.4
Precision Repeatability [b] ± RSD (%)	99.8±0.2	99.6±0.1	99.5±0.27	98.9±0.9	99.0±0.1	99.7±0.6	100.1±0.1
Intermediate precision [b] ± RSD (%)	99.9±0.1	100.2±0.5	99.3±0.3	99.5±0.5	99.8±0.6	99.8±0.4	100.21±0.135
Ruggedness [c] ± RSD (%)	100.9±0.4	100.3±0.4	99.7±0.5	99.9±0.1	98.00±0.981	100.4±0.5	99.9±0.2
Robustness [c] ± RSD (%)	100.8±0.3	99.9±0.8	99.8±0.8	99.7±0.5	99.78±0.630	99.7±0.6	99.9±0.8

[a]n= 6, [b]n = 3x3, [c]n = 3

Different mobile systems of different compositions and ratios were tried for separation of QF and QF deg.

A satisfactory separation of QF from its degradation product was achieved upon using methanol : water (80: 20 v/v) as a mobile phase. QF was completely resolved from its degradation product. Respective R_t values were 3.2 and 1.7 min for QF and QF deg, (Figure 9). This would permit quantitative determination of QF in presence of its acid degradation product.

The proposed method was found to be valid in the range of 1 – 20 µg/ml, and the regression equation was computed and found to be:

A = 0.0495C + 0.0065 r = 0.9996

Where A is the relative peak area at 302 nm, C is the concentration of QF in µg/ml and r is the correlation coefficient.

The linearity was checked and the calibration curve was constructed between the concentration of QF and the relative peak area. The proposed method was successfully applied for the determination of the drug in pure powder form with mean percentages recovery of 99.8 ± 0.7%, (TABLE 1).

To study the method ruggedness, three different concentrations (8, 12, 16 µg/ml) solution of QF were performed in two different laboratories using water system HPLC instrument instead of Agilent 1200, USA. The result in TABLE (1) proved the stability of the method upon change of the instrument.

While for the robustness, determining (8, 12, and 16 µg/ml) solution of QF in methanol: water (90:10 v/v) instead of methanol: water (80: 20 v/v) as a mobile phase was studied, the method demonstrated sufficient stability, (TABLE 1).

System suitability test parameters were calculated to ensure that the system is working correctly during the analysis. However, the calculated Rs value was always above 2. The selectivity factor (α) was also greater than 1, which ensures complete or 100% separation of the drug and the degradation product. The tailing factor was 1.5, which reveals linear isotherm peak elution with-

out tailing[21]. Peak information is given in TABLE 2.

Method (E)

π receptor react with basic nitrogenous group compounds as n-electron donor to form charge transfer complexes or radical anions according to the polarity of the solvent used. QF can act as n-electron donors through the basic nitrogen of amide group with subsequent formation of charge transfer complexes with π-electron acceptor. In this part, the reaction between QF (electron donor) and P-CA (electron acceptor) was studied,optimized and used for quantitative determination QF.

TABLE 2 : Results of system suitability test obtained by applying the proposed HPLC method for the determination of QF in its pure powdered form

Compound	QF	Deg	Reference value[21]
Retention time (min)	3.12	1.70	
Capacity (K´)	5.25	2.40	0.5< K´<10
Selectivity (α)	1.84	-	>1
Resolution (Rs)	5.93	-	>1.5
Tailing factor (T)	1.50	0.85	0.8? T ?1.5
RSD% of retention time	0.003	0.008	
Column efficiency	2021	1130	Increase with efficiency of separation

A variety of electron donating compounds have been reported to yield charge -transfer complexes leads to their utility in the development of simple and convenient colorimetric methods[22,23].

Upon addition of QF solution in acetonitrile to the golden yellow solution of P-chloranilic acid in acetonitrile, a purple color was obtained. This color was suggestive of charge transfer complex formation, which confirmed by the appearance of λ max at 525 nm as shown in Figure (10).

Various parameters affecting the reaction process were studied, the reaction was found to be sensitive to the reagent volume and it was found that 2 ml of P-CA ($2.5M \times 10^{-3}$) was sufficient to give maximum absorbance.

In order to select the most appropriate solvent, the reaction was carried out in different solvents as methanol, ethanol, acetone, acetonitrile, 1, 4-dioxan and 1, 2- dichloromethane. Acetonitrile was found to be ideal solvent. This solvent has high ionizing power which allows complete electron transfer from the donor to the acceptor moiety takes place which is followed by the formation of radical anions[24,25].

Also the stability of the color was studied and it was found that the developed color remain stable at room temperature and revealed no change even after 60 minutes.

Under the optimum experimental conditions the calibration graph was constructed by plotting absorbance measured and the corresponding concentrations.

The regression equation was computed and found to be:

A= 0.0027C-0.0072 r=0.9995

A = the absorbance, C = the concentration in μg/ml, r = the correlation coefficient.

The proposed method was successfully applied for the determination of the drug in pure powder form with mean percentages recovery of 99.9 ± 0.4%, (TABLE 1).

To study the method ruggedness, three different concentrations (80, 160, 240 μg/ml) solutions of QF, were analyzed using Shimadzu UV-Visible spectrophotometer instead of Unicam UV30 spectrophotometer. The results in TABLE (1) proved the stability of the method upon change of the instrument.

While for the robustness, determining the previously mentioned concentrations of QF, using 2.5 ml instead of 2 ml of P-CA and was studied, the method demonstrated sufficient stability, (TABLE 1).

The specificity of the proposed methods was proved by the analysis of a laboratory prepared mixture containing different percentages of the degradation product. Method (A), was found to be specific for QF in presence of up to 70% of its degradation product, while method (B) was specific in presence of up to 90% of its degradation product at 285 nm, and up to 70% at 244 and 344 nm. In methods (C and D), the specificity was achieved in presence of up to 90% of its degradation product. The specificity of method (E) was achieved in presence of up to 50% of its degradate, (TABLE 3).

The usefulness of the proposed methods for the analysis of QF in pharmaceutical preparation was studied by assaying different batches of Seroquel tablets, (TABLE 4). Standard addition technique was also applied to assess the validity of the proposed methods, (TABLE 4).

Results obtained by the proposed methods for the

determination of pure samples of the drug were statistically compared to those obtained by the Manufacture method (3) of the drug and no significant difference was observed, (Table 5).

Linearity of each method was assessed by the determination of the same concentration range as the calibration curves, the mean accuracies are given in Table 1.

The accuracy of the proposed methods was tested

TABLE 3 : Specificity of the proposed methods for the determination of QF in laboratory prepared mixtures containing different concentration of QF and QF deg

Degradation%	Methods (A) and (B)						Method (C)			Method (D)			Method (E)		
	Concentration (µg/ml)						Concentration (µg/band)			Concentration (µg/ml)			Concentration (µg/ml)		
	QF (µg/ml)	QF deg (µg/ml)	Recovery % of method (A) at 266 nm ^{2}D	Recovery % of method (B) at 244 nm ^{1}DD	Recovery % of method (B) at 285 nm ^{1}DD	Recovery % of method (B) at 344 nm ^{1}DD	QF	QF deg	Recovery % of method (C)	QF	QF deg	Recovery % of method (D)	QF	QF deg	Recovery % of method (D)
10	54	6	99.0	99.8	99.0	99.9	18	2	99.7	18	2	100.6	360	40	100.3
30	42	18	99.5	98.9	99.7	99.8	14	6	98.7	14	6	100.9	280	120	100.3
50	30	30	100.2	100.7	100.2	99.5	10	10	99.4	10	10	100.8	200	200	101.0
70	18	42	101.0	99.9	98.7	99.3	6	14	100.1	6	14	100.0	120	280	-
90	6	54	-	-	100.0	-	2	18	98.5	2	18	100.0	40	360	-
Mean			99.9	99.8	99.5	99.6			99.3			100.5			100.5
RSD%			0.9	0.7	0.6	0.3			0.7			0.4			0.4

TABLE 4 : Quantitative determination of QF in pharmaceutical formulation by the proposed methods and results of application of standard addition technique

Pharmaceutical formulation	Method (A)		Method (B)		Method (C)	Method (D)	Method (E)
Seroquel tablets B.N 100120	At 266 nm	At 244 nm	At 285nm	At 344 nm			
Found %*	99.9 ± 0.3%	99.8 ± 0.3	99.3 ± 0.6%	100.0 ± 0.2	99.5 ± 0.3%	99.7 ± 0.6%	100.17 ± 0.650%
Recovery of standard added %*	100.1 ± 0.7%	99.6 ± 0.2%	99.2 ± 0.6%	99.7 ± 0.1%	99.4 ± 0.8%	99.8 ± 0.4%	100.3 ± 0.4%

***Average of three different determinations**

TABLE 5 : Statistical analysis between the results obtained for the determination of QF in pure samples by the proposed methods and that obtained by the manufacturer method[3]

Item	Method (A)	Method (B)			Method (C)	Method (D)	Method (E)	Manufacturer's Method*[3]
		At 244 nm	At 285 nm	At 344 nm				
Mean	99.9	99.9	99.2	99.4	99.9	99.8	99.9	99.3
S.D.	0.6	0.4	0.8	0.8	0.7	0.7	0.4	0.5
R.S.D.%	0.6	0.4	0.8	0.8	0.7	0.7	0.4	0.5
Variance	0.4	0.2	0.6	0.6	0.5	0.5	0.2	0.3
N	6	6	6	6	5	6	6	5
t- test**	1.7 (2.262)	2.0 (2.262)	0.2 (2.262)	0.2 (2.262)	1.5 (2.306)	1.3 (2.262)	2.0(2.262)	-
F-test**	1.3 (6.26)	1.5(5.2)	2.0(6.26)	2.0 (6.26)	1.7(6.4)	1.7 (6.26)	1.5(5.2)	-

***Non aqueous titration (using glacial acetic acid as a solvent and 0.1M perchloric acid as a titrant, the end point was detected potentiometrically); **Figures in parentheses are the corresponding tabulated values at p = 0.05**

by analyzing freshly prepared solutions of QF in triplicate within the linearity range. The recovery percentages (recovery %) and relative standard deviations (RSD) revealed excellent accuracy (TABLE 1).

The repeatability and intermediate precision were evaluated by assaying freshly prepared solutions of the drug in triplicate on the same day and on three successive days respectively at concentrations within the lin-

Review

earity range for the 5 proposed methods. RSD% (TABLE 1) showed the precision and the ruggedness of the methods.

Validation of the proposed methods was made by measuring range, accuracy, precision, repeatability, intermediate precision, linearity, specificity, ruggedness and robustness. Results obtained are depicted in TABLES (1 and 3). These data render the applicability of the proposed methods for the quality control of the drug formulations.

The stability of QF in methanol has been determined by keeping one sample in refrigerator and other in a tightly capped volumetric flask placed at ambient temperature under normal lighting condition. The sample were checked for assay in three successive days of storage and compared with freshly prepared sample by the proposed methods. The RSD% values of assay were found to be below 2.0% in both cases. This indicates that QF is stable in the solution.

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