

## A Simple and Validated HPTLC Method of Evaluation for Quetiapine fumarate in Oral Solid Dosage Form

Ramu Sathiya<sup>a</sup>, Kaliaperumal Krishnaraj<sup>b\*</sup>, Selvadurai Muralidharan<sup>b</sup> and Nithyanantham Muruganatham<sup>b</sup>

<sup>a</sup>Institute of Pharmacology, Madras Medical College, Chennai 600003, India.

<sup>b</sup>TIFAC CORE HD, JSS College of Pharmacy, Rocklands, Ootacamund 643001, India.

*Received: 19/11/2009; Accepted: 23/12/2009*

### Abstract

A simple, sensitive, precise and rapid high performance thin layer chromatographic method of analysis for quetiapine fumarate in pharmaceutical formulation was developed and validated. The method was employed in thin layer chromatographic aluminum plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consist of Toluene: Ethyl acetate: Diethyl amine (5:3:2, v/v/v) as the mobile phase. Densitometric analysis of quetiapine fumarate was carried out in the absorbance mode at 291 nm. The system was found to give compact spots for quetiapine fumarate ( $R_f$  value of 0.54). The linear regression analysis data for the calibration plots showed good linear relationship with  $r^2 = 0.9915$  in the concentration range 25-225 ng per spot. The method was validated for precision, accuracy, recovery and sensitivity. The method has been successfully applied in the analysis of oral solid dosage formulation.

### Keywords:

Quetiapine fumarate; HPTLC; Validation; Estimation

### 1. Introduction

Quetiapine fumarate is a psychotropic agent belonging to a chemical class of dibenzothiazepine derivatives. The chemical designation is 2-[2-(4-dibenzo [*b,f*] [1,4]thiazepin-11-yl-1-piperazinyloxy)-ethoxy]-ethanol fumarate (2:1) (salt). It is present in tablets as the fumarate salt. All doses and tablet strengths are expressed as milligrams of base, not as 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. [1,2,3]. The antagonist activity of quetiapine fumarate at dopamine and serotonin receptors is mediated the antipsychotic effect. Quetiapine fumarate has also an antagonistic effect on the histamine H<sub>1</sub> receptor. This is thought to be responsible for the sedative effect of the drug. It is used to treat psychosis associated with parkinson's disease and chronic schizophrenia. These antipsychotics have a low incidence of extrapyramidal side effects and tardive dyskinesias compared to older antipsychotics [4, 5, 6].

Quetiapine fumarate is well absorbed and extensively metabolised following oral administration. The half life is only 6h. Quetiapine fumarate is approximately 83% bound to plasma proteins. A most common side effect of quetiapine fumarate is sedation. The common side effects are constipation, headache, dry mouth and mild weight gain or weight loss. The

\* Corresponding Author

E-mail: krishnarajsk@gmail.com

ISSN: 1306-3057,

Moment Publication ©2010

less common side effects are dizziness, upset stomach, abnormal liver tests, substantial weight gain or weight loss, increased paranoia and a stuffy nose [5, 6].

The literature survey revealed that methods have been reported for the estimation of quetiapine fumarate in plasma using high performance liquid chromatography (HPLC) method [7,8,9,10,11]. A simple and sensitive UV spectrophotometric method has developed for the determination of quetiapine as fumarate having absorption maximum at 254.7 nm [12]. A spectrophotometric method has developed using capillary zone electrophoretic method for the quality control of quetiapine in commercial formulations [13]. The high performance liquid chromatography-electrospray mass spectrometry method has developed for simultaneous determination of quetiapine and its metabolite in human plasma [14]. The derivative ultraviolet spectrophotometry has successfully been applied to drugs alone or in association. This technique is an alternative method to determine drugs with low specific absorptivity, substances which under the influence of increased background absorption or drugs in association wherein overlaps and absorption addition occur [15]. These studies have led to encourages the method development and investigation of quetiapine fumarate by a new technique.

The major advantage of high performance thin layer chromatographic (HPTLC) [16,17,18] is that several samples can be analyzed simultaneously using a small quantity of mobile phase unlike HPLC. This reduces the time and cost of analysis and possibilities of pollution of the environment. HPTLC also facilitates repeated detection (scanning) of the chromatogram with same or different parameters [19, 20, 21]. Simultaneous assay of several components in a multicomponent formulation is possible.

The aim of present study is to develop and validate an accurate, specific and reproducible HPTLC method for determination of quetiapine fumarate as in marketed oral solid dosage formulation (Qutipin 200, Sun pharmaceutical industries, India).

## **2. Experimental**

### **2.1. Drug and chemicals**

Quetiapine fumarate was obtained as a kind gift sample from Beijing Lunarsun Pharmaceutical Co., Ltd, China, certified to contain 99.98% (w/w). Qutipin tablets (Qutipin 200, Sun pharmaceutical industries, Jammu and Kashmir, India) were procured from the local market in Ooty. All other solvents were HPLC grade procured from SD Fine Chemicals, India.

### **2.2. HPTLC instrumentation and chromatographic condition**

The chromatographic estimation was performed by spotting standards and extracted samples of quetiapine fumarate on a precoated silica gel aluminum plate 60F-254 (thickness 250  $\mu\text{m}$  with 10 x 10 cm, E.Merck, Darmstadt, Germany, supplied by Anchrom Technologies, Mumbai, India) using a Camag Linomat IV sample applicator (Camag, Muttenz, Switzerland) and a 100  $\mu\text{L}$  Hamilton syringe. The samples, in the form of bands of length 8 mm, were spotted 15 mm from the bottom, 15 mm from left margin of the plate and 10mm apart, at a constant application rate of 10 s  $\text{mL}^{-1}$  using nitrogen aspirator. Plates were developed using a mobile phase consisting of Toluene: Ethyl acetate: Diethyl amine (5:3:2, v/v/v). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber (Camag Muttenz, Switzerland) equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The length of chromatogram run was 7 cm. approximately; 10 mL of the mobile phase (5 mL in trough containing the plate and 5 mL in

the other trough) was used for each development, which required 8 min. It results in better apparent resolution with more convenient capability of the detecting device to perform integration of peak area. Subsequent to the development, TLC plates were dried in a current of air with the help of a hair-dryer; the spots come in brown colour. The slit dimension settings of length 4 mm and width 300  $\mu\text{L}$ , and a scanning rate of 20 mm  $\text{s}^{-1}$  was employed. The monochromator bandwidth was set at 20 nm. Densitometric scanning was performed on Camag TLC scanner III in the absorbance mode at 291 nm and operated by Win CATS Planar chromatography version 1.1.3.0. The source of radiation utilized was  $\text{D}_2$  lamp. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was via peak areas with linear regression.

### 2.3. Calibration curves of quetiapine fumarate

Calibration solutions of quetiapine fumarate in methanol containing concentrations of quetiapine fumarate from 25 to 225  $\text{ng mL}^{-1}$  were prepared by individual weighing. Five microlitres from each solution was spotted on the TLC plate to obtain final concentration range from 25 to 225  $\text{ng}$  per spot. Each concentration was spotted two times on the TLC plate. The data of peak area versus drug concentration were treated by linear least-square regression analysis.

### 2.4. Method validation

The developed HPTLC method was validated for the following parameters.

#### 2.4.1. Sensitivity

The sensitivity of the method was determined with respect to limit of detection (LOD), limit of quantification (LOQ), linearity range and correlation coefficient. Solutions containing 25 to 225  $\text{ng mL}^{-1}$  of quetiapine fumarate were spotted on TLC plate. These were calculated by use of the equations  $\text{LOD} = 3 \times \text{N/B}$  and  $\text{LOQ} = 10 \times \text{N/}$  where N is the standard deviation of the peak areas of the drugs ( $n = 3$ ), taken as a measure of the noise and B is the slope of the corresponding calibration plot.

#### 2.4.2. Selectivity

The selectivity of the assay was determined in relation to interferences from formulation ingredients like from tablets.

#### 2.4.3. Recovery studies

Recovery of quetiapine fumarate was determined by spiking quetiapine fumarate in drug to obtain three different concentrations [22] covering the low, medium and higher ranges of the calibration curve. The recovery was calculated by comparing the resultant peak areas with those obtained from pure standards in methanol at the same concentrations.

#### 2.4.4. Precision and Accuracy

Different amount of quetiapine fumarate covering low, medium and higher ranges of the calibration curve were spotted on the TLC plate. These spots were analyzed by using above described HPTLC method. Precision was expressed as the percentage coefficient variation (% C.V) and accuracy was expressed as a percentage (observed concentration  $\times 100$  / theoretical concentration).

### 2.4.5. Reproducibility

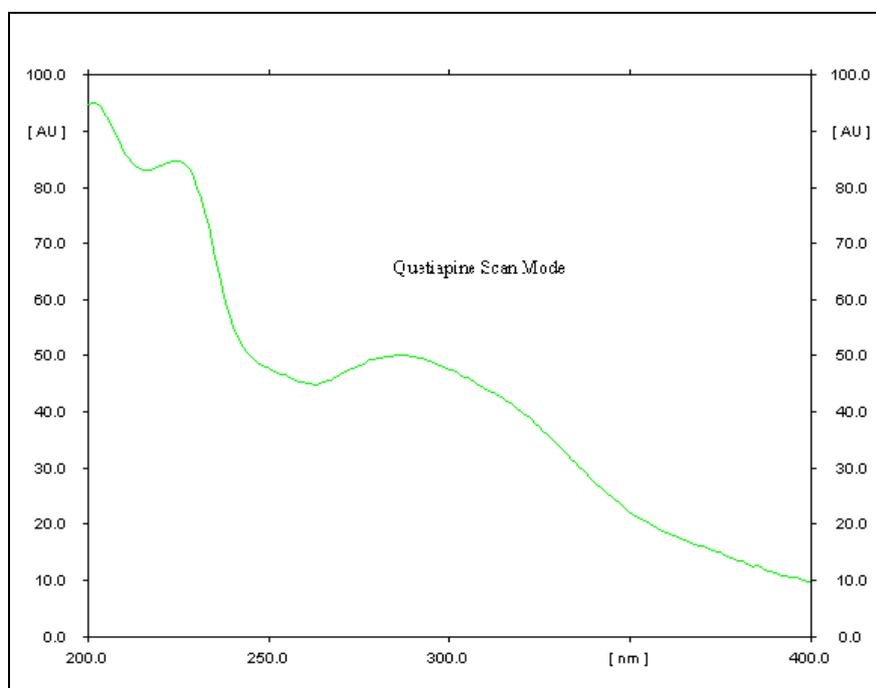
The repeatability was evaluated by analyzing the amount of quetiapine fumarate spotted on TLC plate covering low, medium and higher ranges of calibration curve in replicates ( $n = 5$ ). The intermediate precision was evaluated by analyzing the same amount of analyte over period of 3 days ( $n = 5$ ) and expressed in terms of % C.V.

### 2.5. Analysis of marketed formulation

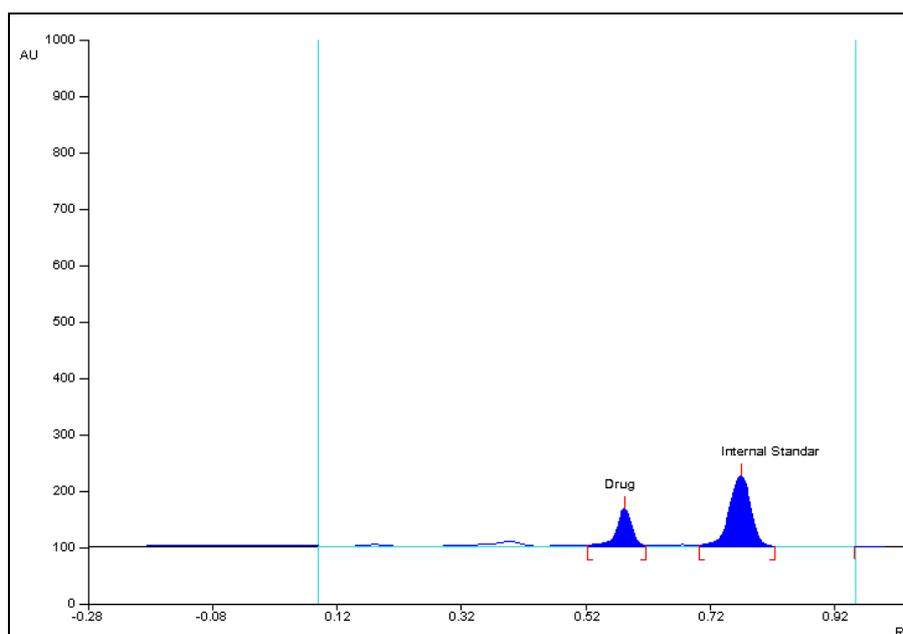
The developed method can be applied in determination of quetiapine fumarate in Qutipin 200 tablet, which is marketed oral solid dosage formulation. Twenty tablets were weighed individually and crushed in a mortar. An accurately weighed quantity of powdered tablets (100 mg) was extracted with methanol. (Qutipin 200, Batch No: AD80781, Sun pharmaceutical industries, India). To ensure complete extraction of the drug, it was sonicated for 30 min. The resulting solution was allowed to settle for about an hour and the solution was filtered through 0.45  $\mu$  membrane. Then it was suitably diluted to give desired concentration. Ten microlitres of the solution was applied on TLC plate followed by development. The analysis was repeated in triplicate. The possibility of excipient interference in the analysis was studied.

### 3. Results and discussion

HPTLC offers several advantages over reported methods. It facilitates automatic application and scanning *in situ*. The composition of the mobile phase for development of chromatographic method was optimized by testing different solvent mixtures of varying polarity. Use of Methanol as single component and short saturation time of 15 min give necklace effect. So Methanol: Chloroform (7:3, v/v), Methanol: Dichloromethane (7:3, v/v), Hexane: Ethyl acetate: Glacial acetic acid (7:2.5: 1, v/v) were tried. The best results were obtained using Toluene: Ethyl acetate: Diethyl amine (5:3:2, v/v/v).



**Fig. 1** Scanned spectrum of quetiapine fumarate



**Fig. 2** Standard Chromatogram of quetiapine fumarate with internal standard

A typical scanned spectrum of quetiapine fumarate is shown in Fig. 1. The present method uses Toluene: Ethyl acetate: Diethyl amine (5:3:2, v/v/v) as the mobile phase for development. The present method is quicker as the time needed for development of plate is reduced considerably to less than half an hour for chamber saturation. The method was successfully used in the analysis of quetiapine fumarate from the tablet dosage forms (Fig. 2) and in case of Qutipin 200 without any interference of the formulation excipients.

### 3.1. Validation

#### 3.1.1. Sensitivity

Under the experimental conditions employed, the lowest amount of drug which could be detected was found to be 5 ng per spot and the lowest amount of drug which could be quantified was found to be 17 ng per spot, with relative standard deviation <6%. The calibration curve was found to be linear in the range from 25 to 225 ng mL<sup>-1</sup> ( $n = 5$ ). Peak area and concentration was subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficients. The regression data as shows a good linear relationship over the concentration range studied.

#### 3.1.2. Recovery studies

Results given in Table 1 have high extraction efficiency of quetiapine fumarate from formulation components. The recovery of quetiapine fumarate ranged from 92.98 to 98.98%, average of 95.98%. This confirms that the proposed method can be used for determination of quetiapine fumarate in tablet formulated in our lab.

**Table 1** Precision and accuracy data of HPTLC method performed on quetiapine fumarate

Parameter	Amount of quetiapine fumarate in ng		
Actual spotted	25	125	225
Amount detected	23.04 ± 1.53	120.39 ± 2.01	223.32 ± 1.66

### 3.1.3. Precision and Accuracy

Five microliter aliquots of samples containing 25, 125 and 225 ng mL<sup>-1</sup>quetiapine fumarate were analyzed according to the proposed method. In order to control the scanner parameters, one spot was analyzed several times. By spotting and analyzing the same amount several times ( $n = 5$ ) the precision of the automatic spotting device was evaluated. The % C.V for the analysis of five replicates indicated good precision for the proposed TLC method as shown in Table 2. The result depicts good accuracy and high precision.

### 3.1.4. Precision

Table 3 shows that reproducibility studies of quetiapine fumarate at different time levels. The percentage C.V of inter day was found to range from 1.86 to 0.71%, averaging to 1.29%. The range from 4.76 to 0.46 % C.V was found to be in intra day studies. These results show that the method has excellent reproducibility.

**Table 2** Accuracy and precision of the assay

Amount of quetiapine fumarate spotted in ng	Amount detected in ng ( $n=5$ )	C.V (%)	Accuracy (%)
25	23.24 ± 1.27	5.48	92.98
125	121.99 ± 1.66	1.36	97.59
225	222.72 ± 1.74	0.78	98.98

**Table 3** Inter and Intra day studies data of HPTLC assay for quetiapine fumarate

Amount of quetiapine fumarate spotted in ng	Amount detected in ng	C.V (%)
Inter day ( $n=5$ )		
25	23.84 ± 0.44	1.86
125	122.20 ± 1.87	1.53
225	224.42 ± 1.59	0.71
Intra day ( $n=5$ )		
25	24.03 ± 1.14	4.76
125	122.80 ± 3.00	0.61
225	225.24 ± 1.04	0.46

### 3.2. Analysis of marketed formulation

The analysis of marketed formulation of quetiapine fumarate tablet showed drug content of 198.56 mg. The applicability of the method was verified by determination of quetiapine fumarate in pharmaceutical preparation. The percent recovery of the proposed method ranges from 93.56 to 101.44% averaging to 97.5%.

### 4. Conclusion

The developed HPTLC method combined with densitometry was found to be suitable for determination of quetiapine fumarate as tablet formulation (Qutipin 200, Sun

pharmaceutical industries, India) without any interference from the excipients. The results prove that the method is repeatable and selective for the analysis of Quetiapine fumarate. These advantages are including low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

### Acknowledgements

The authors are grateful to Department of Phytopharmacy & Phytomedicine, JSSCP, Ooty for providing the facilities which made it possible to undertake the experimental work in its research laboratories.

### References

1. Andrew J C, Jeffrey M G, John A T (2002) Dosing and switching strategies for quetiapine fumarate. *Clin Therap* 24(2): 209-222.
2. Ihor W R and Lisa A A (1997) Overview of the efficacy of Seroquel (quetiapine). *Schi Res* 24 (1-2): 199.
3. King D J and Link C G G (1996) Seroquel (ICI 204636): An atypical antipsychotic results from Phase III. *E Neuro* 6(3): 202.
4. Diletti E, Hauschke D, Steinijans V W (1991) Sample size determination for bioequivalence assessment by means of confidence intervals. *Int J Clin Pharmacol Ther Toxicol* 29(1): 1-8.
5. Carlos F, Martin B, Jennifer H M, Helen W (2009) Pharmacokinetic profiles of extended release quetiapine fumarate compared with quetiapine fumarate immediate release. *Progress in Neur. Psychopharm. Biol Psychi* 33: 199-204.
6. Tonmoy S (2001) Quetiapine-efficacy in different domains. *E Neuro* 11(4): S385-S390.
7. Patricia C D, James W, Gefvert O (1999) Analysis and pharmacokinetics of quetiapine fumarate and two metabolites in human plasma using reversed-phase HPLC with ultraviolet and electrochemical detection. *J Pharm Biomed Anal* 20: 271-282.
8. Mandrioli R, Fanali S, Ferranti A, Raggi M A (2002) HPLC analysis of the novel antipsychotic drug quetiapine fumarate in human plasma. *J Pharm Biomed Anal* 30: 969-977.
9. Julia S, Johannes K, Sebastian H, Christoph H (2006) Automated analysis of quetiapine fumarate and other antipsychotic drugs in human blood by high performance liquid chromatography with column switching and spectrophotometric detection. *J Chrom B* 830(2): 342-348.
10. Barrett M, Holcapek J, Huclova V, Borek D P, Fejt B, Nemeč I J (2007) Validated HPLC-MS/MS method for determination of quetiapine fumarate in human plasma. *J Pharm Biomed Anal* 44(2): 498-505.
11. Bellomario S A, Brown A J, Conlan X A, Barnett N W (2009) Preliminary evaluation of monolithic column high performance liquid chromatography with tris (2,2'-bipyridyl) ruthenium(II) chemiluminescence detection for the determination of quetiapine fumarate in human body fluids. *Talanta* 77(5): 1873-1876.
12. Baby S L P, Vardhan S V M, Ramachandran D, Rambabu C (2009) UV spectrophotometric determination of quetiapine and zonisamide. *Asian J Chem* 21(1): 811-813.

13. Vincenzo P, Roberto M, Anna F, Sandra F, Maria A R (2003) Two different analytical methods for the quality control of quetiapine in commercial formulations. *J Pharm Biomed Anal* 32 (4-5): 1037-1044.
14. Li, Cheng K Y, Li Z, Bai X, Zhang X, Wang B, Li H (2004) Quality control of commercial tablets containing the novel antipsychotic quetiapine. *Acta Pharmacol Sin* 1(25): 110-114.
15. Uslu B, Ozkan S A (2002) Determination of lamivudine and zidovudine in binary mixtures using first derivative spectrophotometric, first derivative of the ratio-spectra and high-performance liquid chromatography–UV methods. *Anal Chim Acta* 466: 175-185.
16. Aman D K, Ravichandran V, Prateek K J, Ram K A (2008) High-performance thin layer chromatography method for estimation of conessine in herbal extract and pharmaceutical dosage formulations. *J Pharm Biomed Anal* 46: 391–394.
17. Neeraj K, Dhaneshwar S R, Himani A, Abhijit K, Bharat P (2005) Application of HPLC and HPTLC for the simultaneous determination of tizanidine and rofecoxib in pharmaceutical dosage form. *J Pharm Biomed Anal* 37: 27-38.
18. Nitin G T, Mangal S N (2007) Validated HPTLC method of analysis for artemether and its formulations. *J Pharm Biomed Anal* 43: 839–844.
19. Sanjay K M, Roop K K, Farhan J A, Shruti C, Kohli K, Talegaonkar S (2007) Application of a validated stability-indicating densitometric thin-layer chromatographic method to stress degradation studies on moxifloxacin. *Anal Chim Acta* 582: 75–82.
20. Kuldeep M P, Subhash L B (2005) High-performance thin-layer chromatographic determination of lamotrigine in serum. *J Chrom B* 823: 152–157.
21. Gunther M, Schmidt P C (2005) Comparison between HPLC and HPTLC-densitometry for the determination of harpagoside from *Harpagophytum procumbens* CO<sub>2</sub>-extracts. *J Pharm Biomed Anal* 37: 817–821.
22. Muralidharan S, Meyyanathan S N, Muruganantham N, Rajan S, Krishnaraj K, Suresh B (2009) Validated HPTLC method of analysis of dexibuprofen in its formulation. *J Plan Chrom* 22: 207–210.