

Research Article



Qualitative, Quantitative Determination and Validation of Free Quetiapine Base Form in Quetiapine Fumarate Tablet Using Nuclear Magnetic Resonance Spectroscopy

Sukhdev Singh*

Shri Jagdish Prasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu, Rajasthan, India.

*Corresponding author's E-mail: sukhdevsingh29@gmail.com

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ABSTRACT

Rapid, specific and accurate proton nuclear magnetic resonance (^1H NMR) method was developed to determine disproportionation of Quetiapine Fumarate in its base form, in pharmaceutical tablet formulation. The method is based on quantitative and qualitative study of Quetiapine Fumarate salt to Base conversion by ^1H NMR and using dimethylsulfoxide (DMSO-d₆) as a solvent. For the disproportionation study, ^1H NMR signals at 6.87 to 6.90 ppm (Triplet) and 6.55 ppm (singlet) corresponding to the analyte proton of Quetiapine Fumarate base and Fumaric acid were used for the study. The method was validated for the parameters of specificity and selectivity, linearity, accuracy and limit of detection (LOD) - limit of quantification (LOQ). The method is non-destructive and can be applied for establishment of disproportionation study of Quetiapine Fumarate in commercial formulation products.

Keywords: Disproportionation, Quetiapine Fumarate, Quantitative nuclear magnetic resonance.

INTRODUCTION

Nuclear Magnetic Resonance (NMR) Spectroscopy is a quantitative spectroscopic tool because the intensity of a resonance line is directly proportional to the number of resonant nuclear. This fact enables accurate and precise quantitative determinations. NMR has been used for quantitative determination of pharmaceutical compounds in different matrices. The high selectivity under appropriate acquisition conditions and the possibility of performing quantitative and qualitative analysis without analyte standards are the most attractive features of this technique. Quantitative determination is normally obtained from the ratio between the integration of a specific signal of the analyte and the internal reference standard.

Quantitative measurement was first described in 1963 by Jungnickel and Forbes and Hollis.¹⁻² Despite limited accuracy, quantitative ^1H NMR find application in various fields of science.³⁻¹¹ The lack of absorbing Chromophores for UV-(ultraviolet) –visible detection and the need for the special chromatographic detectors as well as the difficulties in establishing highly efficient solid or liquid phase extraction procedures, have made qNMR most suitable for sample analysis of many drugs moiety.¹²⁻¹⁴

Quetiapine Fumarate (Figure 1), 11-[4-[2-(2-Hydroxyethoxy) ethyl]-1-piperazinyl]-dibenzo [b, f] [1, 4] thiazepine fumarate salt, is a psychoactive organic compound that acts as an antagonist for multiples neurotransmitter receptors in the brain and acts as an antipsychotic agent.

MATERIALS AND METHODS**Reagents and Standards**

High purity analytical grade substance was used throughout the experiment. Authentic sample of Fumaric acid, Quetiapine Fumarate API and tablet formulation was obtained from Torrent Pharmaceuticals Limited, Ahmadabad, India. Fumaric acid (99.90%) and deuterated dimethylsulfoxide (DMSO-d₆) (99.99%) were purchased from Merck.

Instrumentation

NMR: Bruker AVANCE III 400 MHz for protons quipped with a 5 mm ^1H - ^{13}C dual probehead and 5 mm multinuclear observe (BBO) probehead.

Preparation of experimental solutions**Quetiapine Fumarate Salt Standard Preparation**

Accurately weighed and mixed 10 mg of Quetiapine base with 5 mg of Fumaric acid. The mixture was dissolved in 0.5 ml of DMSO-d₆. Solution was thoroughly mixed till complete dissolution.

Quetiapine Fumarate Base Standard Preparation for Specificity

Accurately weighed 10 mg Quetiapine Fumarate base and transferred to stoppered tube and 0.5 ml of DMSO-d₆ was added. Solution was thoroughly mixed till complete dissolution.

Fumaric acid of Specificity

Accurately weighed and transferred 5 mg of Fumaric acid to stoppered tube and 0.5ml of DMSO-d₆ was added. Solution was thoroughly mixed till completed dissolution.



Placebo solution preparation for Specificity

Accurately weighed and transferred 23.15mg of placebo (mixture of excipients without drug) equivalent to 200mg of Quetiapine base and 663.1mg of total weight of tablet. Solution was thoroughly mixed and transferred in stoppered tube 0.5 ml DMSO-d6 was added.

Tablets Sample Preparation

Five tablets of Quetiapine Fumarate Tablet were weighed and crushed to obtained fine powder. Powder equivalent to 33.15mg of Quetiapine Fumarate was weighed accurately and transferred to stoppered tube and 0.5 ml DMSO-d6 was added. Solution was thoroughly mixed till complete dissolution and supernatant was taken.

NMR Analysis

¹H NMR spectra of preparation as mentioned above were measured using 400MHz, BRUKER –AVINCE III Spectrometer. 16scans were collected for each sample into 32,768 data points using a 30° pulse length, spectral width 20 (PPM), D1 1sec. Acquisition time 3s.

NMR spectra for standard preparation collected in replicate and sample preparation in triplicate. Recorded 1H-NMR under the experimental condition gives as per proposed method of analysis. Integrated analysis of proton signal obtained at 6.87-6.90ppm (Triplet) of Quetiapine base peak and 6.55 ppm of Fumaric acid peak.

RESULTS AND DISCUSSION

NMR Experiments for confirmation of Structure Characterization

¹H-NMR, Deuterium Exchange NMR, ¹³C NMR, DEPT NMR, 2-D ¹H-¹H Correlation spectroscopy (COSY) and 2-D ¹H-¹³C heteronucleur single quantum correlation (HSQC) NMR experiment were performed for confirmation of structure characterization of Quetiapine Fumarate salt and Fumaric acid.

Figure 1 shows the Structure of Quetiapine Fumarate and Fumaric acid with its assignments respectively and ¹H-NMR are provided in Figure 2 and 3 for Quetiapine Fumarate and Fumaric acid respectively.

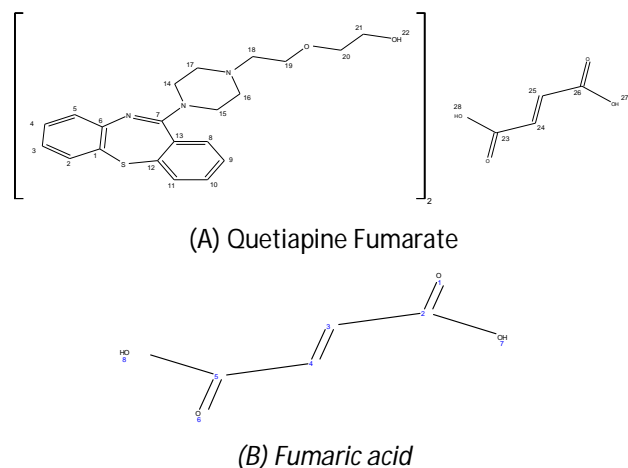
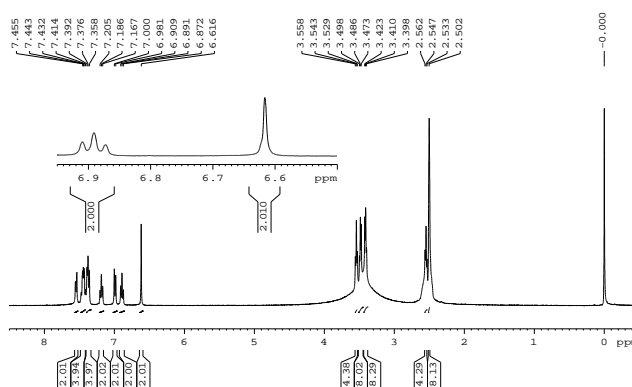
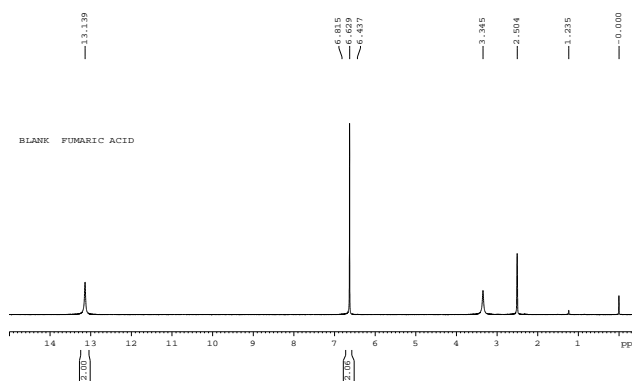


Figure 1: Quetiapine Fumarate and Fumaric acid



Chemical Shift δ (in ppm)	Multiplicity	No. Of proton(s)	Proton assignment
7.53-7.55	Multiplet	2	2-CH
7.43-7.47	Multiplet	4	4,8-CH
7.35-7.39	Multiplet	4	3,11-CH
7.16-7.20	Triplet	2	10-CH
6.98-7.00	Doublet	2	5-CH
6.87-6.90	Triplet	2	9-CH
6.61	Singlet	2	24,25-CH
3.52-3.55	Triplet	4	21-CH ₂
3.47-3.49	Multiplet	8	19,20-CH ₂
3.39-3.42	Multiplet	8	14,15-CH ₂
2.53-2.56	Multiplet	4	18-CH ₂
2.50	Unresolved Multiplet	8	16,17-CH ₂

Figure 2: ¹H NMR Spectra of Quetiapine Fumarate



Chemical Shift δ (in ppm)	Multiplicity	No. Of proton(s)	Proton assignment
13.13	Broad Singlet	2	7,8-OH
6.62	Singlet	2	3,4-CH

Figure 3: ¹H NMR Spectra of Fumaric acid

Calculation

The signals intensity and integration of Fumaric acid at 6.55ppm is the study of how much percentage Quetiapine Fumaric salt convert into free Quetiapine Base.

$$\% \text{ Fumaric acid content} = \frac{I_x}{I_y} \times \frac{C_y}{C_x} \times P \times 100 \dots\dots (1)$$

I_x = Mean internal value of Fumaric acid ¹H signal (Singlet) at 6.55 in test solution

I_y = Mean internal value of Fumaric acid ^1H signal (Singlet) at 6.55 in standard solution

C_y = Concentration of Fumaric acid in standard solution.

C_x = Concentration of Quetiapine Fumarate in test solution.

P = Potency of Fumaric acid standard.

Analytical method validation

The method was validated as per International Conference on Harmonization (ICH) guidelines for parameters like, system suitability, specificity and selectivity, linearity, LOD - LOQ determination and accuracy.¹⁷

System suitability

System suitability – to show that the control measures required have been complied for a particular analysis on a particular day, a system suitability check is required. Such a check on the performance of the spectrometer and method may be used, for example, to ensure that the expected specificity and sensitivity can be achieved. One of the advantages of the use of NMR as a quantitative method is that the sample itself may provide such system suitability test by, for example, making use of line-width or S/N data in the sample spectrum. Because of the high precision and intrinsic accuracy, system precision for NMR is not required. However system precision was performed for every parameter by replicate acquisitions of standard preparation. It was called as system suitability test and checked the compliance of acceptance criteria as mentioned below.

For System suitability check, criteria like - % Relative standard deviation (RSD) of the integral value of analyte signal should not be more than 2.00, Signal to Noise Ratio (S/N) of the analyte signal should be more than 150 and difference of the δ ppm value of analyte signal should not be more than 0.2 ppm were confirm before analysis.^[15-18]

Specificity and selectivity

The selectivity and specificity of proposed method was evaluated through possible interference due to the presence of the excipients in the pharmaceutical formulations.

Specificity study was performed by analyzing the diluents (DMSO-D6), placebo solution preparation, Quetiapine base, Fumaric acid and Quetiapine base spike with Fumaric acid. Also the signals of the analyte proton and Fumaric acid were well separated from other in standard and sample (Figure 4).

Linearity

qNMR as a method itself is linear because the intensity of the response signal is directly proportional to the amount of nuclei contributing to this signal.

Linearity was checked by preparing Fumaric acid at seven different concentration levels ranging from 70% to 140%,

according to the expected content of analyte in test sample. Linearity curve was draw for area of Fumaric acid (number of protons) vs. concentration of Fumaric acid (in ppm). The equation for curve found $y = 1\text{E-}04\text{C} + 0.025$ and correlation coefficient was found 0.994, indicating good linearity of proposed method. (Figure 5)

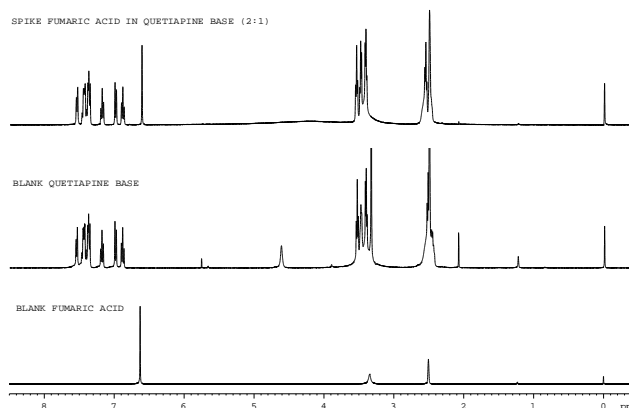


Figure 4: NMR spectra depicting Blank Fumaric acid, Quetiapine Base, Quetiapine Base spike with Fumaric acid

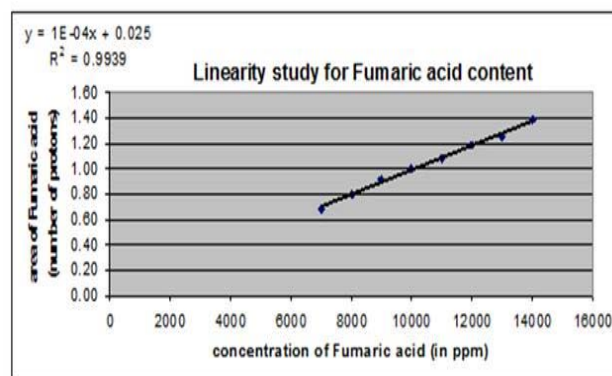


Figure 5: Linearity study for Fumaric acid content

Determination of LOD & LOQ

In the case of NMR with lorentzian line as response signals, the LOD and LOQ have to be calculated by the standard deviation of the response, σ and the slop, S of a calibration curve obtained in Linearity study. The LOD and LOQ were calculated using equation 2.¹⁹

$$\text{LOD} = \frac{3.3 \sigma}{S} \quad \text{LOQ} = \frac{10 \sigma}{S} \quad \dots \dots \dots (2)$$

LOD and LOQ were found to be 0.63 mg and 1.91 mg per ml diluents respectively.

Accuracy

The accuracy of an analytical method expresses the closeness for agreement between an accepted reference value and the value found. The accuracy of an analytical procedure should be established across its range.

Data from nine determinations over three concentration levels covering the specified range was determined. The accuracy was studied 80%, 100% and 120% levels with respect to the sample by preparing the solution in

triplicate at each level. From the results as per Table-1, it was concluded that method for Fumaric acid content was accurate between in the range of 80% to 120% level. % RSD at each level was found to be less than 2.00.

Table 1: Accuracy test results

Accuracy level	FA in mg added (mg)	FA in mg found (mg)	% Assay
80% set-1	8.07	7.96	98.57
80% set-2	8.11	7.91	97.66
80% set-3	8.05	7.88	97.76
100% set-1	10.2	9.85	98.22
100% set-2	10.05	9.86	97.98
100% set-3	10.3	9.87	98.36
120% set-1	12.04	11.72	97.31
120% set-2	12.02	11.77	97.80
120% set-3	12.12	11.91	98.16
Mean			97.98
SD			0.38
%RSD			0.40

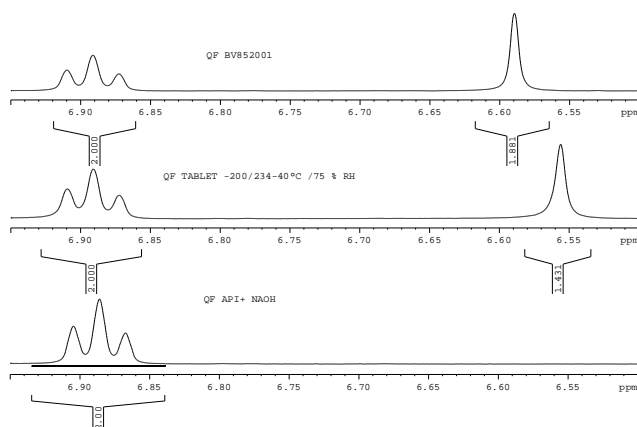


Figure 6: NMR Spectra depicting Quetiapine Fumarate API, QF Tablet Stability sample and API Spiked with NaOH

Study of disproportionation of Quetiapine Fumarate Salt

NMR spectra were collected for Fumaric acid, Quetiapine base and Quetiapine Base spike with Fumaric acid using DMSO-d₆ solvent at same concentration. The Fumaric acid gives signal at about 13.12 ppm (corresponds to acid proton) and also produce singlet at about 6.59 ppm which corresponds to aliphatic proton of formic acid [Figure 4]

Tablet formulation was kept for stability study and after 3M stability period sample was analysed as per proposed method. For excipients compatibility study and salt to base conversion study Quetiapine Fumarate mixed with magnesium oxide (MgO₂) in ratio of 1:0.45, Quetiapine Fumarate mixed with magnesium oxide (MgO₂) in ratio of 1:0.45 and mixture was moistened with 10µl water and Quetiapine Fumarate mixed with 20µl of 1M Sodium hydroxide solution. Integral of NMR signal at about 6.59 ppm where measured for excipients compatibility and salt to base conversion study [Figure 6 & 7]. The proton value

at the signal of Fumaric acid peak i.e. 6.59 ppm evaluated and found that the QF API, QF API spiked with Magnesium Oxide and QF API spiked with Magnesium Oxide in presence Water has 1.92, 1.92 and 0.69 proton value respectively.

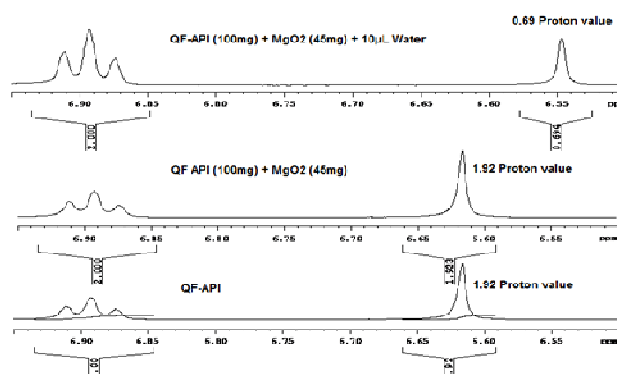


Figure 7: NMR spectra depicting Quetiapine Fumarate API, QF API spiked with Magnesium Oxide and QF API spiked with Magnesium Oxide & Water.

CONCLUSION

The qNMR method employed herein proved to be rapid as well as easy to implement. The different aspects of performance of the method such as linearity and accuracy, satisfies requirements well. It offers an excellent choice over previously described procedures and can be used for routine quality control and stability analysis of Quetiapine Fumarate tablet.

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