Analytical Method Development and Validation for Simultaneous Estimation of 5-Methyl-*N*-{[2-(Morpholin-4-Yl) Quinolin-3-Yl] Methyl}-1,3-Thiazol-2-Amine in Bulk Form

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Abstract- ***A reverse phase high performance liquid chromatographic technique for validating 5-methyl-N-[2-(morpholin-4-yl) quinolin-3-yl] methyl-1,3-thiazol-2-amine (MMQMTA) in its original form has been developed. A HYPERSIL (250 x 4.6 mm, 5 m) ID column was used for the chromatography, and the mobile phase was a 60:40 mixture of methanol and ammonium acetate with a flow rate of 1.0 ml/min. The detection wavelength was 235 nm. MMQMTA had a retention time of 6.812 min. The approach yields linearity responses at concentrations of 4–24 mg/ml of MMQMTA. MMQMTA has a detection limit of 0.021 g/ml and an area of 2.61. Area 6.812 and 0.05 g/ml were reported as the MMQMTA limits of quantification, and the MMQMTA recovery rate was determined to be 99.98%. The technique is helpful for ensuring and monitoring the quality of pharmaceutical and bulk formulations.***

Indexed Terms- ***5-methyl-N-{[2-(morpholin-4-yl) quinolin-3-yl] methyl}-1,3-thiazol-2-amine, MMQMTA, RP-HPLC, validation.***

I. INTRODUCTION

Substituted quinolines are abundant in nature, particularly in a diverse range of plants, soil, and marine microorganisms [1]. Numerous quinoline derivatives display significant biological properties, such as anti-microbial, anti-inflammatory, antimalarial [2], antibacterial, anti-HIV, anti-leukemic, cytotoxicity [3], anti-tumor properties [3, 4], antagonist activity to D1 and NMDA receptors [5], analgesicactivity [6], Parkinson's disease pathogenesis [7], and enzyme inhibitory activities for glucosidases [8] and monoamine oxidases [9], among others [10–13]. A review of the literature found that only a few methods, such as RP-HPLC, HPTLC, UV spectroscopy, and the Densitometric Method, have been described for the study of MMQMTA combinational dosage forms [14].

II. EXPERIMENTAL

- *Reagents and Chemicals:*

MMQMTA was prepared by the described method. Sodium dihydrogen ortho phosphate, water, Methanol, potassium dihydrogen ortho phosphate, ammonium acetate, THF, and dipotassium hydrogen ortho phosphate are HPLC grade chemicals that are used and bought.

- *Instrumentation:*

Chromatographic separation was done on a HPLC, Shimadzu, LC-20AD with an auto sampler, PDA Detector variable wavelength programmable UV/VIS detector, and HYPERSIL (250 x 4.6 mm, 5 m) with a 5m fixed loop.

- *Chromatographic Conditions:*

A solution containing a mixture of ammonium acetate and methanol in 1000 ml of water in the ratio of (60:40) v/v was given with 235 nm detection on a HYPERSIL (250 x 4.6 mm, 5 m) column used for separation [15]. The mobile phase is sonicated for 20 minutes after being filtered using a 0.45 nylon filter.

- *Method Development:*

After experimenting with numerous ratios of ammonium acetate, methanol, and water, ammonium acetate, methanol = 60:40 v/v was chosen as the proper

mobile phase since it provided good resolution, retention time, and acceptable system suitability parameters [16].

III. PROCEDURE

- *Preparation of standard solution*:

The volumetric flask was filled with 20 mg of MMQMTA, which was weighed out in 10 ml of mobile phase, which was then filled up with more mobile phase. 20 mg/mL of MMQMTA was prepared by diluting 1 to 10 mL of the aforementioned stock solution with mobile phase [17-18].

- *Procedure:*

Change the chromatographic conditions, inject the samples into the RP-HPLC, and keep track of the proper peak elution and retention settings when recording the chromatograms and execute the validation parameters in accordance with ICH standards.

- *Linearity:*

In order to create standard stock solutions of MMQMTA, 20 mg of MMQMTA were dissolved in 100 ml of mobile phase. The solution was then diluted to 100ml with a mobile, filtered using a 0.45-micron syringe filter, and sonicated for 5 minutes.

- *Procedure for analysis of a sample:*

For example, standard insufficient mobile phase was used to dissolve 20 mg of MMQMTA to prepare standard stock solutions (mg/ml). Following a 5-minute sonication, the solution was filtered through a 0.45-micron syringe filter and diluted to 100 ml with the mobile phase. MMQMTA dilutions of 25g/ml were repeated five times more.

IV. METHOD VALIDATION

- *System Suitability:*

According to the test procedure, standard solutions were made and then injected into the chromatographic apparatus. The asymmetric factor, theoretical plates, and resolution were examined as system appropriateness parameters [19].

- *Procedure**:***

Five injections of the standard solution were made, and the area of each injection was quantified using HPLC. It was discovered that the percent RSD of the area of five replicate injections fell within the set limits [20].

- *Specificity:*

The method is obviously intended for the detection of analytes in their dosage form because the mobile phase, solvent, and placebo do not affect the analyte peak or its peak purity [21].

- *Standard Sample:*

By dissolving 20 mg of MMQMTA in enough mobile phases, standard stock solutions of MMQMTA (mg/ml) were prepared. After that, the mixture was diluted with the mobile phase to 100 ml and filtered through a 0.45-micron syringe filter before being sonicated for 5 minutes. By mixing 10 ml of the mobile phase with 1 ml of the stock solution, further dilutions of 20 g/ml of MMQMTA were prepared in 5 repetitions[22].

- *MMQMTA sample**:***

By dissolving a weight comparable to 20 mg of MMQMTA and dissolving it in enough mobile phase, a sample stock solution of MMQMTA (g/ml) was prepared. Following that, a 0.45-micron syringe filter was used to filter the solution, which was then sonicated for 5 minutes before being diluted with mobile phase to 100 ml. Further dilutions of 10 g/ml of the mobile phase should be mixed with 1 ml of the stock solution. Further dilutions of 20 g/ml of MMQMTA were prepared in 5 repetitions23.

- *Linearity:*

A linearity response in the range of 24–24 mg/ml of MMQMTA linear regression data was given.

- *Precision:*

By dissolving a weight comparable to 20 mg of MMQMTA and dissolving it in enough mobile phase, a sample stock solution of MMQMTA (g/ml) was prepared. Following that, a 0.45-micron syringe filter was used to filter the solution, which was then sonicated for 5 minutes before being diluted with mobile phase to 100 ml. 24–26. 10 ml of the mobile

phase should be mixed with 1 ml of the stock solution; further dilutions of 20 g/ml of MMQMTA were prepared in 5 repetitions[24-26].

- Accuracy:

Study recovery studies determined the method's accuracy. The reference standards for the pharmaceuticals were added to the formulation (pre-analyzed sample) at levels of 80%, 100%, and 120%. The recovery percentage and mean recovery percentage were calculated for the drug and are displayed in the table following three recovery studies. To assess the accuracy of the approach, recovery tests were carried out by adding standard drug solution to pre-analyzed sample solution at three different levels: 80, 100, and 120 %.

- *Limits of Detection and Limits of Quantification:*

The limits of detection and quantification were obtained using the response standard deviation and calibration curve slope. The LOD for this approach was determined to be 0.021 g/ml and area 2.61 for MMQMTA. The LOQ for MMQMTA for this method was found to be 0.06 g/ml and area 7.91.

- *Robustness:*

By making minor adjustments to chromatographic parameters including the mobile phase ratio, buffer pH, and flow rate, the robustness of the approach was evaluated. The fact that there were no obvious variations in the chromatograms shows that the established RP-HPLC procedure is reliable.

V. RESULTS AND DISCUSSION

Chromatographic separation was accomplished on a Hypersil C18 column. 235 nm, on the basis of the isobathic point, was chosen as the ideal wave length for the determination of MMQMTA. Several experiments were conducted using several mobile phases in various ratios. The ratio of 60:40 ammonium acetate:methanol was chosen because the peaks had high peak symmetry. 5 MMQMTA was shown to have a retention time of 6.812. The retention periods for MMQMTA were much lower than those seen for the drugs in the other mobile phases. According to ICHQ2B criteria from the International Conference on Harmonization, the various analytical performance metrics, such as linearity, precision, accuracy, and specificity, LOD, and LOQ, were calculated. Plotting peak area vs concentration for MMQMTA over the concentration range of 4–24g/mL allowed for the creation of the calibration curve [27-30]. Based on linearity, the correlation coefficient R^2 value for MMQMTA was determined to be 0.999.

Additionally, the system appropriateness, system precision, and method precision of the suggested HPLC method were validated. Less than 2% of RSD was discovered to exist in the drug's peak area. The column performed effectively, as indicated by the discovery that the number of theoretical plates was not less than 2000. MMQMTA has detection limits of 0.021 g/ml and an area of 2.61. MMQMTA's limit of quantitation was reported to be 0.05 g/ml and area of 6.812. The MMQMTA recovery rate was determined to be 99.98%.

- *Accuracy:*

Table-1: Development and validation of an analytical approach for MMQMTA simultaneous estimation in bulk drugs.

% Recovery				
Level (%) / pptn	Smpl Wt (in mg)	Conc (in ppm)	Area (□v)	% Recovery
20_1	4.02	4.02	1154876	98.83
20_2	3.96	3.96	1151152	100.00
20_3	4.01	4.01	1154450	99.04
60_1	12.04	12.04	3474265	99.27
60_2	12.01	12.01	3473840	99.50
60_3	11.95	11.95	3474384	100.02
80_1	16.02	16.02	4665098	100.18
80_2	16.15	16.15	4680942	99.71
80_3	16.06	16.06	4677318	100.19
100_1	20.05	20.05	5837585	100.16
100_2	20.14	20.14	5836985	99.70
100_3	19.98	19.98	5832639	100.43
120_1	24.31	24.31	7139200	101.03
120_2	24.47	24.47	7182099	100.97
120_3	24.56	24.56	7190309	100.72

Overall % Recovery	99.98
Overall STDEV	0.66
Overall % RSD	0.66

- *Precision:*

Table-2: Precision study of MMQMTA

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.32	5791853	99.93
Sample-2	20.36	5796396	99.81
Sample-3	20.42	5817114	99.88
Sample-4	20.40	5830713	100.21
Sample-5	20.30	5780994	99.84
Sample-6	20.40	5805699	99.78
Average	NA	NA	99.91
STDEV	NA	NA	0.16
% RSD	NA	NA	0.16

Observation: The percent RSD of the assay results is within limits, according to test results for MMQMTA and Table-2 in the results was displayed.

- *Specificity:*

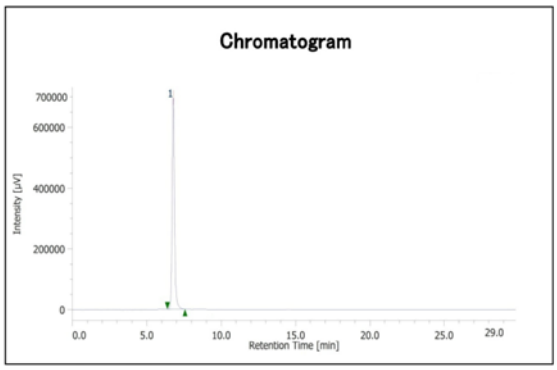

Figure-1: Chromatograph of MMQMTA specificity

Table-3: Specificity of MMQMTA

#	Peak Name	CH	tR [min]	Area [μV sec]	Height [μV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	1	6.812	7144142	696342	100.000	100.000	N/A	14234	N/A	1.154	

Observation: The MMQMTA peak is not being interfered with by diluents or excipient peaks, as can be seen from the data above.

Limit of Detection:

The LOD for this approach was determined to be 1.54 g/ml and area 104.45 for MMQMTA.

Limit of Quantification:

LOQ = $\frac{10\sigma}{S}$

σ = the response's standard deviation

S = the calibration curve's slope

The calibration curve of the analyte can be used to estimate the slope S.

Observation:

MMQMTA LOQ for this approach was discovered to be 0.05 g/ml and area 6.812.

Linearity and Range:

Table-4: Linearity of 5 MMQMTA

Conc ppm	Replications	Peak Area Counts	Means Area
4.024	R1	1154876	1152441.5
	R2	1150007	
8.048	R1	2284211	2283585.5
	R2	2282960	
12.072	R1	3465205	3464652.5
	R2	3464100	
16.096	R1	4618858	4618183.5
	R2	4617509	
20.12	R1	5833185	5825213.5
	R2	5817242	
24.144	R1	7030736	7025546.5
	R2	7020357	

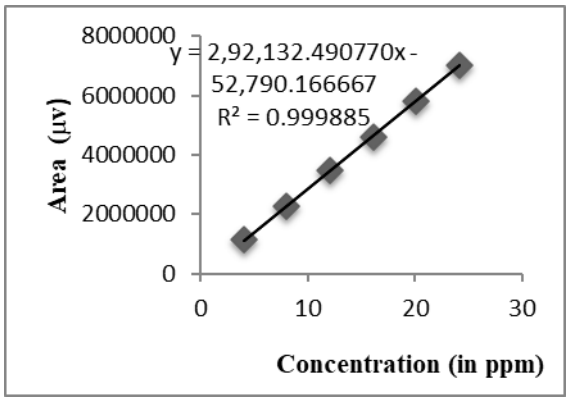

Figure-2: Linearity graph of MMQMTA

Robustness:

Table-7: Result of Robustness study

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.34	6920473	99.46

	% Content	Absolute Difference
Precision	99.91	0.45
Rob_1.05 mL	99.46	

CONCLUSION

It was observed that the method developed for the simultaneous estimation of MMQMTA was straightforward, exact, accurate, and of high resolution. This method can be successfully used for routine analysis in research institutions, quality control departments in intended industries, approved testing laboratories, and bio-pharmaceutical firms thanks to its shorter retention time, which also increases its acceptability and efficiency [30-31].

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