RESEARCH ARTICLE OPEN ACCESS

RP-HPLC ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR NEWLY SYNTHESIZED *N*-{[6-METHOXY-2-(MORPHOLIN-4-YL) QUINOLIN-3-YL]METHYL}-4*H*-1,2,4-TRIAZOL-4-AMINE

Yogita Shinde[1], and Kalpana Patankar-Jain[2]

[1] Chemistry Dept, K. C. College, Churchgate, Mumbai - 400 020.

[2] Dept of Chem, BNN College, Bhiwandi, Dist:Thane-Maharashtra, India.

ABSTRACT

Development of an analytical technique and RP-HPLC validation for N-[6-methoxy-2-(morpholin-4-yl)quinolin-3-yl]methyl-4H-1,2,4-triazol-4-amine (MMQMTA). The HYPERSIL column utilised for the analysis was run on using an acetonitrile:water mobile phase with a ratio of 80:20v/v and a flow rate of 0.5ml/min (UV detection at 340nm). The MMQMTA retention period lasted 7.687 minutes. The linear response of MMQMTA in the concentration range of 4-24 ppm was demonstrated, and its correlation coefficient (or "r" value) was 0.9998. The created method was validated in terms of precision, accuracy, linearity, selectivity, range, and force degradation studies, and it was discovered that the method was precise, accurate, linear, and specific. RSD was discovered to be greater than 2 percent for injector repeatability and inter-assay accuracy. The percentage recoveries for MMQMTA vary from 98.69 to 101.19 percent, with a mean recovery of overall percent of 100.22.

Keywords: MMQMTA, linearity, accuracy, precision, inter-assays precision.

INTRODUCTION

Analytical chemistry is a discipline of research that uses cutting-edge technology to determine the composition[1-5]. to produce accurate and high-quality analytical data[6], play a significant role in the analytical instruments. An accurate assay procedure to ascertain the composition of the formulation is the selection step in the development of analytical methods. The procedure of validating the analytical method involves demonstrating that it is appropriate for use in measuring the concentration in a lab for subsequent samples[7]. Instrumental RP-HPLC analytical methods must be created and applied in GLP and GMP settings[8-11].

EXPERIMENTAL

Standard samples of MMQMTA are utilised by in-house double purification and MMQMTA technical is used by in-house preparation. HPLC/AR grade solvents were employed[12].

Chromatographic conditions

The study was conducted using an HPLC system (HPLC, Shimadzu, LC-20AD with PDA detector) and a HYPERSIL (C18, 5.0 x 250 x 4.6mm) column. Acetonitrile:water (80:20v/v) was used as the mobile phase, and isocratic elution was done with a flow rate of 0.5 ml/min with 340 nm detection. Data processing software called Empower was used for the HPLC system.

Stock solution of standard

By accurately weighing 100 mg of the MMQMTA standard, adding 5 mg of acetonitrile, and diluting it up to 20 mg with acetonitrile, a stock solution of the standard was created (stock solution-I). Transfer 5 ml of the aforementioned solution to a 50 ml volumetric flask and dilute it with acetonitrile as directed by the stock solution-II label.

Stock solution of sample

100 mg of the MMQMTA sample were diluted with acetonitrile to make 10 mL[13]. 5ml of the aforementioned solution was transferred into a 50ml volumetric flask and diluted to the proper strength using acetonitrile.

Calibration curve

Standard solution was pipetted into 100 ml volumetric flasks for MMQMTA. With acetonitrile, the concentration range for MMQMTA was 4, 8, 12, 16, 20 and 24 ppm of volume. Duplicate dilutions of each medication were made separately for each concentration. 20-l injections of each concentration of MMQMTA were made separately from these duplicate solutions into the RP-HPLC equipment and chromatograph under the predetermined conditions. A 254nm UV detector was used to evaluate the MMQMTA[14–15].

Method Validation

The method's numerous parameters, including robustness, force degradation, investigations of the appropriateness of the system, precision, selectivity, accuracy, range, and linearity, have all been validated.

Specificity

By scanning the diluent solution and the standard solution of MMQMTA at a concentration of 20 g/ml, specificity was achieved. To show that there is no interference during the retention time of MMQMTA from any reagent or solvent (mobile phase) blank, derivatized[16] solutions of MMQMTA were injected into the chromatographic system after injecting solvent blank, reagent blank, and sample blank.

Linearity

The concentration of different levels, such as 4, 8, 12, 16, 20 and 24 ppm, was generated from a stock solution as the assay method for linearity test solutions. 20µl of each solution was injected into the HPLC apparatus, and the peak area from the chromatogram that was produced was documented. Least squares linear regression was used to evaluate the peak area versus concentration data. The calibration curve's slope and y-intercept were reported.

Precision

By determining the linearity range of the MMQMTA mixture on different days and on the same day, different analysts, different columns, etc., the suggested method precision (intra-day precision and injector repeatability) fixed concentration of six replicates was established.

Accuracy (Recovery studies)

Comparing the area before and after the addition of the working standard allowed us to calculate the percent recovery. Recovery was carried out the same way for both medicines. This common addition technique was used at levels of 20%, 60%, 80%, 100%, and 120%, and the percentage recovery was calculated.

RESULTS AND DISCUSSION

The MMQMTA was detected at 340 nm utilising a designed and validated RP-HPLC MMQMTA compound resolution method on a HYPERSIL RP C18 column employing an acetonitrile:water (80:20v/v) acetonitrile:water ratio. Overall, the data showed that the excipients did not interact with MMQMTA peaks, showing that the approach is selective. Analyses were finished and divided in within 10 minutes[17].

HPLC method optimization and development:

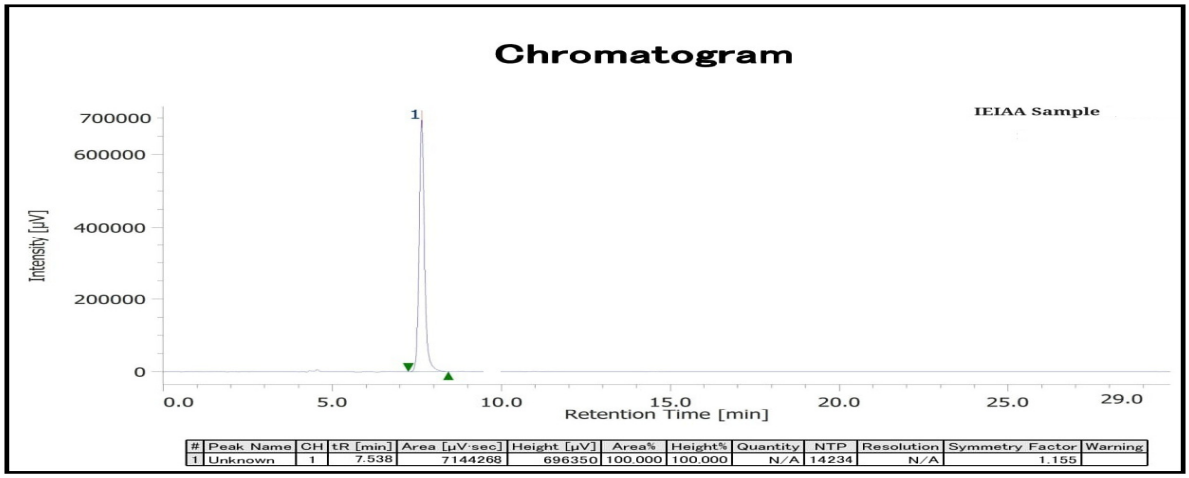
The samples were originally examined in mobile phase with a flow rate of 0.5 mL/min of water:acetonitrile (20:80, v/v). Well-resolved peaks with good sharpness and symmetry are present under these circumstances. In order to achieve the best chromatographic response during the entire investigation, acetonitrile:water mobile phase was selected[18].

System suitability studies of method validation:

Complete testing was done on the system to make sure it was appropriate for the intended purpose. Measurements of the different characteristics revealed a peak at a time of 7.687 min, with an average retention time of less than 2.0, a variable peak area, a tailing factor of less than 2, and more than 2000 observed theoretical plates for the MMQMTA peak. The suggested method's great sensitivity makes it possible to properly detect the peak[19]. In each instance, the MMQMTA was successfully excipients from the peak.

Specificity:

The retention time of MMQMTA was 7.687min, there was no any peak interfering from the blank at the retention time of MMQMTA and hence the determination of MMQMTA proposed method is specific[20-22].



#	Peak Name	CH	tR [min]	Area [μV·sec]	Height [μV]	Area%	Height%	Quantity	NTP	Resolution	Symmetry Factor	Warning
1	Unknown	1	7.538	7144268	696350	100.000	100.000	N/A	14234	N/A	1.155	

Figure-1: Specificity peak purity chromatogram of MMQMTA

Linearity

MMQMTA's linear calibration curve was found to cover the 4–24 ppm concentration range. The calibration curves of the regression equations (Figure-2) were determined to be Y = 329,556.503891x + 628,290.300000 (Figure-2) with a coefficient of correlation of 0.9998, which is equivalent to unity[23], for the MMQMTA data for the peak area in treatment was concentration.

Table-1: Linearity data of MMQMTA [24]

Linearity Sol Level	Conc ppm	Replications	Peak Area Counts	Means Area
L1	4.002	R1	1827272	1848070
		R2	1868868	
L2	8.004	R1	3398969	3348594.5
		R2	3298220	
L3	12.006	R1	4630008	4648431.5
		R2	4666855	
L4	16.008	R1	5923290	5914624.5
		R2	5905959	
L5	20.01	R1	7176955	7176062.5
		R2	7175170	
L6	24.012	R1	8530736	8530546.5
		R2	8530357	

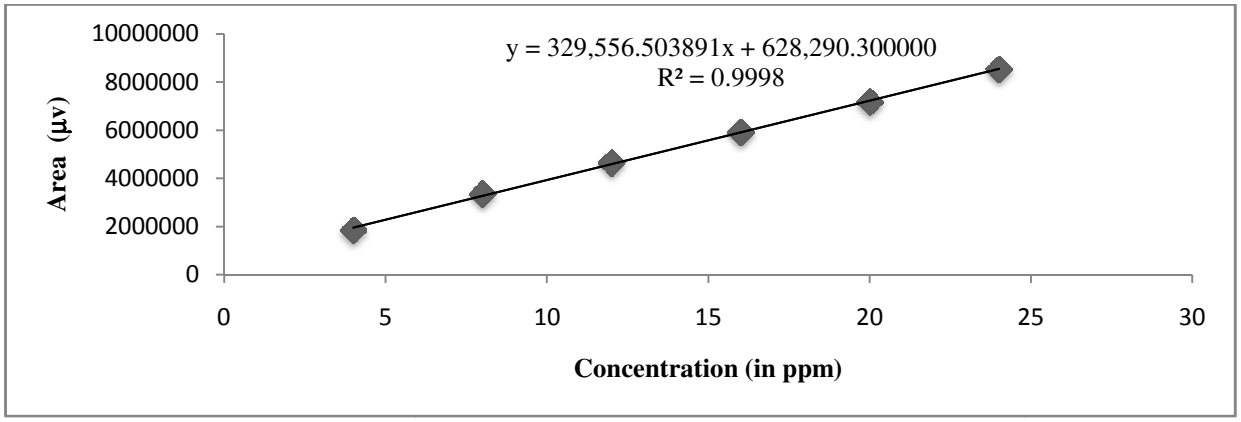


Figure-2: Linearity graph of MMQMTA standard

Precision

The precision (intra-assay and injector repeatability) of the method were determined of the isoeugenolindole-3-aceticacid standard solutions. The % RSD for repeatability and intra-assay precisions was less than 2% found indicated that high degree of precision[25].

Table-2: Injection repeatability (precision) for MMQMTA.

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.02	7144669	99.73
Sample-2	20.06	7144648	99.53
Sample-3	20.02	7134179	99.58
Sample-4	20.09	7143248	99.36

Sample-5	20.03	7103493	99.10
Sample-6	20.07	7134145	99.33
Average	NA	NA	99.44
STDEV	NA	NA	0.22
% RSD	NA	NA	0.22

Table-3: Intra-assay (precision) data of MMQMTA technical.

Sample no.	Conc in ppm	Area (mv)	% Content
Sample-1	20.02	7144547	99.63
Sample-2	20.10	7144478	99.24
Sample-3	20.20	7214122	99.71
Sample-4	20.03	7143214	99.56
Sample-5	20.01	7134704	99.55
Sample-6	20.05	7134670	99.35
Average	NA	NA	99.51
STDEV	NA	NA	0.18
% RSD	NA	NA	0.18

Table-4: Comparison between analyst-1 and 2

	Mean % Content	Absolute Difference
Analyst 1	99.44	-0.07
Analyst 2	99.51	

Accuracy

Less than 2.0 with an overall percent mean recovery of 100.27 for MMQMTA and a recovery resulting percent RSD found to be 98.28 -101.65 percent. This proves that the procedure is free of interference from the blank, whether it be positive or negative. As a result of the aforementioned finding, it was determined that the analyte's recovery data fell within the acceptable range and that the suggested approach is reliable[26].

Table-5: Accuracy data for MMQMTA technical.

Level (%) / pptn	Smpl Wt (in mg)	Conc (in ppm)	Area (mv)	% Recovery	% Mean Recovery	STDEV	% RSD
20_1	4.09	4.09	1468746	100.39	100.27	0.10	0.10
20_2	4.10	4.10	1466249	99.98			
20_3	4.09	4.09	1466978	100.27			
60_1	12.04	12.04	4301247	99.87	99.28	0.70	0.71
60_2	12.01	12.01	4273147	99.47			
60_3	12.00	12.00	4234579	98.65			
80_1	16.27	16.27	5904180	101.45	101.65	0.22	0.22
80_2	16.28	16.28	5906978	101.44			
80_3	16.21	16.21	5902478	101.80			
100_1	20.13	20.13	7182145	99.74	99.85	0.08	0.08
100_2	20.09	20.09	7175255	99.85			
100_3	20.10	20.10	7183676	99.92			
120_1	24.31	24.31	8681325	99.83	100.28	0.32	0.32
120_2	24.27	24.27	8687461	100.07			
120_3	24.16	24.16	8687517	100.53			
Overall % Recovery				100.22			
Overall STDEV				0.82			
Overall % RSD				0.82			

Range:

The range for the MMQMTA is evaluated[26] from 20% i.e. 4 ppm to 120% i.e. 24 ppm.

Table-6: Range for MMQMTA

Solution	20% (4 ppm)	120% (24 ppm)
1	1767456	8696211
2	1767758	8696451
3	1767245	8695478
4	1742242	8697240
5	1768968	8697232
6	1767376	8697254
Average	**1763507.50**	**8696644.33**
STDEV	**10436.76**	**729.02**
% RSD	**0.59**	**0.01**

Force degradation Studies:

Overall, MMQMTA showed to be a stable drug substance in metallic condition (0.05M FeCl3), basic condition (1N NaOH), acidic condition (1N HCl), oxidation condition (3 percent H2O2), photolytic condition (exposure to 1.2 million lux/hour), reduction condition (1 percent Na2S), and thermal degradation conditions of 105°C.

Each degradant peak is clearly differentiated from the blank and main peaks in all of the aforementioned degradation conditions. The relationship between the rise in degradant impurities and the fall in assay value for MMQMTA was satisfactorily determined. The above method for MMQMTA by HPLC is concluded to be a specific and stability indicating method27 based on the aforementioned validation data.

Table-7: Forced degradation calculation of MMQMTA technical

Condition	Smpl Wt (in mg)	Conc (in ppm)	Area (mv)	% Assay	% Total Imp.	Mass Balance
As such	20.19	20.19	7112987	100.48	0.000	NA
0.05M_FeCl3_24 Hrs	20.06	20.06	7002789	99.56	0.131	99.2
1N_NaOH_24 Hrs	20.19	20.19	7092145	100.19	0.539	100.2
1N_HCl_24 Hrs	20.09	20.09	7014789	99.59	0.143	99.3
3% H2O2_24 Hrs	20.11	20.11	7024589	99.63	1.476	100.6
1% Na2S_24 Hrs	20.03	20.03	7103458	101.15	1.044	101.7
Photo @ 1.2 million lux/Hr	19.96	19.96	7008214	100.14	0.899	100.6
Thermal @ 105°C_24 Hrs	20.03	20.03	7102459	101.13	0.000	100.6

Table-8: Force degradation of MMQMTA of impurity profile

% impurity (by Area normalization)								
RT about -->	**Unk @ 2.90**	**Unk @ 3.15**	**Unk @ 3.26**	**Unk @ 3.45**	**Unk @ 3.99**	**Unk @ 6.82**	**Unk @ 8.32**	**Total Imp**
As such	-	-	-	-	-	-	-	0.000
0.05M_$FeCl_3$_24 Hrs	-	-	-	-	-	0.131	-	0.131
1N_NaOH_24 Hrs	0.0812	-	0.204	0.254	-	-	-	0.539
1N_HCl_24 Hrs	-	0.078	-	-	0.065	-	-	0.143
3% H_2O_2_24 Hrs	-	-	1.476	-	-	-	-	1.476
1% Na2S_24 Hrs	-	-	1.044	-	-	-	-	1.044
Photo @ 1.2 million lux/Hr	-	-	-	-	-	-	0.899	0.899
Thermal @ 105°C_24 Hrs	-	-	-	-	-	-	-	0.000

CONCLUSION

The method was found to be specific, accurate, exact, and robust when used with the MMQMTA chemical that was validated, produced, and utilised for MMQMTA determination. Pharmaceutical dose form MMQMTA elutes quickly (within 8 minutes), and no interference was observed. In conclusion, the proposed approach is appropriate due to the high repeatability, accuracy, good selectivity, and sensitivity of MMQMTA for simultaneous determination.

REFERENCES

1. A. Alnajjar, *Journal of AOAC International*, **94(2)**, 498(2011). doi:10.1093/jaoac/94.2.498.
2. S. Sweetman and C. Martindale, The Complete Drug Reference. 34th edition, London: Royal Pharmaceutical Society of Great Britain. 2005; 996.
3. S. Bari, S. Sathe, P. Jain and S. Surana, *Journal of Pharmacy and Bioallied Sciences*, **2(4)**, 372(2010). doi:10.4103/0975-7406.72144.
4. S. Charde, L. Kumar and R. Saha, *Analytical Letters*, **40(11)**, 2128(2007). doi:10.1080/00032710701566636.
5. V. Gurumurthy, R. Deveswaran, S. Bharath, B. Basavaraj, and V. Madhavan, *Invneti Rapid Pharm Ana and Qual Assur*, **2**, 287(2012). doi:10.13005/BPJ/339.
6. N. Raj, S. Anbazhagan, K. Babu, S. Babu and C. Bhimanadhuni, *International Current Pharmaceutical Journal*, **1(11)**, 336-341(2012). doi.org/10.3329/icpj.v1i11.12058.
7. M. Patil, S. Ganorkar, A. Patil, A. Shirkhedkar and S. Surana, *Critical Reviews in Analytical Chemistry*, 1(2020). doi:10.1080/10408347.2020.1718484.
8. A. Kasture and M. Ramteke, *Indian J Pharm Sci*, **68**, 394(2006). doi: 10.4103/0250-474X.26665.
9. M. Swamy, U. Sinniah and A. Ghasemzadeh, *Applied Microbiology and Biotechnology*, **102(18)**, 7775(2018). doi:10.1007/s00253-018-9223-y.
10. R. Ceresole, M. Moyano, M. Pizzorno and A. Segall, *Journal of Liquid Chromatography & Related Technologies,* **29(20)**, 3009(2006). doi:10.1080/10826070600983393
11. G. Tulja Rani, D. Gowri Sankar, P. Kadgapathi, B. Satyanarayana, *Journal of chemistry*, **8**, 1238(2011). doi.org/10.1155/2011/121420.
12. K. Vidhya Bhusari and R. Sunil Dhaneshwar, *ISNR Analytical chemistry*, **1**, 226(2012). doi:10.5402/2012/609706.
13. S. Dinakaran, B. Alluri, K. Annareddy, V. Ayyagari, H. Avasarala, R. Kakaraparthy and R. Gadi, *Journal of Pharmacy Research*, **7(7)**, 666(2013). doi:10.1016/j.jopr.2013.07.012.
14. M. Muchtaridi, M. Prasetio, Nyi Mekar Saptarini and Febrina Amelia Saputri, Rasayan J. Chem, **11 (3),** 973 - 978 (2018). **DOI:** http://dx.doi.org/10.31788/RJC.2018.1132098.
15. J. Bauer, J. Quick, S. Krogh and D. Shada, *Journal of Pharmaceutical Sciences*, **72**, 924(1983). https://doi.org/10.1002/jps.2600720821.
16. E. Ebeid, A. El-Zaher, R. EL-Bagary, and G. Patonay, *Analytical Chemistry Insights*, **33**(2014). doi:10.4137/aci.s13768
17. M. Stephen Walters and B. Dalia Stonys, *J Chromatogr. Sci*, **21**, 43(1983). doi.org/10.1093/chromsci/21.1.43.
18. U. Patil, S. Gandhi, M. Sengar and V. Rajmane, *Journal of the Chilean Chemical Society*, **55(1)** (2010). doi:10.4067/s0717-97072010000100022.
19. R. Mhaske, D. Garole, A. Mhaske and S. Sahasrabudhe, *Application To Commercially Available Drug Products*, **3**, 141(2012). doi:10.1.1.216.4744
20. K. Al Azzam, B. Saad and H. Aboul-Enein, *Biomedical Chromatography*, (2010). doi:10.1002/bmc.1395
21. S. Sa'sa', I. Jalal and H. Khalil, *Journal of Liquid Chromatography*, **11**, 1673(1988). doi.org/10.1080/01483918808076729.
22. S. Deshmukh, *Rasayan J. Chem*, **11 (3)**, 1159 - 1165 (2018). **DOI:** http://dx.doi.org/10.31788/RJC.2018.1134007
23. K. Al Azzam, A. Elbashir, M. Elbashir, B. Saad and S. Abdul Hamid, *Analytical Letters*, **42(10)**, 1458(2009). doi:10.1080/00032710902961065.

24. A. El-Gindy, S. Sallam and R. Abdel-Salam, *Journal of Separation Science*, **31(4)**, 677(2008). doi:10.1002/jssc.200700317.
25. M. Elgawish, S. Mostafa and A. Elshanawane, *Saudi Pharmaceutical Journal*, **19(1)**, 43(2011). doi:10.1016/j.jsps.2010.10.003.
26. J. Sandya Rani and N. Devanna, Rasayan J. Chem, 11 (2), 452 - 459 (2018). **DOI:** http://dx.doi.org/10.31788/RJC.2018.1122079.
27. ICH Validation of analytical procedures: Text and methodology Q2 (R1), International Conference on Harmonization, Geneva, Switzerland. 2005.

