International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 5, 2024

Original Article

QUANTITATIVE DETERMINATION OF METHYL-4-CHLOROBUTYRATE, A POTENTIAL GENOTOXIC IMPURITY, CONTENT IN MOXIFLOXACIN HCL BY GC-EI-MS

K. APARNA[1], K. VIJAYA RACHEL[1*], K. M. V. NARAYANA RAO[2]

[1]Department of Biochemistry/Bioinformatics, GIS, VSP, GITAM University, Visakhapatnam-530045, India. [2]Analytical Research and development, Aragen Life Sciences Pvt Ltd, Hyderabad-5000076, India

*Corresponding author: K. Vijaya Rachel; *Email: vkap@gitam.edu

Received: 24 May 2024, Revised and Accepted: 09 Jul 2024

ABSTRACT

Objective: Methyl-4-Chlorobutyrate (M4CB), a genotoxic impurity, was identified in the active pharmacological components of the fourth-generation fluoroquinolone, moxifloxacin (MXFN). There has not yet been a report on the analysis of the M4CB impurity content in the MXFN molecule. Consequently, a Gas Chromatography-Electron Ionization-Mass Spectrometry (GC-EI-MS) method was established that has the ability to identify and measure M4CB impurity content at ppm level.

Methods: The column exploited in M4CB impurity assay was a Dura Bond 624 (DB-624) type stationary column. Temperatures of 220 °C and 280 °C were consistently maintained at the injection and detection sites, respectively. The helium, as carrier gas, in split mode with ratio of 1:7 was used. The column's flow rate remained steady around 2.0 ml/min. The mass spectrometer was operated in Single Ion Monitoring (SIM) mode at m/z = 74.

Results: The impurity M4CB is generated during the manufacturing process of cyclopropanamine, which is an intermediary molecule in the manufacturing process of MXFN. This new GC-EI-MS approach can measure the M4CB at 0.9452 ppm in MXFN samples with a 500 mg/ml concentration following International Council for Harmonisation (ICH) standards. Very low quantification limits (0.9452 ppm), high linearity (range=0.945 ppm to 5.625 ppm; regression coefficient= 0.9999), and a reasonable recovery range (94.60-94.63%) were all provided by this new validated GC-EI-MS approach. Three batches were analysed for M4CB content by new GC-EI-MS approach and found that none of the batches contained M4CB impurity.

Conclusion: The GC-EI-MS approach has excellent applicability in the quality assurance testing of MXFN for M4CB content since it was adequate in terms of linearity, precision, sensitivity, accuracy, specificity, and robustness.

Keywords: Fluoroquinolone, Moxifloxacin, Methyl-4-Chlorobutyrate, Genotoxic impurity, Quality control, GC-EI-MS

DOI: https://dx.doi.org/10.22159/ijap.2024v16i5.51551 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

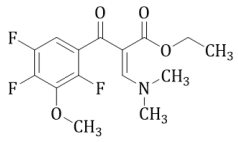
INTRODUCTION

A fluoroquinolone of the fourth generation called moxifloxacin (MXFN) has extended action against g-positive microbes, including variants of *Streptococcus pneumoniae* that are multidrug tolerant [1]. Acute bacterial sinusitis, community-acquired pneumonia, and acute complications of chronic bronchitis are among the respiratory path diseases that MXFN effectively treats [2-4]. Additionally, MXFN is suggested for the medical management of moderate infections of the skin and its supporting structures. MXFN shown to have a lower propensity than certain other fluoroquinolones to induce phototoxic and excitatory impacts on the central nervous network [5].

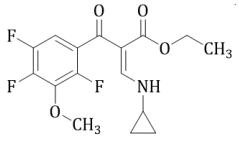
The majority of pharmaceutical drugs are created either by complete synthesis or by altering a naturally existing substance. In both circumstances, a diverse set of reactive reagents are employed [6, 7]. As a result, it is common for small quantities of those reagents or side products to be detectable as impurities in the ultimate active ingredient or ultimate medicinal product. These impurities may be hazardous, causing genotoxicity as well as carcinogenicity. The health risk posed by the appearance of small compounds as impurities in the ultimate active ingredient has become a growing concern for pharmaceutical firms, patients, regulatory agencies, in addition to doctors [8]. Thus, pharmaceutical regulatory organisations like the "Food and Drug Administration" and the "European Medicines Agency" have expressed their concern pertaining to the existence of genotoxic impurities in the ultimate active ingredient or ultimate medicinal product, which possibly will let down human health negatively [9, 10].

We have identified the production of three genotoxic contaminants, GTS-STG-1A (fig. 1), GTS/STG-1B (fig. 1) and methyl-4-chlorobutyrate (M4CB, fig. 1) during MXFN synthesis. According to an *in silico* toxicological examination using Vega software, we have noticed that M4CB, GTS-STG-1A and GTS/STG-1B as genotoxic impurities. In our earlier investigation study work, it was explained how LC-MS/MS may be used to monitor GTS-STG-1A and GTS/STG-1B in MXFN [11]. Since the impurity M4CB is a volatile aliphatic molecule that is not chromophoric, Gas Chromatography-Electron Ionization-Mass Spectrometry (GC-EI-MS) is the most appropriate methodology for determining it. This study mainly focuses on the establishment and comprehensive validation of a GC-EI-MS approach for the measurement of impurity M4CB concentration in MXFN.

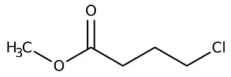
Djurdjevic *et al.* used RP-HPLC to investigate four synthesis-connected impurities in MXFN tablets and MXFN infusion and measured each of them as 0.1% of the overall drug [12]. The RP-HPLC was adopted by Vankalapati *et al.* to monitor MXFN-related compounds in MXFN therapeutic products [13]. Cai *et al.* observed ten MXFN-related impurities by the way of High-Performance Liquid Chromatography-Ultra Violet (HPLC-UV) technique, and eight MXFN-related impurities implementing highly precise molecular mass paired with multiple-phase mass spectrometric measurements [14]. Li *et al.* exploited the Liquid Chromatography/Mass Spectrometry (LC/MS/MS) technology for conducting a pharmacokinetic investigation of MXFN-N-sulfate following a one-time oral dosage of MXFN in rats [15]. The M4CB impurity was not identified/quantified in which ever of the analytical approaches [12-15] that have been reported for determining specific MXFN-related substances. Since M4CB is a genotoxic impurity, it is essential to identify and measure the M4CB content in the MXFN molecule. The analysis of the M4CBimpurity content in MXFN will, therefore be done using the GC-EI-MS technique. Following International Council for Harmonisation (ICH) standards, a comprehensive validation of the approach was performed.



Ethyl-3-dimethylamino)-2-(2,4,5-tri fluoro-3-methoxybenzoyl) acrylate (GTS-STG-1A)



Ethyl-3-(cyclopropylamino)-2-(2,4,5-trifluoro-3-methoxybenzoyl) acrylate (GTS/STG-1B)



Methyl-4-chlorobutyrate (M4CB)

Fig. 1: IUPAC name and structures of GTS-STG-1A, GTS/STG-1B and methyl-4-chlorobutyrate

MATERIALS AND METHODS

Chemicals

In this study, 1,3-dimethyl-2-imidazolidinone (given by "Honeywell research chemicals", India), M4CB (given by "Sigma Aldrich", India) and MXFN batch samples (given by "Aragen Life sciences private limited", India) were used.

Optimized conditions and instruments

Chromatographical analysis of M4CB impurity was accomplished by "Aligent" Gas chromatograph 7890B separating module having fitted by a 7693A module autosampler, 7697A module Head Space, 5977A module Mass selective detector and Open Lab 2. X module software. The column exploited in M4CB impurity assay was a Dura Bond 624 (DB-624) type stationary column (length measure of 30 m; identification measure of 0.32 mm; particle measure of 1.5 µm). At the points of injection and detector, respectively, the temperatures of 220 °C and 280 °C were maintained. The helium, as carrier gas, in split mode with ratio of 1:7 was used. The column's flow rate remained steady around 2.0 ml/min. The temperature rise programme that was predetermined included: 80 °C (set for 2 min), raised by 10 °C/min to 210 °C (set for 1 min), and raised by 25 °C/min to 240 °C, which was upheld for 5 min. Gas flow parameters in the detector included 40 ml/min for hydrogen flow, 400 ml/min for air flow, and 25 ml/min for carrier gas (helium) flow.

Headspace analysis criteria were outlined as follows: maintained 90 °C (near oven), 160 °C (near Loop), and 165 °C (transfer line); maintained 33 min (for cycle time), 0.05 min (for loop equilibration), 2.0 min (for pressure equilibration), 5.0 min (for vial equilibration) and 3.0 min (for injection). Maintained pressure of 14 psi (at fill) and 4 psi (at final loop).

According to the information provided, the ion source used electron impact ionization in Selective Ion Monitoring (SIM) manner with high resolution and maintained 70 eV (electron energy), 230 °C (temperature near source), 150 °C (temperature near Quadruple), 74/500 mS (dwell time), 5.6 min (solvent delay), 14 min (MS off) and 15 (gain factor).

Solutions

Stock M4CB solution were prepared at 470 ppm level of quantity by dissolving 23.5 of M4CB in 1,3-dimethyl-2-imidazolidinone. Working M4CB solution was made at 3.75 ppm level of quantity through apt dilution of stock M4CB solution with 1,3-dimethyl-2-imidazolidinone. Added 0.5 ml of milli-Q-water plus 0.5 ml of working M4CB solution (3.75 ppm) to every one of the 20 ml headspace vials before sealing with a septum and crimping the vials right away.

For blank solution, added 0.5 ml of Milli Q water plus 0.5 ml of 1,3-dimethyl-2-imidazolidinone to every one of the 20 ml headspace vials before sealing with a septum and crimping the vials right away.

In each of the 20 ml headspace vials, for the test MXFN sample, 500 mg of the test MXFN sample and 0.5 ml of 1,3-dimethyl-2-imidazolidinone were carefully transferred before being immediately sealed using septum and crimped.

Calibration curve for M4CB

The stock M4CB solution (470 ppm) was consecutively diluted with 1,3-dimethyl-2-imidazolidinone to produce M4CB samples with 0.945 ppm to 5.625 ppm concentration range. The produced M4CB samples (range: 0.945 ppm to 5.625 ppm) were evaluated by methodology in section titled "optimized conditions and instruments". A curve of calibration for M4CB and regression analysis was generated employing the data on M4CB peak area and concentration that was gathered.

M4CB content assay in test MXFN sample

Analyzed either one or two injections of sample blank, six injections of working M4CB solution, either one or two injections of a sample blank again and finally one injection of test MXFN sample using methodology in section titled "optimized conditions and instruments". Using either the calibration curve, the regression equation, or the subsequent formula, computed the total quantity of M4CB (in ppm) in the test MXFN sample.

Methyl-4-chlorobutyrate content (ppm) =

$$\frac{(\text{Average Area in sample} - \text{Blank area})}{(\text{Average area from standard} - \text{Blank area})} \times \frac{\text{wt. of the standard in mg}}{50} \times \frac{2.0}{50} \times \frac{5.0}{50} \times \frac{1.0}{\text{wt. of the sample in mg}} \times 10^6$$

RESULTS AND DISCUSSION

Formation of impurity

The "Aragen Life Sciences" established the MXFN (API) production method (fig. 2), which was validated and used for producing the MXFN monohydrochloride. The same was submitted to Certification of Suitability (CEP) and World Health Organization (WHO). An intermediary molecule in the manufacturing process of MXFN API is cyclopropanamine. The impurity M4CB is generated during the manufacturing process of cyclopropanamine (fig. 3). The M4CB is produced from dihydrofuran-2(3H)-one through hydrolysis with acid catalyst, followed by chlorination with $SoCl_3$ and esterification with methanol.

Optimization of GC-EI-MS conditions forM4CB content assay

Chromatographical analysis of M4CB impurity was bring-about by "Aligent" Gas chromatograph 7890B separating module having fitted by a 5977A module mass selective detector and Open Lab 2. X module software. The split mode where a 1:7 ratio was applied to get the optimal sensitivity. At 220 °C, the injection point temperature got established. Helium was employed as a make-up gas maintained an average rate of flow near 25 ml/min while the detector was operated at an even temperature of 280 °C. The column exploited in M4CB assay was a DB-624 type stationary column (length measure of 30 m; identification measure of 0.32 mm; particle measure of 1.5 µm). The programme optimized for the column temperatures were 80 °C to 210 °C at raise of 10 °C/min and then to 240 °C at raise of 25

°C/min which was upheld for 5 min at 240 °C. The maximum sensitivity for detection was obtained by GC-EI-MS with SIM at *m/z* = 74. The optimised GC-EI-MS configurations showed higher specificity as well as sensitivity for M4CB impurity (fig. 4).

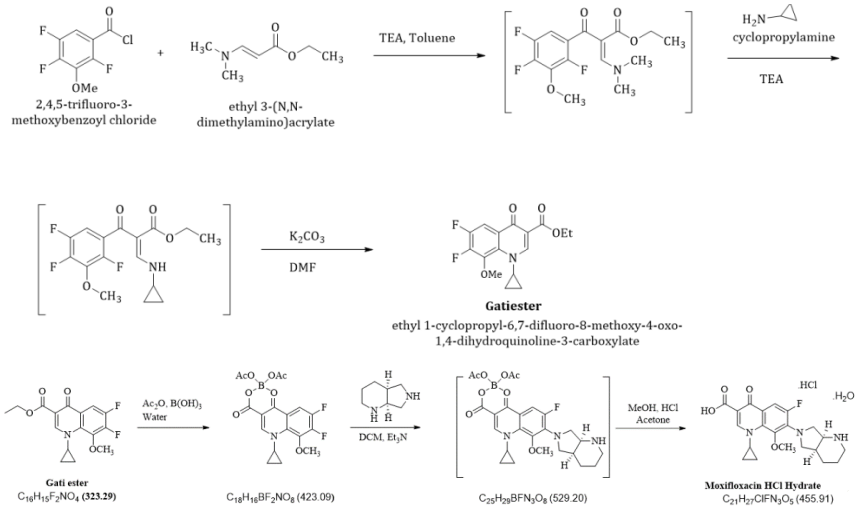


Fig. 2: MXFN molecule manufacturing pathway

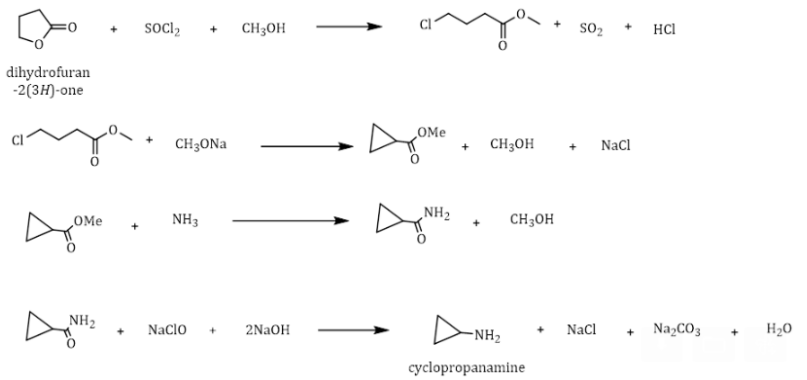


Fig. 3: Methyl-4-chlorobutyrate generation during manufacturing process of cyclopropanamine

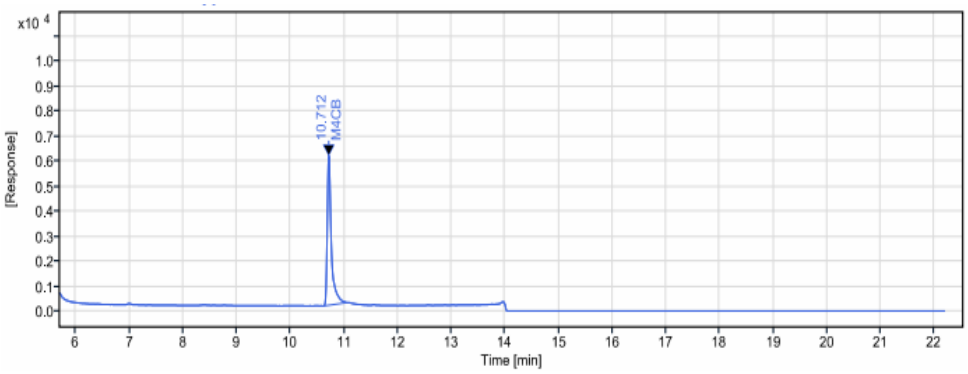
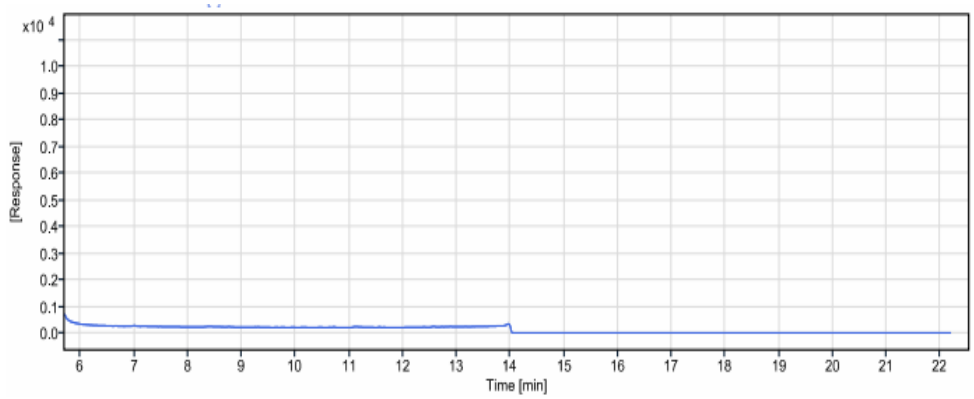


Fig. 4: M4CB chromatogram with optimized M4CB content assay conditions

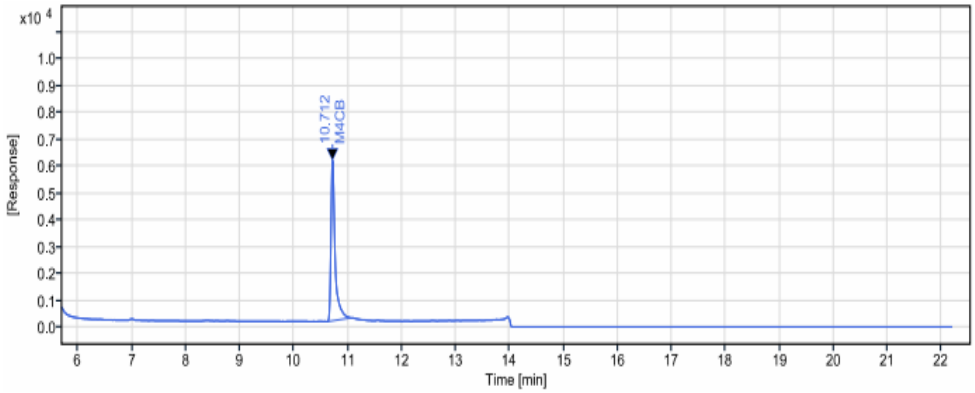
Validation

Specificity

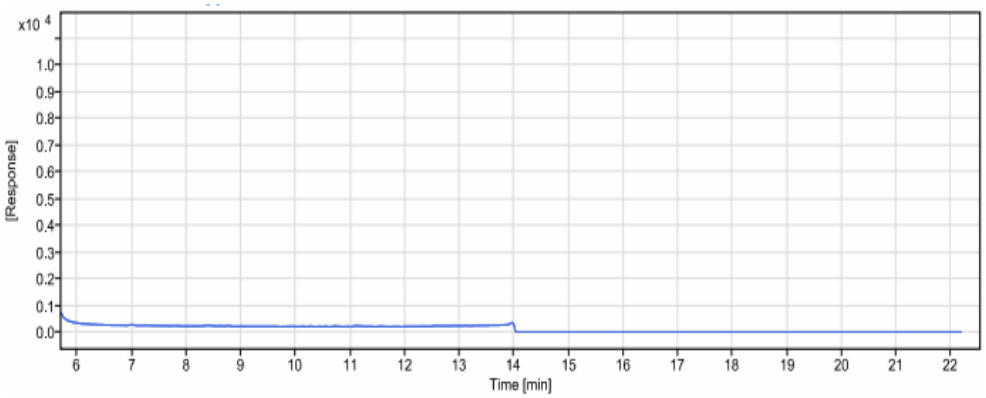
The sample blank, the standard M4CB solution (3.75 ppm), the test MXFN sample, and the specificity solution (test MXFN spiked with M4CB at 3.75 ppm) were all assessed to confirm the specificity. As demonstrated in fig. 5, by analysing the four respective sample chromatograms, the specificity of the M4CB assessment was validated. Other than the test MXFN and blank solution chromatograms, the other two solution chromatograms had an M4CB peak. No additional peaks were found in the chromatogram acquired with specificity solution or the chromatogram with standard M4CB solution around the 10.712 min where the M4CB is eluted.



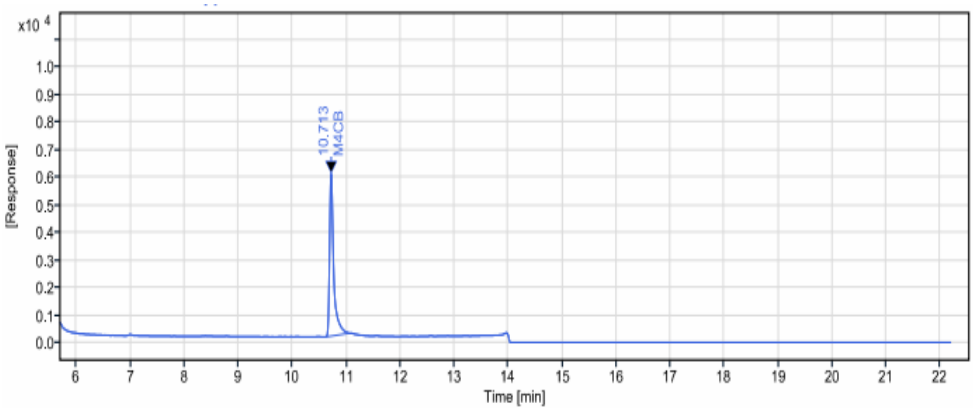
Sample blank



Standard M4CB solution



Test MXFN sample

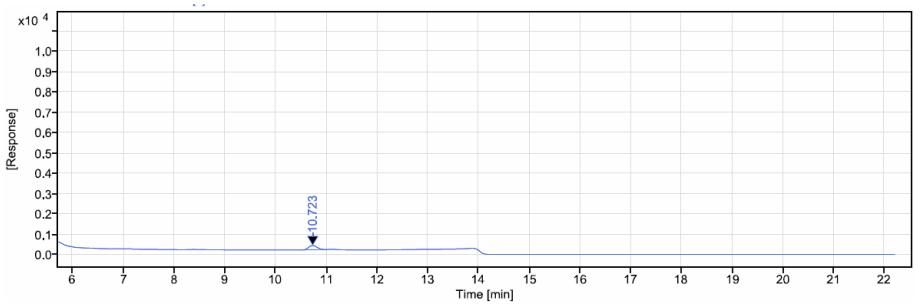


Specificity solution

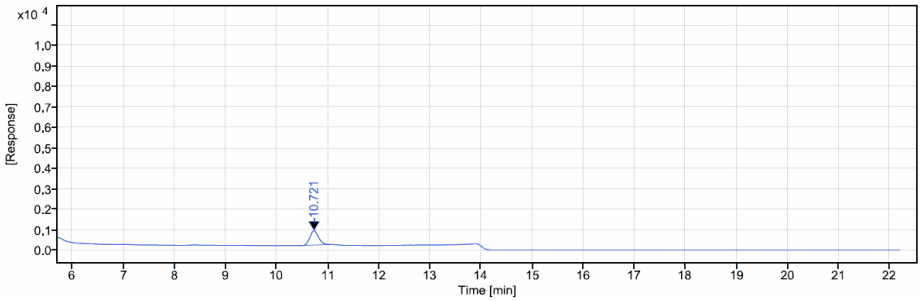
Fig. 5: Specificity-relevant representative chromatograms

LOD and LOQ

The LOD was observed to be 0.2836 ppm M4CB with S/N (signal response: noise response) of 13.8 while it was assessed at a level wherein S/N is>3. The LOQ was assessed at a level wherein S/N is>10 and % RSD for peak response is beneath 15% for six repeated assessments. A 0.9452 ppm M4CB standard was infused and assessed. Six injections had a % RSD of 4.19% and a S/N of 45.5. As a result, a 0.9452 ppm LOQ for M4CB was shown. Fig. 6 displays the characteristic LOD and LOQ level M4CB chromatograms.



LOD



LOQ

Fig. 6: LOD and LOQ-relevant representative chromatograms

Linearity

Six standard M4CB solutions prepared in 1,3-dimethyl-2-imidazolidinone solvent were applied as injections to assess the technique's linearity in the concentrations that ranged from 0.945 ppm to 5.625 ppm (LOQ to 150% test quantity level). The regression line was: M4CB area response = 8024.4 × M4CB concentration-100.58 (fig. 7).

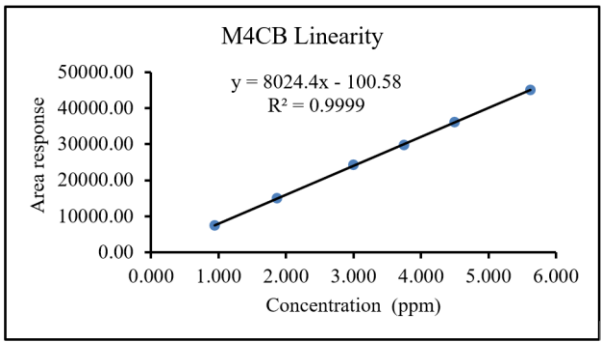


Fig. 7: M4CB linearity

Method precision

Six repetition injections of the M4CB-spiked MXFN sample at quantities of 3.75 ppm and 0.9452 ppm were carried out to show repeatability. The findings from the calculation of the peak area's % RSD are outlined in table 1. The observed % RSD in the M4CB-spiked MXFN sample was 4.194% for 0.9452 ppm test level and 2.041% for 3.75 ppm test level.

Accuracy

Three duplicates of the MXFN sample with additional M4CB concentrations equal to the LOQ (0.98 ppm), 50% (1.875 ppm), 100% (3.78 ppm) as well as 150% (5.625 ppm) test quantity level were analysed to fig. out the accuracy. The observed recovery (table 2) in the M4CB-spiked MXFN sample was 96.62% for 50% (1.875 ppm) test quantity level, 94.63% for 100% (3.78 ppm) test quantity level, 97.45% for 150% (5.625 ppm) test quantity level and 94.60% for LOQ (0.98 ppm) test quantity level.

Robustness

The technique parameters, which included the flow rate, gain factor, and injector temperature, were substantially modified in order to gauge the robustness. Working M4CB sample (3.75 ppm) was used for this. Calculations were made for percentage relative

difference in M4CB quantity between the optimized condition and each adjusted condition (table 3). The percentage relative difference values ranged from-15.80% to+16.77% for all the conditions under investigation.

Table 1: Method precision findings

Precision sample	M4CB area at	
	0.9452 ppm level	3.75 ppm level
1	7289.15	34422.4
2	7869.73	33225.6
3	7202.63	34314.4
4	7534.3	34556.6
5	7771.21	35080.2
6	7107.7	35190.9
Mean*	7462.453	34465.01
±S. D*	312.973	703.465
(%) R. S. D	4.194	2.041

*Data are expressed as mean±SD, n=6; M4CB-Methyl-4-Chlorobutyrate; RSD – relative standard deviation

Table 2: Method accuracy findings

Weight of the MXFN (mg)	Area of M4CB solution	M4CB (ppm)	After sample correction (ppm)	M4CB added (ppm)	Recovery (%)	Average*±SD
500.18	7569.93	0.89	0.89	0.98	90.74	94.60±4.102
500.08	8250.33	0.97	0.97		98.91	
500.20	7856.56	0.92	0.92		94.17	
500.24	14020.63	1.75	1.75	1.875	93.56	96.62±2.785
500.29	14578.10	1.82	1.82		97.28	
500.48	14837.35	1.86	1.86		99.01	
500.47	34422.39	3.62	3.62	3.78	95.86	94.63±1.840
500.52	33225.62	3.50	3.50		92.52	
500.64	34314.41	3.61	3.61		95.53	
500.12	42663.38	5.43	5.43	5.625	96.47	97.45±3.427
500.23	44781.74	5.70	5.70		101.26	
500.41	41845.23	5.32	5.32		94.62	

*Data are expressed as mean±SD, n=3; M4CB-Methyl-4-Chlorobutyrate; SD – standard deviation

Table 3: Method robustness findings

Condition	Value	M4CB (ppm)
Optimized conditions	See section titled "Optimized conditions and instruments"	4.21
High Flow	2.2 ml/min	4.21
Mean*±SD		4.21±0.00
%Relative difference		0.00
Low Flow	1.8 ml/min	4.88
Mean*±SD		4.55±0.474
%Relative difference		-15.80
Low Injection temperature	215 ℃	3.33
Mean*±SD		3.77±0.622
%Relative difference		10.99
High Injection temperature	225 ℃	3.82
Mean*±SD		4.02±0.276
%Relative difference		9.25
High Gain	17	2.66
Mean*±SD		3.44±1.096
%Relative difference		16.77
Low Gain	15	2.93
Mean*±SD		3.57±0.905
%Relative difference		10.55

*Data are expressed as mean±SD, n=2; M4CB-Methyl-4-Chlorobutyrate; SD – standard deviation

M4CB stability

The stability of M4CB in spiked MXFN sample was examined by keeping the solution at ambient thermal condition (25 ℃) to see how stable the M4CB impurity would be in the diluent (1,3-dimethyl-2-imidazolidinone). The MXFN sample that was previously spiked with M4CB was kept for a full day and analysed at 0 h, 8 h, 18 h and 24 h. Calculations were made for percentage relative difference (table 4) in M4CB quantity between the 0 hr time and after each interval time (8 h, 18 h and 24 h). The percentage relative difference values ranged from-16.92% to+8.22% for 0 h, 8 h, 18 h and 24 h time investigations.

Table 4: Stability of M4CB at room temperature

S. No.	Weight of the MXFN (mg)	Area of M4CB solution	M4CB (ppm)
Initial	500.24	32753.98	3.83
After 8 h	500.33	30065.64	3.52
Mean*±SD			3.68±0.219
%Relative difference			8.22
After 18 h	500.38	38308.04	4.48
Mean*±SD			4.16±0.460
%Relative difference			-16.92
After 24 h	500.16	36574.86	4.31
Mean*±SD			4.07±0.339
%Relative difference			-12.62

*Data are expressed as mean±SD, n=2; M4CB-Methyl-4-Chlorobutyrate; SD – standard deviation

Table 5: System suitability assessments for GC-EI-MS analyzer

System suitability done prior to	Peak area*	±SD*	% RSD
Specificity test	33369.8	2435.29	7.298
Linearity test	33396.5	2078.15	6.223
Method precision test	35875.9	1628.51	4.539
Accuracy test	35856.4	1619.34	4.516
Sensitivity test	33428.07	2400.60	7.181
Robustness	32780.1	1789.99	5.461
Solution stability test	33394.2	2347.13	7.029
Batch analysis	33297.4	2497.77	7.501

*Data are expressed as mean±SD, n=6; RSD – relative standard deviation; M4CB-Methyl-4-Chlorobutyrate; SD – standard deviation

Batch analysis

Using the MXFN batches MF2TEST01, MF2TEST02, and MF2TEST03, batch analyses were conducted. These batches were analyzed for M4CB content using methodology in section titled "Optimized conditions and instruments". None of the batches analyzed contained M4CB.

System suitability

Prior to doing analysis on a GC-EI-MS analyzer, system appropriateness is carried out to demonstrate that the system is operating flawlessly in determining M4CB content in MXFN molecule. System suitability must be completed prior to each sample analysis. To assess the suitableness of the GC-EI-MS system, six injections were carried out with a freshly prepared M4CB solution (3.75 ppm) and assessed using the suggested GC-EI-MS conditions. For impurity M4CB peak areas, mean and % RSD's was computed (table 5). The peak area exhibits a % RSD of range 4.516% to 7.501%.

DISCUSSION

The GC-EI-MS approach is effective for both quantitative plus qualitative analysis and has proven widely used in the petroleum sector. Other areas where it has proved successful include environmental assessment, food-related applications, toxicological as well as forensic applications, and forensic science [16]. The combination of GC along with MS offers high-resolution separations enabling extremely selective and sensitive identification, which is crucial in quantitative trace assessment [17-20].

M4CB impurity carries a structural alert for the potential impurity in MXFN molecule [21]. As per ICH M7, M4CB impurity must be managed to be less than 3.75 ppm in MXFN molecule. There is occasionally a possibility that chlorinated organic molecules have genotoxic effects [22, 23]. Due to the fact that M4CB is a chlorinated compound, it may have genotoxic properties.

We established an GC-EI-MS method in this study to identify and measure M4CB in the MXFN molecule. The identification along with measurement of M4CB in MXFN samples were validated using the GC-EI-MS method applying ICH criteria [24-26]. In this GC-EI-MS method, the calibration plot for M4CB demonstrated outstanding linearity with an R^2 reading of 0.9999. Peak responses from M4CB are extremely repeatable, with RSD values of 4.194% at 0.9452 ppm level and 2.041% at 3.75 ppm level. With % RSD <5.0%, precision with regard to peak areas was demonstrated. With higher recoveries of M4CB, the approach was stated to be more accurate. The percentage relative difference values for M4CB amounts that were acquired independently of the operator were underneath 17%, which is within the permitted range of 20% and supports the robustness. The stability investigation revealed that M4CB remains persistent in the diluent for a full day when preserved at ambient thermal condition (25 °C). The outcomes of the system suitability assessment serve to both confirm the methodology and guide further investigations, guaranteeing the GC-EI-MS system's ability to generate precise as well as accurate data over an extended period of time. The system suitability evaluates were determined to yield values that met the acceptance requirements.

The ingredients and manufacturing conditions employed in the synthesis of MXFN were taken into consideration while evaluating potential genotoxic contaminants. Next, the control plan is developed in compliance with ICH M7 [27]. The sample detection findings obtained from the implementation of GC-EI-MS method to monitor the M4CB impurity revealed that the impurity was not found in any of the three separate batches of MXFN investigated. This certifies the MXFN molecule's safety.

CONCLUSION

The presented work presented a precise analytical technique for measuring M4CB concentration in MXFN using GC-EI-MS technique in SIM manner at extremely low levels. The devised GC-EI-MS approach is highly sensitive, specific, specific, linear, and accurate for determining M4CB quantity in MXFN, as indicated by method validation findings. Also established is the stability of M4CB in diluent, which is proven to be stable for up to 24 hr at ambient thermal condition. Additionally, the GC-EI-MS method's remarkable effectiveness at low quantities was successfully applied to quantify M4CB in bulk company batch samples of MXFN. The M4CB content in MXFN may thus be more accurately evaluated using this approach during quality control testing. In addition, the source of M4CB generation during MXFN synthesis was explored.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Conceptualization – K. Aparna and K. M. V. Narayana Rao; Investigation – K. Aparna; Supervision – K. Vijaya Rachel; Writing

original draft – K. Aparna, K. Vijaya Rachel; Review and writing-K. Aparna; Editing – K. Vijaya Rachel, K. M. V. Narayana Rao.

CONFLICTS OF INTERESTS

Declared none

REFERENCES

1. Greenberg RG, Landersdorfer CB, Rivera Chaparro N, Harward M, Conrad T, Nakamura A. Population pharmacokinetics of moxifloxacin in children. Paediatr Drugs. 2022;24(2):163-73. doi: 10.1007/s40272-022-00493-3, PMID 35284983.
2. Obrink Hansen K, Hardlei TF, Brock B, Jensen Fangel S, Kragh Thomsen M, Petersen E. Moxifloxacin pharmacokinetic profile and efficacy evaluation in empiric treatment of community acquired pneumonia. Antimicrob Agents Chemother. 2015;59(4):2398-404. doi: 10.1128/AAC.04659-14, PMID 25666151.
3. Anon. Current management of acute bacterial rhinosinusitis and the role of moxifloxacin. Clin Infect Dis. 2005;41 Suppl 2:S167-76. doi: 10.1086/428057, PMID 15942883.
4. Chuchalin A, Zakharova M, Dokic D, Tokic M, Marschall HP, Petri T. Efficacy and safety of moxifloxacin in acute exacerbations of chronic bronchitis: a prospective multicenter observational study (Avanti). BMC Pulm Med. 2013;13:5. doi: 10.1186/1471-2466-13-5, PMID 23343427.
5. Wang H, Liu H, Lou M, Xu L, Zhang W, Jing L. Comprehensive clinical evaluation of moxifloxacin: a retrospective study. Med (Baltim). 2023;102(22):e33896. doi: 10.1097/MD.0000000000033896, PMID 37266643.
6. Szekely G, Amores de Sousa MC, Gil M, Castelo Ferreira F, Heggie W. Genotoxic Impurities in pharmaceutical manufacturing: sources regulations and mitigation. Chem Rev. 2015;115(16):8182-229. doi: 10.1021/cr300095f, PMID 26252800.
7. Reddy AV, Jaafar J, Umar K, Majid ZA, Aris AB, Talib J. Identification control strategies and analytical approaches for the determination of potential genotoxic impurities in pharmaceuticals: a comprehensive review. J Sep Sci. 2015;38(5):764-79. doi: 10.1002/jssc.201401143, PMID 25556762.
8. Giordani A, Kobel W, Gally HU. Overall impact of the regulatory requirements for genotoxic impurities on the drug development process. Eur J Pharm Sci. 2011;43(1-2):1-15. doi: 10.1016/j.ejps.2011.03.004, PMID 21420491.
9. Alsante KM, Huynh Ba KC, Baertschi SW, Reed RA, Landis MS, Furness S. Recent trends in product development and regulatory issues on impurities in active pharmaceutical ingredient (API) and drug products. Part 2: safety considerations of impurities in pharmaceutical products and surveying the impurity landscape. AAPS PharmSciTech. 2014;15(1):237-51. doi: 10.1208/s12249-013-0061-z, PMID 24363207.
10. Lovsin Barle E, Winkler GC, Glowienke S, Elhajouji A, Nunic J, Martus HJ. Setting occupational exposure limits for genotoxic substances in the pharmaceutical industry. Toxicol Sci. 2016;151(1):2-9. doi: 10.1093/toxsci/kfw028, PMID 27207978.
11. Aparna K, Rachel KV, Narayana Rao KM. A rapid sensitive and accurate LCMS method for the determination of mutagenic impurities in moxifloxacin HCl. Rasayan J Chem. 2023;16(3):1780-8. doi: 10.31788/RJC.2023.1638349.
12. Djurdjevic P, Ciric A, Djurdjevic A, Stankov MJ. Optimization of separation and determination of moxifloxacin and its related substances by RP-HPLC. J Pharm Biomed Anal. 2009;50(2):117-26. doi: 10.1016/j.jpba.2009.03.029, PMID 19464135.
13. Vankalapati KR, Algete P, Boodida S. A rapid RP-HPLC stability indicating method development and validation of moxifloxacin hydrochloride related substances in finished dosage forms. Biomed Chromatogr. 2021;35(11):e5192. doi: 10.1002/bmc.5192, PMID 34110029.
14. Wu CS, Jia ZX, Ning BM, Zhang JL, Wu S. Separation and identification of moxifloxacin impurities in drug substance by high-performance liquid chromatography coupled with ultraviolet detection and fourier transform ion cyclotron resonance mass spectrometry. Chin Chem Lett. 2012;23(10):1185-8. doi: 10.1016/j.cclet.2012.09.001.
15. Li J, Yuan Y, Fan R, Su Q, Wang S, Zhou T. A Simple LC/MS/MS method for the determination of moxifloxacin n-sulfate in rat plasma and its application in a pharmacokinetic study. J AOAC Int. 2015;98(4):921-6. doi: 10.5740/jaoacint.14-254, PMID 26268973.
16. Krone N, Hughes BA, Lavery GG, Stewart PM, Arlt W, Shackleton CH. Gas chromatography-mass spectrometry (GC/MS) remains a pre-eminent discovery tool in clinical steroid investigations even in the era of fast liquid chromatography-tandem mass spectrometry (LC/MS/MS). J Steroid Biochem Mol Biol. 2010;121(3-5):496-504. doi: 10.1016/j.jsbmb.2010.04.010, PMID 20417277.
17. Apridamayanti P, Pratiwi L, Sari R. Gas chromatography study of n-hexane and chloroform fractions of ethanol extract of *Melastoma malabathricum l.* Int J Pharm Pharm Sci. 2022;14(3):40-6. doi: 10.22159/ijpps.2022v14i3.43801.
18. Raja EF, Paul JJP. GAS chromatography mass spectroscopy analysis and prediction of bioactivities in the chloroform extract of halymenia dilatata zanardini (red algae) collected from mandapam tamil nadu india. Asian J Pharm Clin Res. 2021;14(12):60-3. doi: 10.22159/ajpcr.2021.v14i12.43068.
19. Madriwala B, Jays J. Analytical method development and validation for estimation of residual solvents in gliclazide using gas chromatography. Int J Curr Pharm Sci. 2022;14(4):68-73. doi: 10.22159/ijcpr.2022v14i4.1998.
20. Chen X, Gu X, Yang J, Jiang Z, Deng J. Gas chromatography mass spectrometry technology: application in the study of inflammatory mechanism in COVID-19 patients. Chromatographia. 2023;86(2):175-83. doi: 10.1007/s10337-022-04222-3, PMID 36718226.
21. Ganesh P, RajuChBV N, Suman G. Rama Devi d, Basavaiah K. Quantitative determination of methyl-4-chlorobutyrate in nucleoside reverse transcriptase inhibitors by GCMS using electron ionization. World J Pharm Pharm Sci. 2023;12(12):716-26. doi: 10.20959/wjpps202312-26108.
22. Marques Dos Santos M, Cheriaux C, Jia S, Thomas M, Gallard H, Croue JP. Genotoxic effects of chlorinated disinfection by products of 1,3-diphenylguanidine (DPG): cell based *in vitro* testing and formation potential during water disinfection. J Hazard Mater. 2022;436:129114. doi: 10.1016/j.jhazmat.2022.129114, PMID 35739694.
23. Wu B, Zhang Y, You Y, Liang Y. Genotoxicity of chlorinated hydrophobic organic compounds extracted from a source of drinking water. Ecotoxicology and Environmental Safety. 2023;267(15):115598. doi: 10.1016/j.ecoenv.2023.115598.
24. ICH Guidelines, Validation of analytical procedures technical requirements for registration of pharmaceuticals for human use: text and Methodology. Vol. Q2. Geneva, Switzerland; 2005. p. R1.
25. Sharma S, Goyal S, Chauhan K. A review on analytical method development and validation. Int J App Pharm. 2018;10(6):8-15. doi: 10.22159/ijap.2018v10i6.28279.
26. Pekamwar SS, Kalyankar TM, Tambe BV. Validated RP-HPLC method for simultaneous estimation of cefixime and moxifloxacin in combined pharmaceutical dosage form. Int J Pharm Pharm Sci. 2014;6(11):84-8.
27. ICH Guidelines, Assessment and control of DNA reactive (mutagenic) IMPURITIES in pharmaceuticals to limit potential carcinogenic risk. Vol. M7. Geneva, Switzerland; 2017. p. R1.