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ULTRA-TRACE DETERMINATION OF METHYL METHANE SULFONATE IN APIS USING A VALIDATED ANALYTICAL METHOD BY AUTO LIQUID SAMPLER MODE IN GC-FID CHROMATOGRAPHIC TECHNIQUE

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ABSTRACT
Methyl Methane Sulfonate (MMS) is a potential genotoxic impurity, which could be generated during the synthesis of an active pharmaceutical ingredient (API) and therefore requires sensitive and reliable analytical methods for patient safety and regulatory compliance. For MMS determination, a number of techniques based on liquid chromatography–mass spectrometry (LC–MS/MS) and gas chromatography–mass spectrometry (GC–MS) have been reported. A simple, rapid, sensitive and cost-effective gas chromatography–flame ionisation detection (GC–FID) technique by ALS mode of injection has been developed and validated for the assessment of MMS in calcium dobesilate API utilising an Auto Liquid Sampler (ALS). Optimization of the chromatographic conditions was performed systematically by trying different stationary phases, injection modes, carrier gas flow rates and oven temperature programs. The DB-624 capillary column was found to give the best chromatographic performance in the separation of MMS from residual solvents and API-related matrix components, with good peak symmetry and reproducibility. The established technique has been validated in accordance with ICH Q2(R2) recommendations for specificity, linearity, limit of detection, limit of quantification, precision, accuracy, and system adaptability. The approach demonstrated an excellent linear range ($R^2 = 0.9997$), high sensitivity for trace-level measurement, commendable precision (RSD < 2%), and satisfactory recovery, making it appropriate for quantitative analysis. The validated approach was effectively utilised for the examination of many production batches of Calcium Dobesilate API, in which MMS was undetected. The novelty of this study is the development of a validated GC–FID method through ALS mode of injection, which represents a practical and economical alternative method to mass spectrometry-based methods for routine monitoring of MMS, which is a time-saving process. The proposed method can be adopted for pharmaceutical quality control, process monitoring, batch release testing and regulatory compliance to ICH M7 recommendations.

KEYWORDS: Gas Chromatography – Flame Ionisation Detector[GC–FID], Auto Liquid Sampler[ALS], ICH Q2(R2), ICH M7, Methyl methanesulfonate, Calcium Dobesilate, Pharmaceutical quality control, Genotoxic impurity, Method validation.

1. INTRODUCTION

Methyl methanesulfonate (MMS) is a potent, direct-acting alkylating agent used in various research and synthesis of chemical substances to pull the reaction forward to obtain the desired product. It is also used in microbiology to damage DNA, causing mutations by adding methyl groups to DNA bases (like guanine and adenine), leading to replication blocks and cell death, and is classified as a probable human carcinogen (Group 2A) and suspected reproductive toxicant [1]. A colorless liquid, it is eminently reactive and is used in various organic synthesis processes and molecular biology to induce DNA damage for genetic studies, but it's noxious and requires immense approach in handling due to its genotoxic, mutagenic and carcinogenic potential among users [1-2]. The structure of methyl methane sulphonate is given in Fig. 1.

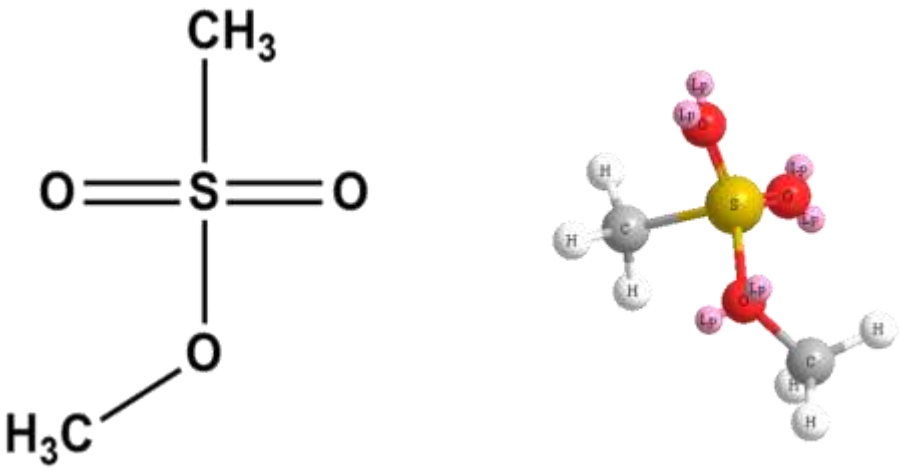


Fig. 1. Structure of Methyl Methane Sulfonate [3]

Methyl methane sulfonate is a genotoxic impurity in active pharmaceutical ingredient (API) drugs; this work primarily aims to establish a method to detect and quantify this impurity [4]. This is especially important during the production of the API drug Calcium Dobesilate, which is currently the subject of API research. It is a laborious process to eliminate all of these contaminants from the product. Hence, following ICH requirements, it is essential to significantly reduce the amounts of methylmethanesulfonate contaminants in Calcium Dobesilate to the lowest possible extent. To save time and money compared to the analytical method, quality control labs testing MMS in Calcium Dobesilate it is imperative need to develop a new and valid strategy for detecting and quantifying trace contaminants from a literature review and analytical study [5].

The USFDA and EMA guidelines have established the genotoxic/carcinogenic impurity level as the "threshold of toxicological concern" (TTC). An exposure of 1.5 g/day to mutagenic contaminants is considered a minor risk. The anticipated daily dosage of a patient may be used to determine the ppm threshold of genotoxic contaminants in the TTC-derived medication. The recommended maximum dosage of TEN is 40 milligrams per day.

$$\text{Concentration limit of PDIs (ppm)} = \frac{\text{TTC (µg/day)}}{\text{Dose (g/day)}} = \frac{1.5}{0.040} = 37.5 \text{ ppm}$$

If methyl methane sulfonate is an impurity that may be introduced into active pharmaceutical ingredient manufacturing, this study is ready to build a method to quantify and qualify it in accordance with the recommendations of ICH guidelines [14]. In order to keep the active pharmaceutical ingredients' ICH quality, a control plan must be set up during the last stages.

Pharmaceutical products may contain trace quantities of pollutants that are not associated with the therapeutic benefits of the active pharmaceutical ingredient (API). These impurities may originate from degradation products formed inside the formulation or from intermediates generated during manufacture [6]. The ICH Q3A (R2) guideline "Impurities in New Drug Substances" and the ICH Q3B (R2) guideline "Impurities in New Drug Products" have traditionally regulated the safety evaluation of these impurities. Based on the maximum daily dose, these standards set threshold limits for contaminants above which a safety qualification is necessary [7]. API genotoxicity threshold of toxicological concern (TTC) [8]. An in silico investigation using the ICH M7 guideline classified alkyl camphorsulfonates generated by reacting camphorsulfonic acid salts with methanol, ethanol, and isopropyl alcohol, popular API manufacturing solvents. Two sensitive, reproducible, and precise analytical procedures utilising GC-FID and GC-MS were established through analytical Quality By Design (QbD) methodologies to quantify three alkyl camphorsulfonates in active pharmaceutical ingredients, adhering to the TTC PGI control limit [9]. Genotoxic pollutants derived from alkyl sulfonate esters are significant issues. Nine prevalent sulfonate ester medications can be detected via gas chromatography triple-quadrupole mass spectrometry (GC–MS/MS). To quantify these nine GTIs, multiple reaction monitoring (MRM) mode was employed to investigate three solvents: acetonitrile (ACN), dichloromethane (DCM), and ethyl acetate (EtOAc) [10].

Genotoxic impurities (GIs) are chemical substances that have a DNA-interaction feature which can ultimately lead to cancer. Their presence in a variety of drug compounds prompted regulatory bodies to set guidelines for monitoring, controlling, and limiting their level in different drug products [11].

2. Objective of the Study

The objective of this study was to create and validate a straightforward, rapid, sensitive, and cost-effective GC-FID method by ALS mode of injection for quantifying methyl methanesulfonate (MMS) as a genotoxic contaminant in

Calcium Dobesilate API during regular pharmaceutical quality control, in accordance with ICH M7 and ICH Q2 guidelines.

3. MATERIALS AND METHODS

3.1 Chemicals and Reagents

Methyl methanesulfonate (MMS) reference standard was purchased from Sigma-Aldrich (St. Louis, MO, USA). Calcium Dobesilate active pharmaceutical ingredient (API) samples were sourced from an in-house manufacturing facility. HPLC-grade acetonitrile and analytical reagent-grade dichloromethane were bought from Rankem (India) and Azytus (India), respectively. Most of the chemicals and solvents used were analytical grade and used as received.

3.2 Instrumentation

Chromatographic analysis was conducted with a gas chromatograph, Shimadzu Nexis GC-2030 (Shimadzu Corporation, Kyoto, Japan), which is fitted with an Auto Liquid Sampler (ALS) and Flame Ionisation Detector (FID) [12-22]. LabSolutions software was used for instrument control, data collection and chromatographic processing. Samples were weighed on a calibrated analytical balance (Shimadzu, 0.01 mg), and the dissolution of samples was facilitated by an ultrasonic bath.

The chromatographic method was developed by systematically comparing different capillary columns comprising various stationary-phase polarities such as DB-WAX, DB-23, DB-17, DB-35, DB-1, DB-5 and DB-624. Other solvent compositions, injection methods (Headspace, Auto Liquid Sampler), carrier gas flow rates, split ratios and oven temperature programs were also evaluated to obtain the best chromatographic performance [12-13][15-20][22].

Of the columns tested, the DB-624 (6% cyanopropylphenyl–94% dimethylpolysiloxane) column showed the best chromatographic performance with symmetrical peak shape, higher theoretical plate count, minimal peak tailing and complete separation of methyl methanesulfonate from residual solvents and API related matrix components. Because of its excellent selectivity and reproducible retention of volatile analytes, the medium-polar stationary phase of the DB-624 column is widely used for the separation of volatile organic compounds (VOCs) and pharmaceutical impurities. Thus, the DB-624 column was chosen to be further optimized and validated.

3.3 Optimized Chromatographic Conditions

Table 1. The operating conditions for GC-FID have been optimized.

Parameter	**Condition**		
Instrument	Shimadzu Nexis GC-2030 Gas Chromatography System		
Mode of Operation	GC-FID: Gas Analysis with Flame Ionisation		
Sensor	(FID): Flame Ionization Detector		
Detector Temperature	260°C		
Column	DB-624 (3.0 µm film thickness, 30 m × 0.53 mm I.D.)		
Carrier Gas	Nitrogen		
Carrier Gas Flow Rate(CGFR)	3.0 mL min^{-1}		
Injection Volume	2.0 µL		
Injector Temperature	220°C		
Split Ratio	1:1		
Injection Mode	Auto Liquid Sampler (ALS)		
Hydrogen Flow Rate	30 mL min^{-1}		
Air Flow Rate	300 mL min^{-1}		
Makeup Gas	“Nitrogen (20 mL min^{-1})”		
Total Run Time	50 min		
phase	**Hold Time (min)**	**Ramp Rate (°C min^{-1})**	**Temperature (°C)**
Initial	10.0	—	40
Ramp 1	5.0	4	160
Ramp 2	2.3	30	240

3.4 Preparation of Standard Solution

A reference standard weighing 25.0 mg was precisely measured and transferred to a 100 mL volumetric flask to create a stock solution of methyl methanesulfonate. Approximately 50 mL of diluent (acetonitrile: dichloromethane 70/30 (v/v)) was introduced, and the mixture was agitated until fully dissolved, subsequently diluted to volume with the identical diluent. A standard working solution was created by putting 7.5 mL of the stock solution into a 100 mL volumetric flask and diluting it with the appropriate solvent. This section outlines the preparation of the sample solution in the laboratory. The calcium dobesilate API was precisely measured into a 10 mL volumetric flask, followed

by the addition of 5 mL of the diluent. The solution was sonicated for complete dissolution and subsequently diluted to volume with the identical diluent. The solution was meticulously mixed, filtered (if necessary, using a 0.45 µm PTFE membrane filter), and subsequently placed into the GC-ALS vials for analysis. Replicated specimens were utilised for all sample solutions.

3.5 Analytical Procedure

A blank diluent was injected before the analysis to ensure that the system was stable and there were no interfering peaks. Six replicate injections of the working standard solution were made to assess system suitability. Once good system suitability results were obtained, duplicate sample preparation was performed under the optimized chromatographic conditions. Methyl methane sulfonate was quantified by comparison of the peak areas of the sample chromatogram and the standard chromatogram (external standard method) [12-22].

3.6 Validation of Methodology

The established GC-FID method was validated in accordance with the ICH Q2(R2) guidelines for analytical procedure validation. The validation metrics included specificity, limit of detection (LOD), limit of quantification (LOQ), linearity, system precision, method precision, accuracy (recovery), and system appropriateness. This method was subsequently verified and employed for the routine analysis of MMS in Calcium Dobesilate API.

4. RESULTS AND DISCUSSION

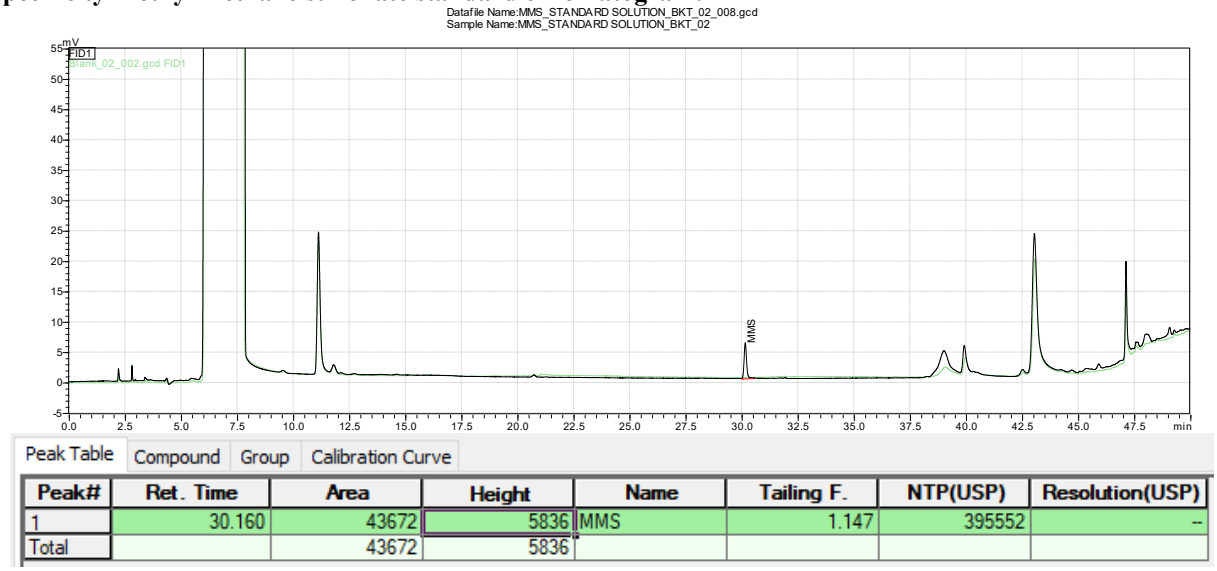
4.1 Validation

The proposed Gas Chromatography method was validated in accordance with ICH guidelines to ensure the reliability, consistency, and accuracy of the analytical results [12-13] [16-20].

4.2 Specificity

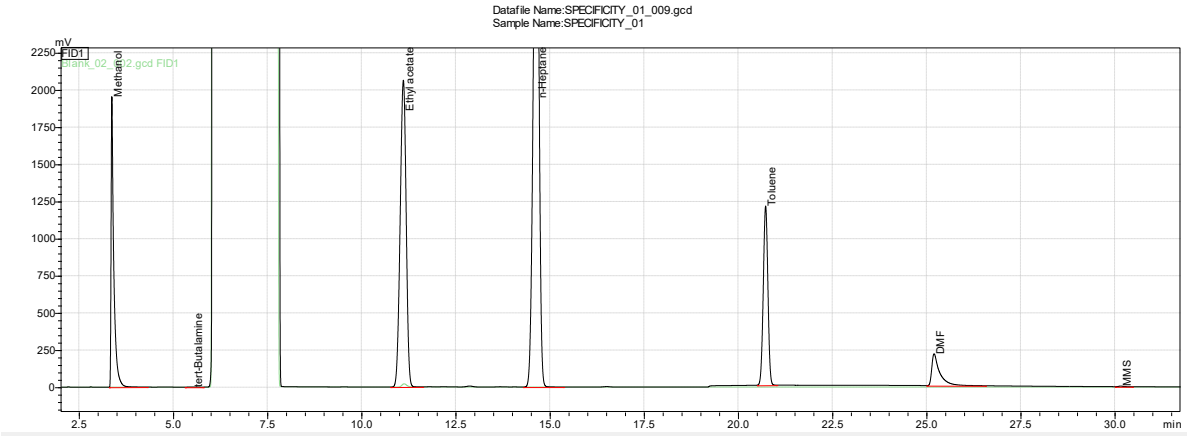
To determine specificity, in this study, methyl methane sulfonate was injected separately at a standard level, eluting at 30.160. In the Mix of organic solvents used in the calcium dobesilate was also tested, where the MMS elutes at 30.160 separately and is spiked in each testing sample independently; in the unspiked sample, it elutes at 30.160 retention time, and in the spiked sample, it elutes at 30.151 retention time. Along with the methylmethanesulfonate peak, the solvents utilized in this API procedure do not co-elute or merge with it. With respect to the sequence of elution, the following solvents are utilized in this process: methanol (3.379), dichloromethane (6.094), acetonitrile (7.810), ethyl acetate (11.124), n-Heptane (14.643), toluene (20.695), dimethylformamide (25.283), and tertiary butyl amine. Based on the specificity results for monomethyl sulphonate peaks in individual, mixed, and standard spiked samples, it can be deduced that the specified parameters, such as resolution (16.148), tailing factor (1.072), number of theoretical plates (392121), and peak retention time (PT), are all important. The Specificity reference chromatogram is depicted in Fig. 2.

Specificity Methyl methane sulfonate standard chromatogram:



Peak#	Ret. Time	Area	Height	Name	Tailing F.	NTP(USP)	Resolution(USP)
1	30.160	43672	5836	MMS	1.147	395552	--
Total		43672	5836				

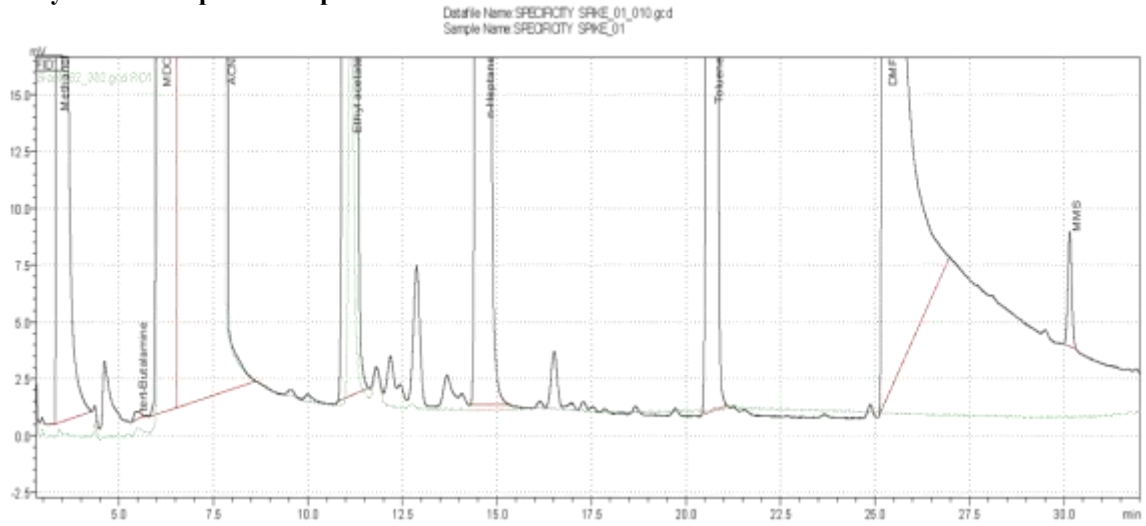
Specificity Chromatogram Of Standard Mix Solvents Solution In Calcium Dobesilate:



Peak Table | Compound | Group | Calibration Curve

Peak#	Ret. Time	Area	Height	Name	Tailing F.	NTP(USP)	Resolution(USP)
1	3.378	9650703	1948404	Methanol	2.473	16908	--
2	5.505	6179	388	tert-Butalamine	1.272	4034	9.442
3	11.117	22753504	2062468	Ethyl acetate	0.945	22736	17.495
4	14.647	41889328	4082609	n-Heptane	0.932	46139	12.435
5	20.734	10218487	1204381	Toluene	0.994	132881	24.337
6	25.206	3082618	213549	DMF	2.504	96530	16.203
7	30.160	33751	4406	MMS	1.207	374136	18.990
Total		87634570	9516206				

Specificity Standard Spiked Sample of Mix Solvents Solution in Calcium Dobesilate:



Peak Table | Compound | Group | Calibration Curve

Peak#	Ret. Time	Area	Height	Name	Tailing F.	NTP(USP)	Resolution(USP)
1	3.379	7162331	1420699	Methanol	2.612	16724	--
2	5.447	4132	396	tert-Butalamine	1.421	2701	7.897
3	6.094	1409464002	55810972	MDC	--	6	0.128
4	7.810	5257682804	89402320	ACN	--	186	0.286
5	11.124	22056762	1997656	Ethyl acetate	0.932	22702	2.562
6	14.643	36311908	3567206	n-Heptane	0.940	46826	12.434
7	20.695	9032969	1022466	Toluene	0.969	127463	24.089
8	25.283	2552670	130976	DMF	3.825	60717	14.284
9	30.151	36523	5045	MMS	1.072	392121	16.148
Total		6744304101	153357735				

Fig. 2. Specificity reference chromatogram

4.3 Limit of Quantification (LOQ) and Limit of Detection (LOD)

The Methyl methane sulfonate solution was quantitatively diluted stepwise with the diluent. These diluted solutions were then separately injected into the auto-liquid sampler under GC conditions. The Limit of Quantification (LOQ) and Limit of Detection (LOD) were clearly established, with the LOQ and LOD corresponding to predetermined concentrations (%) that yielded signal-to-noise ratios of ≥10 and ≥3, respectively. The LOD and LOQ values (Table 1) and chromatogram in Fig. 3 and Fig. 4 demonstrated the sensitivity of the GC method for analysing methyl methane sulfonate at low levels of concentrations.

Table 1. GC methodology sensitivity results

Content	LOQ		LOD	
	S/N Proportion	(ppm)	S/N Proportion	(ppm)
Methyl methane sulfonate	20.78	6.29	4.39	2.07

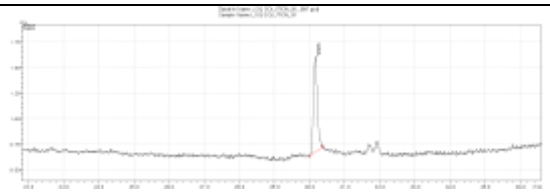


Fig. 3. LOQ Chromatogram

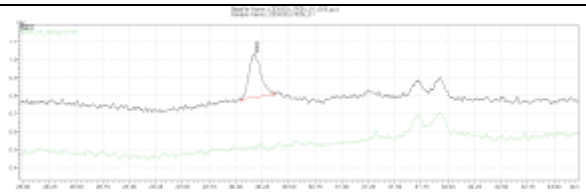


Fig. 4. LOD Chromatogram

4.4 LOQ Precision:

The LOQ Precision is carried out to demonstrate that the MMS is ultraprecise, definite, and repetitive at low levels. From the obtained data, it is evident that LOQ level precision data indicates it can be quantified even at low levels [6.29 ppm] based on its symmetrical and consistent results. The Relative standard deviation obtained from the six replicates is 1.45%, which indicates the stability of MMS at low ppm levels from its stipulated standard level.

LOQ Precision	
Standard Name	**Methyl Methane Sulfonate**
No. of Injection	**LOQ Area**
Inj-1	7288
Inj-2	7114
Inj-3	7109
Inj-4	7120
Inj-5	7113
Inj-6	7338
Average	**7180**
SD	**104.03**
% RSD	**1.45**

*%RSD not more than 15.0%

4.5 Linearity

Five different concentration solutions containing Methyl methane sulfonate impurities were prepared in concentration ranges of 20% [7.47 ppm], 50% [18.68 ppm], 80% [29.88 ppm], 100% [37.35 ppm], 120% [44.82 ppm] and 150% [56.03 ppm], respectively. These solutions were analyzed twice. Calibration curves for Methyl methane sulfonate were developed by plotting the mean area obtained against its concentrations. The regression equations and linearity correlation coefficients for Methyl methane sulfonate obtained are shown in Table 2. The correlation coefficients, all greater than 0.99, confirmed the linearity of the GC method (Fig. 5).

Table 2. GC methodology linearity and line equation results

Parameter	Methyl methane sulfonate	Specifications
Linearity Range	7.47 to 74.70 %	-
Y-Intercept	-2.58	-5 to +5 %

Slope	-1136.26	Observed Value
% Y-Intercept	-2.58	-5 to +5
Correlation Coefficient(R)	0.9998	NLT 0.99
Regression Coefficient(R^2)	0.9997	NLT 0.99

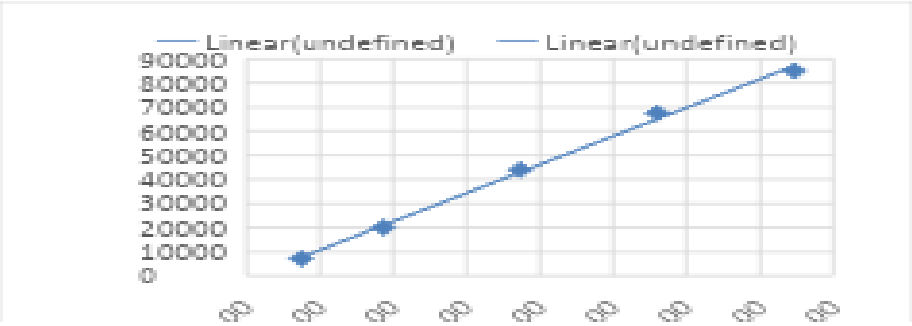


Fig. 5. Linearity graph of Methyl methane sulfonate

4.6 System precision

The Methyl methane sulphonate working standard solution was injected under the proposed GC conditions in six replicates to quantitate its precision, repeatability and endurability. The peak area for Methyl methane sulfonate was recorded at the 30.16 Retention time. The Relative standard deviation (RSD%) for the six standard areas from the six replicates was recorded to be 0.63%, which is within the specified limit of ICH Guidelines in the determination of GTI impurities (Fig. 6 and Table 3). These results demonstrate the system's precision in quantifying Methyl methane sulfonate Impurity.

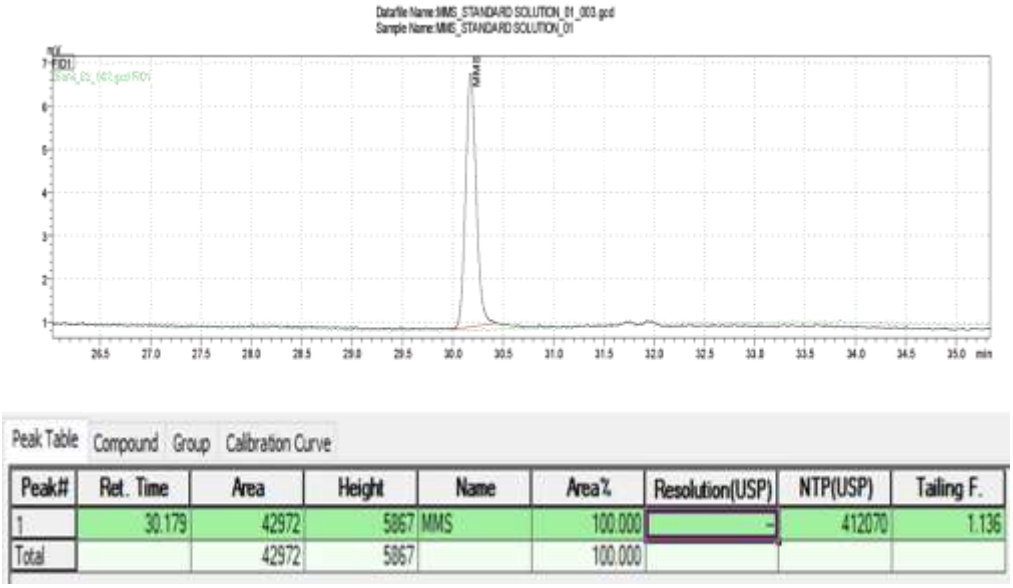


Fig. 6. System precision chromatogram of Methyl methane sulfonate Impurity

Table 3. System precision findings

System precision		
Standard Name	**Methyl Methane Sulfonate**	
No. of Injections	**Area**	**BKT standard Area**
Inj-1	42972	43894
Inj-2	42960	43672
Inj-3	42963	44645
Inj-4	42382	44941

Inj-5	42500	
Inj-6	42950	
Average	**42788**	**43388**
SD	**271.33**	**872.92**
% RSD	**0.634**	**2.012**

***%RSD not more than 15.0%**

4.7 Method precision

Working standard solutions of Methyl methane sulfonate, both as a pure sample and as a sample spiked with API, were separately injected and analyzed under the proposed GC conditions in six replicates. The concentrations of Methyl methane sulfonate GTI were determined. The per cent relative standard deviation (RSD) from the six replicates is 0.63% (Table 4). These results demonstrate the GC method's precision in analyzing the Methyl methane sulfonate impurity in the API products.

Table 4. GC methodology precision results

Samples	Methyl methane sulfonate precision data					
	System Precision		Method Precision Spiked		Method Precision in pure sample	
	Peak area	Amount obtained (ppm)	Peak area	Amount obtained (ppm)	Peak area	Amount obtained (ppm)
1	42972	37.3500	41354	36.0984	Absent	NIL
2	42960	37.3500	41037	35.8217	Absent	NIL
3	42963	37.3500	41439	36.1726	Absent	NIL
4	42382	37.3500	42172	36.8124	Absent	NIL
5	42500	37.3500	39870	34.8030	Absent	NIL
6	42950	37.3500	40330	35.2045	Absent	NIL
Average	**42788**	**37.35**	**41354**	**36**	-	-
SD	**271.33**	**0.00**	**41037**	**0.72**	-	-
RSD	**0.634**	**0.00**	**41439**	**2.01**	-	-

4.8 Recovery

A recovery analysis was conducted in accordance with the specified ICH principles to assess the Recovery, also known as Accuracy. In accordance with the sample concentration and the specified ppm limit, standard solutions of methyl methane sulphonate were made at three levels of limit of detection (LOD): 50%, 100%, and 150% of the specification limit, as per the references provided by the ICH recommendations. Table 5 shows the results of the accuracy verification process, which involved injecting the spiked sample solutions three times. There was confidence in the GC method's accuracy in analyzing the API medication sample's methylmethanesulfonate content because the recovery of methylmethanesulfonate from four different levels was satisfactory.

Table 5. GC methodology Accuracy results

Accuracy level	Amount Spiked up (%)	Amount Retrieved (%)	Recovery (%)	Average of Recovery (%)
Methyl methane sulfonate recovery				
LOQ	7.4700	6.2413	83.6	83.7
	7.4700	6.2274	83.4	
	7.4700	6.2885	84.2	
100%	37.3500	36.0984	96.6	95.9
	37.3500	35.8217	95.9	
	37.3500	36.1726	96.8	
150%	56.0250	58.6649	104.7	105.4
	56.0250	59.2201	105.7	
	56.0250	59.2751	105.8	

*Recovery limit is 80% to 120% for 100% and 150% accuracy level. LOQ level is 70% to 130%.

4.9 System Suitability

To ensure that the GC system is adequate and performs well when analysing Methyl methane sulfonate in API, system suitability testing is carried out. Calculated the percent relative standard deviations of Calcium Dobesilate API was calculated, and several factors, such as Number of Theoretical Plates, Tailing-factor, and Resolution for Methyl methane sulfonate, were determined to determine if the system was suitable. Six independent injections of a 37 ppm Methyl methane sulfonate in API working solution were used to determine the peak locations. The range of the ICH limit is well-covered by the resolution of 16.15 between Methyl methane sulfonate and Dimethyl formamide, Tailing factor is 1.147, and the number of Theoretical plates is 395552, as shown in Table 6.

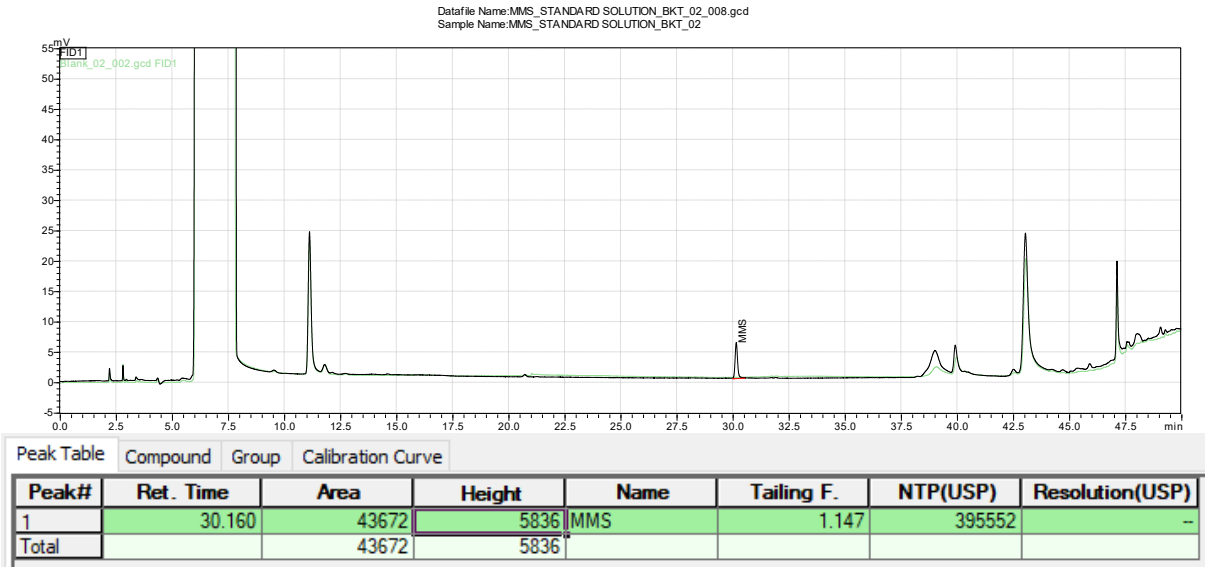


Table 6. GC methodology System suitability results

Parameter	Methyl methane sulfonate	
	Standard area	Bracketing Standard area
System suitability evaluation throughout the analysis		
Average*	42788	43388
SD	271.33	872.92
%RSD	0.634	2.012
System Suitability Results		
Criteria	Observation	Limits as per USP
Retention Time	30.160	Observed Value
Resolution between MMS and dimethylformamide	16.15	Not Less than 2.0%
Tailing Factor	1.147	0.8-1.8
No. Of Theoretical Plates	395552	Not Less than 2000

4.10 Application of GC methodology developed.

All three lots of Calcium Dobesilate were tested for methyl methanesulfonate (MMS) using the established and validated gas chromatography method. The results showed that methyl methanesulfonate was not detected in any of the tested Calcium Dobesilate batches, as presented in Table 8.

5. SUMMARY

A rapid, straightforward, and cost-effective GC-FID technique utilising an Auto Liquid Sampler (ALS) was successfully developed and validated for the quantification of Methyl Methanesulfonate (MMS), a putative genotoxic contaminant in the active pharmaceutical ingredient (API) Calcium Dobesilate. During the method development phase, diverse chromatographic columns, injection procedures, and experimental settings were meticulously evaluated to achieve effective separation and dependable detection of MMS. The optimised technique exhibited strong performance on a DB-624 capillary column and successfully met ICH Q2 validation criteria. The validated method was deemed suitable for the selective determination of MMS within the API matrix and was employed for the analysis of production batches of Calcium Dobesilate without issues. The established GC-FID approach is feasible, cost-effective, and readily implementable in standard quality control laboratories compared to more complex analytical techniques. The approach has been demonstrated to be a reliable analytical instrument for monitoring the MMS in pharmaceutical manufacturing, hence ensuring the quality and safety of pharmaceutical products and facilitating regulatory compliance.

6. CONCLUSION

This study demonstrates the development and validation of a powerful GC-FID method using the Auto Liquid Sampler (ALS) technique for the determination of the potential genotoxic impurity MMS in Calcium Dobesilate active pharmaceutical ingredient. The scientific value of this work is the development of a simple, low-cost, reliable analytical system that allows the routine monitoring of MMS with an abundant instrument such as a GC-FID through simple ALS mode of operation, the latter being a more complex analytical method. The validated method was found to be suitable for quality control applications since it successfully detected MMS in pharmaceutical samples with good reliability and met the recommendations of ICH Q2 and ICH M7 guidelines. In an industrial context, the method can be easily applied in pharmaceutical manufacturing laboratories to ensure product quality, patient safety, and batch release testing, process monitoring and impurity control. Additionally, this analytical approach can be adapted for the analysis of other genotoxic impurities in various active pharmaceutical ingredients, presenting a useful procedure for the analysis of other pharmaceutical impurities and regulatory compliance in the future.

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Conflicts of interest

The authors have no conflicts of interest regarding this investigation**.**

REFERENCES

1. Luo X, Liu L, Rong H, Liu X, Yang L, Li N, Shi H. ENU-based dominant genetic screen identifies contractile and neuronal gene mutations in congenital heart disease. Genome Medicine. 2024 Aug 12;16(1):97. https://doi.org/10.1186/s13073-024-01372-x

2. Mustafa M, Ali A, Siddiqui SA, Mir AR, Kausar T, Nayeem SM, Abidi M, Habib S. Biophysical characterization of structural and conformational changes in methylmethane sulfonate-modified DNA leading to the frizzled backbone structure and strand breaks in DNA. Journal of Biomolecular Structure and Dynamics. 2022 Nov 2;40(16):7598-611.

3. National Centre for Biotechnology Information. PubChem compound summary.

4. Panchakarla RK, Ravi PR, Kondapalli VG. Liquid chromatography-tandem mass spectrometric method for trace quantification of ethyl methanesulfonate: a genotoxic impurity in dapoxetine hydrochloride. Future Journal of Pharmaceutical Sciences. 2021 Sep 23;7(1):191. https://doi.org/10.1186/s43094-021-00338-9

5. Zheng Z, Huang X, Wang N, Wang T, Zhou L, Xu Z, Chen G, Cao W, Hao H. Hydration mechanism and its effect on the solubility of aripiprazole. Pharmaceutical Research. 2024 Jan;41(1):113-27. https://doi.org/10.1007/s11095-023-03618-6

6. Preibisch S, Preibisch, McDermott. API Development. Berkeley, CA: Apress; 2018. DOI: 10.71448/bcds2341-4

7. Honma M. Guidelines for the assessment and control of mutagenic impurities in pharmaceuticals. Genes and Environment. 2025 Dec 23. https://doi.org/10.1186/s41021-025-00349-5

8. Sayyed FH, Rathod N, Mishra VK, Nalawade V, Roy B. Identification, trace level quantification, and in silico assessment of potential genotoxic impurity in Famotidine. Drug and Chemical Toxicology. 2024 Sep 2;47(5):564-72.

9. Lee K, Yoo W, Jeong JH. Determination of Three Alkyl Camphorsulfonates as Potential Genotoxic Impurities Using GC-FID and GC-MS by Analytical QbD. Separations. 2022 Sep 5;9(9):246. https://doi.org/10.3390/separations9090246

10. Liu Z, Fan H, Zhou Y, Qian X, Tu J, Chen B, Duan G. Development and validation of a sensitive method for alkyl sulfonate genotoxic impurities determination in drug substances using gas chromatography coupled to triple

quadrupole mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 2019 May 10;168:23-9.10.1016/j.jpba.2018.12.044
11. VANDAMME SS, SELVAKUMAR K, RAJESH R. A REVIEW ON ANALYTICAL CHALLENGES IN MONITORING AND CONTROLLING GENOTOXIC IMPURITIES. Asian J Pharm Clin Res. 2020;13(10):10-5. http://dx.doi.org/10.22159/ajpcr.2020.v13i10.38847
12. Maniavannan M, Ilayaraja P, Parthiban P. Trace-level analysis of genotoxic sulfonate ester impurities in teneligliptin by GC-MS. Journal of Applied Pharmaceutical Science. 2022 Nov 5;12(11):052-6010.7324/JAPS.2022.121106
13. Li M, Gu C, Luo L, Zhou J, Liu J, Zheng F. Determination of trace methanesulfonates in drug matrix using derivatization and headspace single drop microextraction followed by high-performance liquid chromatography with ultraviolet detection. Journal of Chromatography A. 2019 Apr 26;1591:131-7. 10.1016/j.chroma.2019.01.038
14. Amberg A, Andaya RV, Anger LT, Barber C, Beilke L, Bercu J, Bower D, Brigo A, Cammerer Z, Cross KP, Custer L. Principles and procedures for handling out-of-domain and indeterminate results as part of ICH M7 recommended (Q) SAR analyses. Regulatory toxicology and pharmacology. 2019 Mar 1;102:53-64. 10.1016/j.yrtph.2018.12.007
15. Cassidy B, Bloomingdale T, Carmody J. Navigating ICH Q2 (R2) compliance in analytical method validation: A gap analysis toolkit to streamline risk assessment and change management. Journal of Pharmaceutical Sciences. 2025 Jun 1;114(6):103749. https://doi.org/10.1016/j.xphs.2025.103749
16. Latimer GW, Horwitz W. Official methods of analysis. Maryland: AOAC International. 2019.
17. Wang D, Luo L, Peng Y, Wang J, Liang J, Xu D, Feng H, Zeng L, Wang J. Detection of two genotoxic impurities in drug substance and preparation of imatinib mesylate by LC–MS/MS. Chromatographia. 2020 Jul;83(7):821-8. https://doi.org/10.1007/s10337-020-03903-1
18. Pounikar AR, Umekar MJ, Gupta KR. Genotoxic impurities: an important regulatory aspect. Asian J Pharm Clin Res. 2020 Jun 7;13(6):10-25.
19. Balaji N, Sultana S. Development and Validation of Sensitive GC and GC-MS Methods for the Detection and Quantification of Alkyl Mesylates in Pharmaceutical Active Ingredients. Recent Developments in Chemistry and Biochemistry Research Vol. 6. 2024 Sep 23:140-54. https://hal.science/hal-05137878
20. Pena-Pereira F, Wojnowski W, Tobiszewski M. AGREE—Analytical GREEnness metric approach and software. Analytical chemistry. 2020 Jun 15;92(14):10076-82. https://doi.org/10.1021/acs.analchem.0c01887
21. Liu Z, Fan H, Zhou Y, Qian X, Tu J, Chen B, Duan G. Development and validation of a sensitive method for alkyl sulfonate genotoxic impurities determination in drug substances using gas chromatography coupled to triple quadrupole mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 2019 May 10;168:23-9. 10.1016/j.jpba.2018.12.044
22. Jin B, Guo K, Zhang T, Li T, Ma C. Simultaneous Determination of 15 Sulfonate Ester Impurities in Phentolamine Mesylate, Amlodipine Besylate, and Tosufloxacin Tosylate by LC-APCI-MS/MS. Journal of Analytical Methods in Chemistry. 2019;2019(1):4059765.https://doi.org/10.1155/2019/4059765