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Determination of sulfur in topical formulations by GC-MS

Selen Al[1], Aykut Kul[1*] and Ahmet Olcay Sagirli[1]

1Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul University, 34452 Istanbul, Türkiye

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Abstract: A simple, sensitive, and validated gas chromatography–mass spectrometry (GC–MS) method was developed for the quantitative determination of sulfur in topical formulations. The separation was achieved on a TR-5MS capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness) using helium as the carrier gas at a flow rate of 1 mL/min. The oven temperature was maintained at 190 °C, and the total analysis time was 13 min. Detection was performed in selective ion monitoring (SIM) mode at m/z 64. The injection volume was 1 µL. The method exhibited excellent linearity within the concentration range of 0.75–15.00 µg/mL. The lower limit of detection (LOD) and quantification (LOQ) were found to be 0.25 µg/mL and 0.75 µg/mL, respectively. Mean recovery values ranged from 98% to 102%, with intra- and inter-day precisions (RSD) not exceeding 2%. Method validation was conducted in accordance with the ICH Q2(R1) guidelines. Furthermore, measurement uncertainty was evaluated following the EURACHEM approach. The validated method was successfully applied to the determination of sulfur in commercial topical formulations, demonstrating its suitability for routine quality control analysis.

Keywords: Sulfur; topical formulations; method validation; GC-MS; uncertainty assessment.

1. Introduction

Scabies is a contagious parasitic dermatitis affecting both humans and animals, caused by the Sarcoptes scabiei mite [1]. It affects approximately 455 million people worldwide each year and remains a major public health problem in underdeveloped and developing regions [2]. The ideal antiscabietic drug should be effective, easy to apply, non-irritating, non-toxic, and cost-efficient [3].

Permethrin is currently the first-line topical treatment option for scabies in Europe and the United States [4]. However, sulfur—a heteroatom with a long history of use in medicinal chemistry—continues to play an important role in dermatological therapy [5]. Topical formulations containing 6–33% sulfur are recommended in the European guidelines for scabies treatment and are considered safe for use in pregnant women and infants [6]. Itching may persist for 2–4 weeks after treatment, and the absence of nocturnal itching and new active lesions after one week indicates clinical recovery [7]. Notably, scabies treatment with sulfur-based topical formulations has been reported to achieve a higher recovery rate compared to permethrin-based preparations [8].

Chromatographic techniques are widely employed for the separation and quantification of pharmaceutical compounds [9]. According to the literature, various methods have been developed for the determination of sulfur using high-performance liquid chromatography (HPLC) [10–16] and gas chromatography–mass spectrometry (GC–MS) [17–21]. However, these methods have primarily been applied to environmental matrices rather than pharmaceutical formulations. Although several GC–MS methods have been reported for sulfur analysis, they have focused exclusively on geological,

* Corresponding author E-Mail: aykut.kul@istanbul.edu.tr

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environmental, fuel, or industrial samples, with no application to topical pharmaceutical dosage forms. Therefore, the environmental GC–MS studies cited here represent the only available instrumental approaches and were included purely for methodological context. The lack of validated GC–MS methods for sulfur determination in dermatological products clearly highlights the analytical gap and supports the novelty of the present study.

To the best of our knowledge, no validated analytical method has yet been reported for the determination of sulfur in topical dosage forms. Therefore, this study presents the first validated GC–MS method for the quantitative determination of sulfur in topical formulations in accordance with the International Council for Harmonisation (ICH) Q2(R1) guideline [22]. Furthermore, the measurement uncertainty of the developed method was evaluated following the EURACHEM/CITAC guide [23, 24]. The aim of this study was to develop a novel, simple, and selective GC–MS method for the determination of sulfur in pharmaceutical dosage forms.

2. Experimental

2.1. Materials

The sulfur reference standard was purchased from Sigma-Aldrich (Taufkirchen, Germany). The topical pharmaceutical preparation Wilkinson pomade® (containing 12.50 mg sulfur per 100 mg formulation) was obtained from Mega-Farma (Türkiye). All other reagents and solvents of analytical grade were supplied by Merck (Germany).

2.2. Standard Solutions

Standard solutions of sulfur were prepared at seven concentration levels (0.75, 5.00, 7.50, 10.00, 12.50, 13.75, and 15.00 µg/mL). Elemental sulfur was dissolved in dichloromethane with 15 minutes of ultrasonic agitation to ensure complete solubilization, and this solvent also allowed full dissolution of placebo components. During ultrasonic extraction, the bath temperature was maintained below 25 °C to minimize solvent evaporation and potential sulfur loss due to the volatility of dichloromethane. Each solution was freshly prepared before analysis using suitable dilutions to prevent crystallization or precipitation of sulfur. All standard solutions were vortex-mixed and visually inspected to confirm homogeneity before GC–MS injection.

2.3. GC-MS Conditions

Analyses were performed on a Shimadzu GC-2010 Plus GC–MS system (Shimadzu Scientific Instruments, Columbia, MA, USA). The separation of sulfur was achieved on a TR-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness; Restek, USA) using helium as the carrier gas at a flow rate of 1.0 mL/min. The injector was operated in split mode (20:1) using a deactivated quartz wool-packed liner.

The oven temperature was maintained at 190 °C for 13 minutes. The interface and ion source temperatures were set at 200 °C and 190 °C, respectively. Electron impact ionization (EI) was performed at 70 eV with a scan range of m/z 40–400 at 0.2 scans s^{-1}. Quantification was carried out in selective ion monitoring (SIM) mode, monitoring m/z 64 for sulfur (Figure 1).

2.4. Method Validation

The developed GC–MS method for the quantitative determination of sulfur was validated in accordance with the ICH Q2(R1) guideline [22]. The validation parameters evaluated included system suitability, specificity, accuracy, precision, sensitivity, linearity, stability, and robustness. System suitability was confirmed by six replicate injections of the sulfur standard solution, and chromatographic performance was found to be within acceptable limits based on theoretical plate number, resolution, and tailing factor values. The method demonstrated high specificity, as no interfering peaks were observed at the retention time of sulfur in blank, placebo, or standard chromatograms.

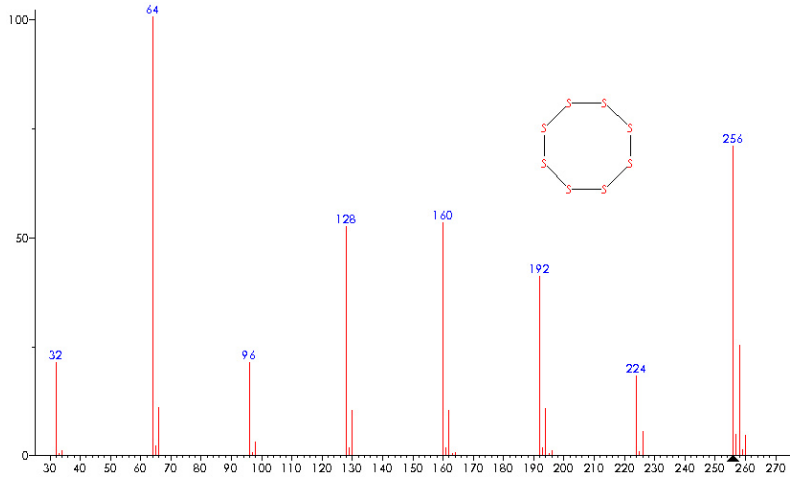

Figure 1. Full-scan mass spectrum of sulfur

Accuracy was evaluated at three concentration levels (0.75, 12.50, and 15.00 µg/mL) by spiking placebo samples, and the mean recoveries were within the acceptable range of 98–102%. The precision of the method was assessed through intra-day and inter-day repeatability studies, each performed at the same three concentration levels and analyzed in six replicates on three separate days. The calculated relative standard deviation (RSD) values were below 2%, confirming the method's reproducibility.

Sensitivity was determined by calculating the limits of detection (LOD) and quantification (LOQ), corresponding to signal-to-noise ratios of 3 and 10, respectively. The LOD and LOQ values (0.25 and 0.75 µg/mL) were experimentally determined based on signal-to-noise ratios obtained directly from GC–MS data, in accordance with the ICH Q2(R1) guideline. These limits were derived from replicate injections of the lowest calibration standards, confirming the instrumental sensitivity and reliability of the method. The method exhibited linearity over the concentration range of 0.75–15.00 µg/mL with a correlation coefficient (R^2) greater than 0.99, indicating a strong linear relationship between concentration and peak area.

The stability of both standard and sample solutions was evaluated by reanalyzing them after 24 hours under ambient conditions, and no significant differences were observed, confirming solution stability within this period. Finally, robustness testing demonstrated that minor deliberate variations in chromatographic parameters—such as flow rate (±0.1 mL/min) and oven temperature (±1 °C)—did not significantly affect the analytical results, indicating the reliability of the developed GC–MS method under routine laboratory conditions.

2.5. Sample Preparation

Twenty topical formulations containing sulfur were individually weighed (12.50 mg sulfur per 100.00 mg formulation) into 10 mL volumetric flasks. Approximately 9 mL of dichloromethane was added and the mixture was sonicated for 15 min to ensure complete dissolution. The final volume was adjusted to 10 mL with dichloromethane, mixed, and filtered. An aliquot of 10 µL of the filtrate was diluted with 900 µL of ethanol, vortexed, and 1 µL was injected into the GC–MS system. A 1:100 dilution with ethanol was performed prior to injection to reduce viscosity and matrix load, ensuring proper vaporization and compatibility with the GC inlet.

2.6. Uncertainty Assessment

The main sources of uncertainty in the validated method were evaluated in accordance with the EURACHEM/CITAC Guide. Uncertainty components arising from the purity of the reference standard, weighing, calibration curve parameters, recovery, and repeatability were identified as the main contributors. The combined standard uncertainty was calculated using the root-sum-of-squares approach according to the following expression:

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$$u = \sqrt{(u_{standard})^2+(u_{weighing})^2 (u_{calibration})^2+(u_{recovery})^2+(u_{repeatability})^2}$$

In this equation, $u_{standard}$ represents the uncertainty due to the purity of the reference material, $u_{weighing}$ corresponds to the weighing process, $u_{calibration}$ is related to the slope of the calibration curve, $u_{recovery}$ reflects the variability observed in recovery studies, and $u_{repeatability}$ denotes within-run analytical precision. The expanded uncertainty (U) was then calculated at a 95% confidence level using the equation:

$$U = k.u$$

where k is the coverage factor, taken as 2 to correspond to an approximate 95% level of confidence. The reported expanded uncertainty (U) is traceable to SI units through the certified purity of the reference standard and the calibration of the analytical balance, ensuring full metrological traceability of the measurement results. The overall uncertainty budget was established in line with the recommendations of the EURACHEM/CITAC Guide [23,24], and detailed numerical calculations are provided in the Supplementary Information.

3. Results and discussion

3.1. Method Optimization

According to the literature, various organic solvents have been employed for the extraction of sulfur, including chloroform [10–12,15,17], acetone [13,14], cyclohexane [18], carbon disulfide [19], and toluene [20]. In some studies, binary solvent systems such as chloroform–cyclohexane [16] and acetone–hexane [21] have also been utilized.

In the present study, several organic solvents—methanol, ethanol, acetonitrile, acetone, ethyl acetate, hexane, chloroform, and dichloromethane—were systematically evaluated for their extraction efficiency of sulfur from topical formulations. Among these, hexane, chloroform, and dichloromethane were found to effectively dissolve the formulations and enable efficient sulfur extraction. The recovery obtained using hexane was 91%, whereas recoveries above 95% were achieved with chloroform and dichloromethane. Considering both extraction performance and safety, dichloromethane was selected as the optimal solvent due to its lower toxicity compared to chloroform and its superior recovery efficiency.

Using this optimized solvent system, a robust GC–MS method was successfully developed for the determination of sulfur in topical formulations, providing excellent resolution and well-defined peak separation for the analyte.

3.2. Validation of the Method

Previous studies on the determination of sulfur have primarily focused on environmental matrices, and none of these reported methods were fully validated in accordance with international guidelines [10–21]. The present study represents the first comprehensive validation of a GC–MS method for sulfur determination in topical formulations, performed in accordance with the ICH Q2(R1) guideline [22].

Under the optimized chromatographic conditions, sulfur was eluted at 11.50 minutes. The tailing factor and theoretical plate number were found to be 1.4 and 66,060, respectively, indicating excellent peak symmetry and efficient column performance. No endogenous or formulation-related interferences were observed at the retention time of sulfur, confirming the method's specificity. No interfering signals were detected at m/z 32 or 48, confirming the selectivity of the method for sulfur monitoring at m/z 64 (Figure 2).

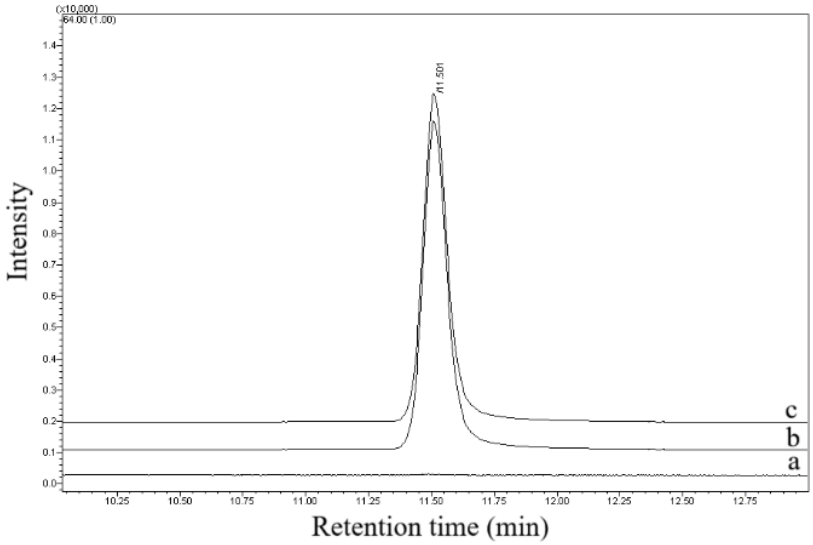

Figure 2. Chromatograms of the placebo (a), sulfur standard (b), and pharmaceutical dosage form (c)

Accuracy was evaluated at three concentration levels (0.75, 12.50, and 15.00 µg/mL), with recovery values ranging between 98% and 102%, demonstrating the reliability of the method for quantitative analysis. The detailed accuracy data are presented in Table 1.

Table 1. Accuracy results

	Concentration (µg/mL)			**Recovery (%)**		
	0.75	**12.50**	**15.00**	**100.00**	**100.00**	**100.00**
1	0.76	12.53	14.99	101.30	99.60	99.90
2	0.75	12.40	15.20	100.00	100.20	101.30
3	0.76	12.35	15.17	101.70	99.20	101.10
4	0.75	12.33	14.88	100.30	98.80	99.20
5	0.76	12.40	15.11	101.70	98.60	100.70
6	0.76	12.41	15.08	101.30	99.20	100.50
Mean	0.76	12.40	15.07	101.05	99.27	100.46
SD	0.01	0.07	0.12	0.73	0.58	0.79
RSD (%)	0.718	0.556	0.787	0.718	0.579	0.787

Precision was confirmed through repeatability and reproducibility studies, where relative standard deviation (RSD) values were ≤2% across all concentration levels, as summarized in Table 2. These results collectively indicate that the method possesses high sensitivity and precision. The slightly higher %RSD observed at the lowest concentration level (0.75 µg/mL) was attributed to the expected variability near the LOQ, which is consistent with typical analytical performance for trace-level determinations.

Table 2. Precision results

	Repeatability Concentration (µg/mL)			**Reproducibility Concentration (µg/mL)**		
	0.75	**12.50**	**15.00**	**0.75**	**12.50**	**15.00**
1	0.75	12.51	15.03	0.75	12.52	15.00
2	0.77	12.48	15.10	0.77	12.41	15.09
3	0.76	12.49	15.22	0.78	12.57	15.13
4	0.77	12.42	14.94	0.76	12.52	14.94
5	0.75	12.61	15.03	0.75	12.44	15.21
6	0.77	12.57	14.92	0.76	12.39	14.92
Mean	0.76	12.51	15.04	0.76	12.48	15.05
SD	0.01	0.07	0.11	0.01	0.07	0.11
RSD (%)	1.29	0.541	0.732	1.54	0.575	0.758

Linearity was established within the concentration range of 0.75–15.00 µg/mL, yielding the calibration equation $y = 6532x + 3524$ with a correlation coefficient (R^2) greater than 0.99. Calibration curves were constructed using unweighted least-squares regression, as no heteroscedasticity was observed across the calibration range.

The slope reproducibility was excellent across replicate calibrations, confirming the stability and reliability of the calibration function.

The intercept value accounted for less than 5% of the analytical response at mid-range concentrations and did not introduce any systematic bias; therefore, it was retained in the calibration model. The lower limit of quantification (LOQ) and the lower limit of detection (LOD) were determined as 0.75 µg/mL and 0.25 µg/mL, respectively, confirming the high sensitivity of the method.

Stability testing revealed no significant changes in the analytical response after 24 hours, indicating that both standard and sample solutions remained stable during the analysis period. Thus, stability was not considered a critical parameter (Table 3).

Table 3. Result of stability study

Time (h)	**Standard area**	**Sample area**
0	88253	86535
24	87073	87339
Change %	-1.36%	+0.92%

Furthermore, robustness testing showed that deliberate small variations in chromatographic parameters, such as flow rate and oven temperature, did not produce significant changes in assay results, confirming the robustness of the developed method (Table 4).

Table 4. Result of the robustness study

Flow rate (mL/min)	**Recovery (%)**
0.9	99.51
1.0	99.87
1.1	100.23
RSD %*	0.36
Column temperature (°C)	**Recovery (%)**
189	101.24
190	100.17
191	99.93
RSD %*	0.69

*RSD % values represent recovery variation.

Compared with previous GC–MS studies on environmental matrices—which typically reported LOD/LOQ values in the low µg/mL range and precision of ~1–5 RSD %—the present method achieved an

LOD of 0.25 µg/mL and an LOQ of 0.75 µg/mL with intra- and inter-day precision ≤2 RSD%, while complying with ICH Q2(R1). This performance, obtained directly in a topical pharmaceutical matrix, supports the method's suitability for routine quality control of sulfur-containing products.

3.3. Application to the Analysis of Pharmaceutical Dosage Forms

The proposed GC–MS method was successfully applied to the quantitative determination of sulfur in Wilkinson pomade®. The sulfur content in the analyzed topical formulation was found to be 12.57 mg per 100.00 mg of pomade, corresponding to 100.56% of the labeled claim. These results demonstrate the accuracy and applicability of the developed method for routine quality control analysis of sulfur-containing topical products.

3.4. Uncertainty Assessment

The combined and expanded uncertainty values are presented in Table 5, while detailed calculation steps are provided in the Supporting Information. The uncertainty contribution from the $u_{weighing}$ parameter was found to be negligible; therefore, it was not included in the table and was omitted from the overall uncertainty calculation.

Table 5. Data of combined and expanded uncertainty

Analyte	$u_{standard}$	$u_{calibration}$	$u_{wecovery}$	$u_{repeatability}$	$u_{combined}$	$U_{expanded}$ (k = 2)
Sulfur[a]	0.289	0.930	0.236	0.221	1.026	2.052

[a]Uncertainty for 12.52 µg/mL; 95 % confidence level; U % values reported.

4. Conclusions

A new GC–MS method was successfully developed and validated for the determination of sulfur in topical formulations. The method demonstrated excellent linearity, accuracy, precision, and robustness, with RSD % values below 2% for all validation parameters. The low detection and quantification limits confirmed the high sensitivity of the method, while the absence of interference at the retention time of sulfur ensured specificity. The expanded uncertainty value (U = 2.052, k = 2) indicated reliable measurement performance with 95% confidence. The method was effectively applied to the analysis of Wilkinson pomade®, yielding 100.56% of the labeled sulfur content. These results prove that the developed GC–MS method is specific, sensitive, accurate, reproducible, and suitable for routine quality control of sulfur-containing topical pharmaceutical dosage forms. Additionally, the method offers practical applicability for routine batch release testing and stability studies within the pharmaceutical quality control workflow.

ORCID
Selen Al: 0009-0009-2014-1998
Aykut Kul: 0000-0002-1195-0939
Ahmet Olcay Sagirli: 0000-0002-0434-2150

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