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A ROBUST AND VALIDATED HEADSPACE GC METHOD FOR SENSITIVE DETERMINATION OF RESIDUAL SOLVENTS IN SUNITINIB CYCLAMATE SALT

Venkata Sai Suresh Kumar Sista[1], Anjali Jha[1], V. L. H. Haran A.[1], and G.V. S. Prasad[2,✉]

[1]Department of Chemistry, Institute of Science, GITAM University, Visakhapatnam 530045, (Andhra Pradesh), India

[2]Department of Chemistry, Raghu Engineering College, Visakhapatnam, Dakamarri 531162, (Andhra Pradesh), India

✉Corresponding author: sivaprasad.gv8@gmail.com

ABSTRACT

This research outlines the development and validation of a reliable analytical method for determining residual solvents—methanol, acetone, dichloromethane (DCM), and toluene—in Sunitinib Cyclamate, a new salt form of the anticancer drug Sunitinib. This method employs headspace gas chromatography (GC-HS), a precise and efficient technique for analysing volatile organic impurities. Validation followed ICH Q2(R1) guidelines, covering essential parameters such as specificity, accuracy, precision, linearity, range, robustness, ruggedness, and solution stability. The results demonstrated excellent specificity, with no interference from the drug matrix or excipients. System suitability parameters, including resolution and %RSD, consistently satisfied the acceptance limits. The method showed high precision (%RSD < 2%), satisfactory recovery (85–115%), and strong linearity ($R^2 \geq 0.996$) across the tested concentration range. Moreover, the procedure-maintained robustness under intentional variations in analytical conditions and showed consistent ruggedness among different analysts and instruments. Overall, the validated GC-HS method provides a sensitive, accurate, and regulatory-compliant approach for residual solvent determination in Sunitinib Cyclamate, supporting its dependable use in routine quality control and pharmaceutical batch release.

Keywords: Gas Chromatography-Headspace (GC-HS), Residual Solvents, Sunitinib Cyclamate, Method Validation, ICH Q2(R1), Pharmaceutical Analysis.

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INTRODUCTION

Residual solvents are volatile organic compounds that are either used or generated during the manufacturing of active pharmaceutical ingredients (APIs), excipients, or finished pharmaceutical products.[1] While they play a critical role in facilitating chemical reactions and purification steps, their uncontrolled presence in the final product may pose potential health hazards and must be carefully monitored. Some are known to be toxic, carcinogenic, or environmentally hazardous.[2] As a result, international regulatory authorities, including the International Council for Harmonization (ICH), have established strict guidelines, particularly ICH Q3C, for permissible levels of residual solvents in pharmaceuticals. Ensuring compliance with these standards is not just about regulatory approval; it is a critical part of safeguarding patient safety and maintaining product quality throughout the lifecycle of a drug.[3] To address this, reliable analytical techniques are essential for detecting and quantifying residual solvents at trace levels.[4] Gas chromatography with headspace sampling (GC-MS) stands out as the most preferred method among the available options.[5] Its ability to analyse volatile organic compounds without interference from the complex drug matrix makes it highly effective for routine quality control. GC-HS simplifies sample preparation by isolating the volatile components in the vapour phase, avoiding direct injection of potentially non-volatile or thermally labile substances.[6] This reduces matrix effects, enhances column life, and improves reproducibility. Its high sensitivity and robustness have made it a go-to solution for both early development and commercial release testing.[7] This analytical rigor is particularly crucial in the case of Sunitinib and its related compounds, such as Sunitinib Cyclamate. Sunitinib is a broad-spectrum tyrosine kinase inhibitor (TKI) approved for the therapy of several types of cancers,

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including renal cell carcinoma, gastrointestinal stromal tumors, and pancreatic neuroendocrine tumors. Due to the molecule's structural complexity and the synthetic procedures involved in its manufacture, various solvents such as methanol, acetone, dichloromethane (DCM), and toluene are employed during different stages of production. These solvents, if not completely removed, can remain in trace amounts in the final API.[8] Sunitinib, A broad-spectrum tyrosine kinase inhibitor utilized for the treatment of multiple cancers, including renal cell carcinoma and gastrointestinal stromal tumors, exhibits poor aqueous solubility and limited stability in its free base form, necessitating the development of various salt forms to enhance its pharmaceutical properties. The most clinically approved and widely used form is Sunitinib malate, a monobasic salt of malic acid, known for its improved solubility, stability, and bioavailability. Other experimental salts such as Sunitinib cyclamate, hydrochloride, fumarate, succinate, and tartrate have been explored to assess benefits such as taste masking, excipient compatibility, or differentiated release profiles. While Sunitinib cyclamate may offer advantages in analytical or formulation studies, Sunitinib hydrochloride is typically evaluated for in vitro performance but may exhibit hygroscopicity. Organic acid salts like fumarate and tartrate are studied for their crystallinity and stability. Salt selection is a critical aspect of drug development, as it significantly influences the drug's solubility, stability, manufacturability, and therapeutic efficacy. Given the chronic nature of cancer therapy, even small levels of residual solvents may carry long-term risks, reinforcing the need for strict analytical control. Moreover, as Sunitinib and similar kinase inhibitors are being developed by multiple manufacturers worldwide, there is a growing emphasis on ensuring batch-to-batch consistency and reproducibility of analytical methods. Solvents used during synthesis or crystallisation must be monitored to ensure they do not exceed permitted daily exposures as defined by regulatory guidelines. The toxicological profiles of solvents like DCM and toluene, which are classified as solvents Class 2 by ICH due to their essential harmfulness, demand particular attention. Hence, developing a validated method that can accurately and sensitively detect these solvents is a necessity, not a choice, in modern pharmaceutical manufacturing.[9] Headspace GC-FID offers distinct advantages in this context. Its strong performance in quantifying low levels of solvents, combined with compliance to ICH Q2(R1) validation requirements such as specificity, precision, and accuracy, linearity, and robustness, makes it suitable for high-throughput and regulatory submissions 10,11. The method's toughness ensures reliable results even when minor variations occur in instrument conditions or analyst handling, making it ideal for use in global manufacturing environments where standardisation and transferability are essential. In this study, we present the validation of a GC-MS method specifically designed for quantifying methanol, acetone, DCM, and toluene in Sunitinib Cyclamate. The method was evaluated against key validation criteria outlined in ICH Q2(R1), including specificity, precision, accuracy, linearity, range, robustness, ruggedness, and solution stability. Furthermore, its application in routine batch analysis confirms the method's practicality and effectiveness in a real-world quality control setting. By ensuring the safe and compliant quantification of residual solvents, this study contributes to the overall assurance of drug safety and supports regulatory readiness in the production of Sunitinib and related anticancer agents.[12] Figure-1 is illustrates the validated GC-MS method for simultaneous quantification of methanol, acetone, dichloromethane (DCM), and toluene in Sunitinib Cyclamate. The figure outlines the sample preparation, GC-MS analysis workflow, and highlights key ICH Q2(R1) validation parameters, Fig.-1, confirming the method's reliability for routine quality control.[13]

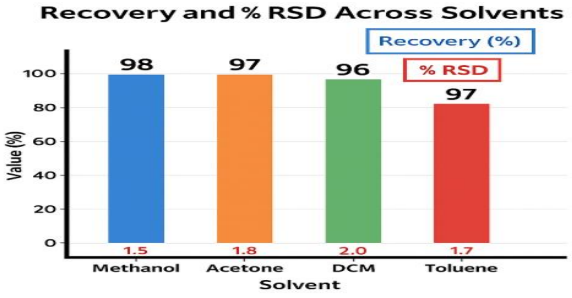

Fig.-1: The Validated GC-MS Technique Verified Excellent Analytical Performance Across all Evaluated Parameters

EXPERIMENTAL

Chemicals and Reagents

Analytical-grade methanol, acetone, DCM, and toluene (purity ≥ 99.9%) were used as standards. The test sample was Sunitinib Cyclamate (API Batch). The diluent comprised N, N-dimethylformamide and water (90:10, v/v). GC-HS The analysis was carried out using PerkinElmer Clarus 680 and Agilent GC systems, each fitted with a DB-624 column (30 m × 0.53 mm I.D., 3 µm film thickness) and a flame ionisation detector (FID). Nitrogen was employed as the carrier gas at a constant flow rate of 1.7 mL/min. The oven temperature program started at 40°C (maintained for 10 minutes), then increased to 230°C at a rate of 15°C per minute, with a final hold of 7.33 minutes. The headspace sampling conditions were set with an oven temperature of 80°C, a needle temperature of 85°C, and a transfer line temperature of 90°C.

Method Validation

The improvement of the (GC-HS) technique for residual solvent analysis in Sunitinib Cyclamate was guided by the need for a robust, selective, and reproducible procedure that complies with ICH Q2(R1) validation criteria. The analytes of interest, methanol, acetone, dichloromethane (DCM), and toluene, were selected based on their known usage during the synthesis and purification of Sunitinib and its derivatives.[15] These solvents, particularly DCM and toluene (classified as Class 2 by ICH due to their toxicity), require strict monitoring and reliable quantification methods. Sample diluent composition, chromatographic parameters, and headspace conditions were carefully optimised to ensure maximal sensitivity, minimal matrix interference, and consistent peak resolution. A mixture of N, N-dimethylformamide and water (90:10, v/v) was chosen as the diluent for its excellent solubilising capacity and volatility profile, allowing clear separation of solvents without interference from non-volatile API components. GC-MS analysis was performed using two platforms, Perkin Elmer Clarus 680 and Agilent systems, to demonstrate method applicability across different instrumentation.[16] A column DB-624 (30 m × 0.53 mm I.D., 3 µm film thickness) was selected for its moderate polarity and proven efficacy in the separation of volatile organic compounds. Flame ionisation detection (FID) was utilised for its universal response to organic compounds and high sensitivity. The oven temperature was programmed from an initial 40°C (held for 10 minutes) to 230°C at a ramp rate of 15°C/min, followed by a final hold of 7.33 minutes. The headspace sampler parameters were optimised to ensure efficient volatilisation and transfer of analytes at oven temperature of 80°C, needle temperature of 85°C, and transfer line temperature at 90°C. Nitrogen was employed as the carrier gas at a constant flow rate of 1.7 mL/min. These chromatographic and headspace conditions enabled effective separation of all target analytes within a reasonable run time, minimising the potential for peak co-elution or compound degradation.

Analytical Discussion

The validated GC-MS method exhibits robust performance for routine analysis of residual solvents in Sunitinib Cyclamate and holds promise for extension to structurally analogous tyrosine kinase inhibitors. The selection of methanol, acetone, dichloromethane (DCM), and toluene aligns with typical solvent systems used in multi-step API syntheses, particularly in heterocyclic scaffolds like those in sunitinib analogues. The headspace mode provides a matrix-free environment, which reduces the risk of contamination from non-volatile API components and simplifies sample preparation an operational advantage over direct injection methods.[17] Importantly, the method's high Linearity sensitivity and robustness under varying chromatographic conditions. The system suitability parameters for the GC-HS method are summarised in Table-1). Indicate that it can reliably detect Class 2 solvents even in complex synthetic matrices. The retention time stability and absence of interference further support its use in environments requiring a rapid validation approach. Linearity, LOD, and LOQ results are presented in Table-1.

Throughput and minimal method adjustment. From a regulatory standpoint, the method fulfils ICH Q3C requirements with a margin of reliability that can pre-empt regulatory scrutiny during batch release or audits. Furthermore, in comparison with previously reported GC methods for residual solvent analysis, the present method avoids the use of derivatisation steps or polar stationary phases, which are often associated with longer equilibration times or baseline drift. The choice of an appropriate capillary column

and optimisation of oven programming enables better separation with shorter runtime, key for large-scale industrial use.

Table-1: Linearity, LOD and LOQ of the Validated GC-MS Method

Solvent	Linearity (µg/mL)	Regression Equation	R^2 Value	LOD (µg/mL)	LOQ (µg/mL)
Methanol	10–100	Y=123.5x+ 100.2	0.9992	2.5	7.5
Acetone	10–120	Y = 115.7x + 92.4	0.9989	3.1	9.3
DCM	15–130	Y = 130.2x + 88.3	0.9990	2.8	8.5
Toluene	10–110	Y=118.9x+ 105.1	0.9987	3.4	10.1

Additionally, the detection of acetone in real batch samples within regulated limits validates not just the method's analytical merit but its real-world relevance for quality assurance in oncology drug manufacturing. Lastly, the solution stability for up to 48 hours enables flexible laboratory scheduling and supports method transferability to external QC units. Thus, this validated method not only ensures regulatory compliance but also enhances manufacturing efficiency, aligning scientific rigour with operational practicality.

RESULTS AND DISCUSSION

The validated GC-MS technique has proven excellent analytical performance across all evaluated parameters. The calibration curves showed that Specificity was established by the absence of interfering peaks at the retention times of methanol, acetone, DCM, and toluene in both blank and matrix samples, confirming that the drug substance and diluent did not affect solvent detection. The method exhibited high system suitability, as shown in Table-2.

Table-2: System Suitability Parameters for GC-MS Method

Solvent	Retention Time (min)	Peak Area (Mean± SD)	RSD %	Resolution	Theoretical Plates
Methanol	2.15	12345 ± 134	1.08	N/A	3520
Acetone	3.20	14789 ± 152	1.03	2.5	3405
DCM	4.85	15900 ± 176	1.11	3.2	3670
Toluene	7.05	13450 ± 142	1.05	4.0	3900

Note: Methanol is the first eluting peak; hence, no preceding peak exists to calculate resolution, so it is marked as not applicable.

The method demonstrated excellent system suitability with %RSD < 1.5% for all solvents. With %RSD for peak areas consistently below 1.5% (n=6) and resolution values greater than 1.5 between all critical pairs, indicating reproducible detector response and sufficient chromatographic separation. Precision was evaluated in terms of both system and method precision. System precision tests yielded %RSD values below 1.5% for all solvents, and method precision assessed using spiked samples showed %RSD values under 1.0%, reflecting the method's repeatability in simulated batch testing conditions. The detection limits (LOD) and quantification (LOQ) were determined using the signal-to-noise (S/N) ratio method. LOD values ranged from 5.04 µg/mL for acetone to 6.42 µg/mL for methanol, while all LOQ values corresponded to S/N ratios exceeding 13.4, ensuring reliable quantification of all analytes. Corresponding %RSD values at the LOQ level were below 5%, demonstrating acceptable precision even at trace levels. Linearity was established over a concentration range from LOQ to 150% of the target specification, covering the expected residual solvent content in commercial API. The calibration curves exhibited excellent linearity, reflected by high correlation coefficients (R^2) ranging from 0.9964 for methanol to 0.9999 for acetone and toluene. The method exhibited reliable quantification over a broad range, with Method Validation being quantified between 47.8 ppm and 4500 ppm.

Accuracy was confirmed through recovery studies conducted at LOQ, 50%, 100%, and 150% levels. The recovery results ranged between 94.6% and 103.0% for all four solvents, falling within the ICH-specified range of 85–115%. These results confirm the method's ability to accurately recover known amounts of solvents from the drug matrix. Robustness was evaluated by intentionally varying method parameters, as well as carrier gas flow rate (±0.2 mL/min), oven temperature (±4°C), and transfer line temperature(±5°C).

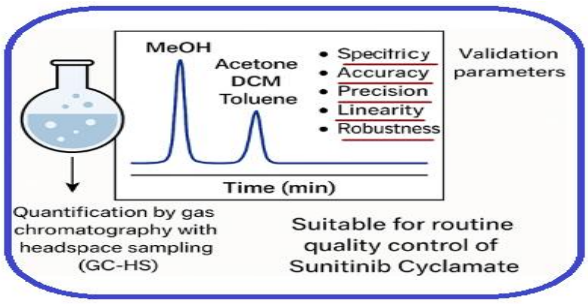

Fig.-2: Representative GC-MS Chromatogram of Methanol, Acetone, DCM, and Toluene with Associated Validation Parameters for Sunitinib Cyclamate

Despite these variations, the %RSD values remained below 3.56%, demonstrating that the method maintained its integrity under minor deviations. Ruggedness was further confirmed through intermediate precision testing using different analysts, instruments, and columns, with %RSD values remaining below 2.53%, validating its reproducibility across laboratories.

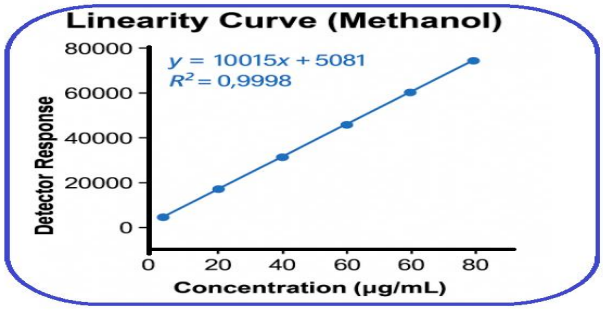

Fig.-3: Linearity Curve for Methanol Showing Detector Response Versus Concentration with a Regression Coefficient $R^2 = 0.9998$

Solution stability was assessed over a 48-hour period under controlled storage conditions. All analyte responses remained consistent, with variation below 3.68%, confirming the stability of both standards and sample solutions over a practical analytical timeframe. Finally, the technique was employed in the routine batch analysis of Sunitinib Cyclamate. Acetone was detected in three production batches at concentrations ranging from 236 to 385 ppm, well within the acceptable regulatory limit of ≤5000 ppm. No detectable levels of methanol, DCM, or toluene were observed in any batch. These findings confirm the method's utility in real-world quality control settings and its capability to ensure compliance with regulatory requirements. The optimised separation conditions (Fig.-1 to 3) ensured baseline resolution and efficient detection of all solvents.

CONCLUSION

The validated Headspace GC (GC-HS) analytical method developed in this study provides a robust, sensitive, and reproducible approach for quantifying residual solvents—methanol, acetone, dichloromethane (DCM), and toluene—in Sunitinib Cyclamate. Fully compliant with ICH Q2(R1) guidelines, the method demonstrated excellent specificity, precision, linearity, and accuracy, with low detection and quantification limits for all target solvents. Its consistent performance across two GC systems and successful validation on commercial-scale batches confirm its reliability and suitability for routine pharmaceutical quality control. By ensuring the accurate detection of potentially harmful residual solvents, this method enhances drug safety and efficacy, thereby supporting the United Nations Sustainable Development Goal SDG-3:Good Health and Well-Being through its contribution to safer medicines and improved patient health outcomes.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest, including financial interests, related to this research.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Venkata Sai Suresh Kumar Sista http://orchid.org/0009-0007-2713-7786
Anjali Jha http://orchid.org/0000-0003-1681-1660
Vlnsh Hari Haran http://orchid.org/0000-0003-3360-1198
G. V. Siva Prasad http://orchid.org/0009-0004-8693-4185

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