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HEADSPACE GAS CHROMATOGRAPHY USING FLAME IONIZATION DETECTOR FOR THE VALIDATION OF VOLATILE MIGRANTS AMOUNT IN STOPPERS OF ONCOLOGY DRUG PRODUCT SYSTEMATIC APPROACH

Gorre Vijaya Chandra, Lakshmi Bavisetti and D. Suryakala and G.V. Padmakar Rao

Department of Chemistry, GITAM (Deemed to be) University, Visakhapatnam-530045, (Andhra Pradesh), India

✉ Corresponding author: lbaviset@gitam.edu

ABSTRACT

Headspace gas chromatography (HS-GC) methods are highly effective for quantifying volatile migrants in stoppers, as these volatile compounds often contain harmful solvents that require stringent control. However, no HS-GC methods have until now been reported for measuring volatile migrants in the stoppers of oncology drug products. Hence, this study aims to address this gap in the literature by developing a reliable and systematic approach for the detection of volatile migrants in stoppers. A method for analyzing the volatile migrants in stoppers was developed and validated. The analytical method used to determine the volatile migrants amount in stoppers for Melphalan Hydrochloride for Injection 50mg/vial and Sterile Diluent for Melphalan for Injection 10mg/vial by the Headspace Gas Chromatography Flame Ionization Detector method. The validation was performed on Agilent DB-624(30m×0.25 mm×1.4µm) chromatographic column, Inlet Temp 240°C, Split ratio 50:1, Injection volume 1mL, Temperature program 40°C hold 6min,15°C/min heat to 150°C, hold 3min, 20°C/min heat to 240°C, hold 10min, FID Temperature 250°C, Line Temperature 90°C and carrier gas,N_2.The validation results of volatile migrants amount in stoppers were linear over the Toluene concentration from 0.0104 µg/mL to 1.2541µg/mL (r = 1.00) and the Toluene recoveries and precision ranged from 97% to 102% (RSD < 10%, n =6). This method was developed and validated successfully, and all the results met the acceptance criteria. Hence, this method is suitable for testing the volatile migrants amount in stoppers of Melphalan Hydrochloride for Injection 50mg/vial and Sterile Diluent for Melphalan for Injection 10mg/vial.

Keywords: Volatile, Migrants, Headspace Gas Chromatography, Validation, Stoppers and Toluene.

INTRODUCTION

The stability and safety of pharmaceutical products, particularly in oncology are paramount considerations in drug development. Packaging components, such as stoppers, play a crucial role in maintaining the integrity of drug formulations. Understanding and quantifying volatile migrants in these stoppers are essential for ensuring the quality and efficacy of oncology drug product injections. Volatile migrants refer to substances that can migrate from the packaging material into the drug product, potentially impacting its stability and safety.[1] In the context of oncology drug product injections, where precision and reliability are critical, identifying and quantifying volatile migrants becomes imperative. This study investigates the volatile migrants in stoppers designed for oncology drug product injections. Employing analytical techniques, particularly Headspace Gas Chromatography with Flame Ionization Detector (HS-GC-FID), we aim to develop a robust method for detecting and quantifying volatile migrants.[2] This technique offers the advantage of high sensitivity and selectivity, making it suitable for the nuanced analysis required in pharmaceutical research.[3] As the pharmaceutical industry strives for continuous improvement and adherence to regulatory standards, comprehensive studies on volatile migrants contribute significantly to the overall understanding of product safety.[3] By elucidating the nature and quantity of volatile migrants in stoppers, this research seeks to enhance the reliability of drug formulations and consequently the therapeutic outcomes for patients undergoing oncological treatments. In the subsequent sections of the research work, all the materials and methods employed for the analysis present our findings and engage in a detailed

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discussion of the implications of volatile migrants in the context of oncology drug product injections. This study addresses current gaps in the literature and provides valuable insights for pharmaceutical researchers, regulatory bodies and manufacturers committed to ensuring the highest standards of pharmaceutical quality and safety.[4-5] Nicholas B. Tiscione *et al.*[6] described that volatiles are regularly dreary as opiates. Processes utilized to identify were usually non-specific if analyzed concurrently by either ethanol or requiring a further procedure, which can be mass spectrometry for different reagents or chemicals, subjected to this process validation and qualitative identification. This process aims to identify qualitatively, including the effects of the matrix and other parameters. Bruno M. Carvalho *et al*[7], described this process for the validation of HS-GC-FID for determining different reagents that commence with lipid oxidation in the samples, which are collected from the cat. The evaluation of the matrix effect on the values 88 - 109% is the mean recovery observed. RSD value is less than that of 6.95%, 0.44 to 20.88%, and from 0.45 to 20.52%, which are both precisions. These values are finally concluded that high potential, which grants measurement of products and substances of lipid oxidation, which is available in samples of cat food directly. Francisco Pena-Pereira *et al*[8], proposed that CECs, which are present in the various samples that are collected from the environment, have grown into an energetic as well as demanding problem. The analytical methodologies involving microextraction access utilized to enrich CECs are generally concluded. At last, challenging aspects with respect to the miniaturized analytical processes to measure CECs are assessed. M.L. Alonso *et al*[9], proposed that two plasticizer metabolites are analyzed by MHS-SPME, which was coupled by GC–MS. For this, different parameters were assessed using samples collected from the urine. They concluded that the enhanced levels of 2-ethylhexanol are identified as $p < 0.05$. Freitas e*t al*[10], described that PAEs were a class of chemicals that are generally utilized as plasticizers. Different methods for the extraction, purification, and quantification of these analytes were displayed. Corrias, F. *et.al*[11], used 2,4-TDI and 2,6-TDI, which are identified both in the adhesive and in the wine. This process is subjected to validation under Eurachem CITAC guidelines. LOD as well as LOQ to 2,6 TDI along with 2,4 TDI are 0.42 and 0.39 µg/L, and 1.72 and 1.57 µg/L. Nathaly Reyes-Garcés *et al*[12], discussed SPME, which is a versatile, non-exhaustive sample preparation tool that has been demonstrated to be well-suited for facile and effective analysis of a broad range of compounds in a plethora of studies.

EXPERIMENTAL

Materials and Methods

Methanol was purchased from Fisher, UK; Toluene was purchased from Tianmo Quality Testing, China; All reagents were analytical grade; Melphalan Hydrochloride for Injection, 50mg/vial and Sterile Diluent for Melphalan HCl for Injection, 10mL/Vial in this study were obtained as gift samples from Kindos pharmaceuticals Co., Ltd.

HS-GC-FID Apparatus and Conditions

HS-GC-FID was achieved on an Agilent 7890 B-Agilent 7697A(Agilent Technologies).The separation was done on a Agilent DB-624 30m×0.25mm×1.40µm GC Column(Agilent, USA),Analytical Balance was Mettler-Toledo XP205,Incubation Temperature 80°C,Incubation Time 20min,Transfer line Temperature 90°C,Analysis Time 40min,Inlet Temperature 240°C,Carrier velocity 1.5mL/min, Split ratio 50:1, Injection volume 1mL, Carrier Gas N_2, Hydrogen flow 30mL/min, Make-up gas N_2, 25mL/min, FID Temperature 250°C,Air flow rate 300mL/min, Temperature program 40°C hold for 6min, 15°C/min heat to 150°C, hold 3min, 20°C/min heat to 240°C, hold 10min.

Standard and Sample Preparation

For this analysis, diluent is used as Methyl alcohol.

Standard stock solution A is prepared by weighing about 50mg of Toluene into a 50 mL standard flask, which contains about 20 mL diluent, dissolving and diluting to volume with diluent, and mixing well. (about 1.0mg/mL). Standard stock solution B is prepared by transferring 2.5 mL of the standard stock solution A to a 50 mL standard flask, diluting to volume with diluent, and mixing well. (about 50µg/mL). Standard stock solution C is prepared by transferring 1 mL of the standard stock solution B to a 100 mL standard flask, diluting to volume with diluent, and mixing well. (about 0.5µg/mL). Standard solution is prepared by transferring 2.00 mL of diluent to a 10 mL headspace vial, adding 40µL standard stock solution B, and

mixing well. (about 1.0µg/mL). The system suitability solution is prepared by transferring 2 mL of diluent to a 10 mL headspace vial, adding 40µL standard stock solution C and mixing well. (about 0.01µg/mL). The sample solution is prepared by taking one bottle of Melphalan Hydrochloride for Injection, adding 10 mL of diluent of Melphalan Hydrochloride for Injection to dissolve and mixing well. Transferred 1 mL of the above solution to a 20 mL standard flask, diluted to volume with purified water and mixed well. transfer 2 mL to a 10 mL headspace vial and seal. Finally, Blank Solution is prepared by transferring 2 mL of purified water to a 10 mL headspace vial, adding 40 µL of Methyl alcohol, sealing and mixing well.

RESULTS AND DISCUSSION

Method Validation

The method was validated according to the International Conference on Harmonization guidelines.[13] The method was validated for System suitability, specificity, accuracy, precision, linearityand range, limit of quantification (LOQ) and solution stability.

System Suitability

The method should be suitable for its intended use and should be evaluated during routine use to ensure continued performance. System suitability was demonstrated prior to each experiment. The standard solution was used to evaluate the systems suitability. The S/N(signal-to-noise) of the Toluene peak from system suitability was determined. The %RSD of peak areas of the Toluene peak from six replicate injections and bracketing standard solutions was determined. The system suitability requirement was met for all experiments. The %RSD of the Toluene peak area from the six replicate injections of the standard solution was less than 15%. The %RSD of Toluene peak area for all injections of standard solution (including Bracketing) was less than NMT 15%. The s/n for the Toluene peak in the system suitability solution is less than 10. The chromatograms of the system suitability solution are shown in Fig.-1. The test results were summarized in Table-1.

Table-1: System Suitability Results –Area RSD of Standard Solution

Inj. No.	Retention Time(min)	Toluene Peak Area
1	10.560	0.8688
2	10.560	0.8574
3	10.560	0.8314
4	10.563	0.8628
5	10.563	0.8655
6	10.563	0.8406
Average	10.561	0.8544
Stdev	0.002	0.015
%RSD	0.0	1.8
S/N	N/A	18

RSD=Relative standard deviation; S/N=Signal to Noise

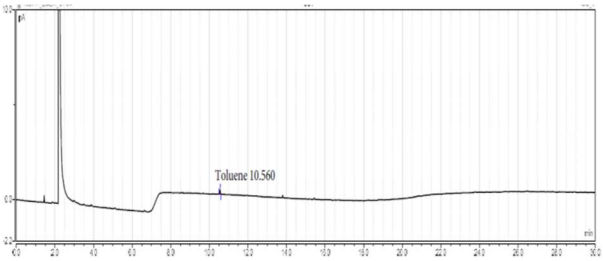

Fig.-1: System Suitability Solution Chromatogram

Specificity

The method should be specific to the analyte of interest and should not respond to other substances in the sample matrix. Blank solution, standard solution, sample solution and sample spike solution were injected into the HS-GC-FID system, and chromatograms were recorded to evaluate the specificity. The blank solution had no interference at the retention time of Toluene and another compound peak from the sample solution and the sample spike solution. The retention time of the Toluene peaks in the sample spike solution

corresponds to that in the standard solution. The recovery of Toluene in the spike sample solution is between 90%-110%. Hence, the specificity requirement was met. Hence, the method is found to be specific. The chromatograms of blank solution, standard solution, sample solution and spike sample solution are shown in Fig.-2,3,4 and 5. The test results are summarized in Table-2.

Table-2: Specificity Test Results

Name	Retention Time(min)	Theoretical Spike Concentration(µg/mL)	Amount (µg/mL)	%Recovery of Toluene
Blank	N. D.	N/A	N/A	N/A
Standard solution	10.56	N/A	N/A	N/A
Sample solution	N. D.	0.0	N.D	N/A
Sample spike solution	10.56	0.9926	1.0145	102%

N.D: Not Detected; N/A: Not Applicable

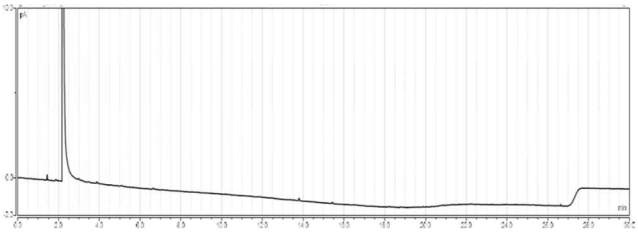

Fig.-2: Blank Solution Chromatogram

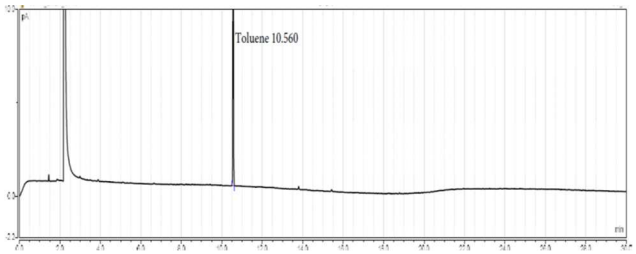

Fig.-3: Standard Solution Chromatogram

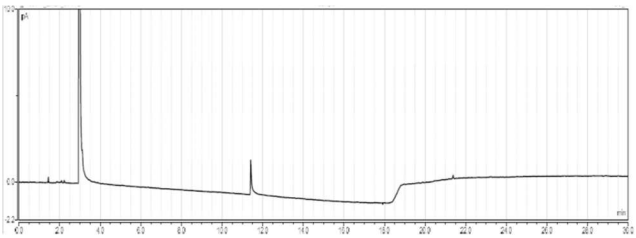

Fig.-4: Sample Solution Chromatogram

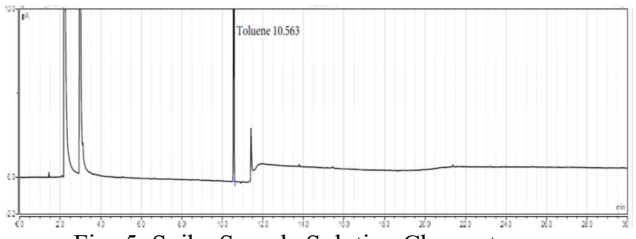

Fig.-5: Spike Sample Solution Chromatogram

Accuracy

The method should measure the analyte of interest with acceptable accuracy. Accuracy can be determined by comparing the results obtained from the method to a known reference standard or by performing a recovery study.

Precision

The method should be reproducible and precise. Precision can be evaluated by analyzing multiple samples and determining the variation in the results. Accuracy and Precision: Accuracy and Precision were

evaluated by analyzing the precision sample solution six times. The recovery of six analyzed results for Toluene is between 90%-110%, and the %RSD (n=6) of recovery of six analyzed results for Toluene is less than 15%. The mean, standard deviation, and confidence interval of recovery for Toluene (n=6). Hence, the method is Accurate and Precise. The chromatograms of accuracy and precision solutions are shown in Fig.-6. All test results meet the acceptance criteria and are summarized in Table-3.

Table-3: Repeatability and Accuracy Test Results

Name	Level	Theoretical Spike Concentration(µg/mL)	Amount (µg/mL)	%Recovery of Toluene
Sample solution	N/A	N/A	N. D	N/A
1	100%	0.9926	1.0107	102%
2			0.9775	98%
3			0.9606	97%
4			0.9699	98%
5			0.9880	100%
6			0.9711	98%
Recovery Mean(n=6)				99%
Recovery SD(n=6)				1.8348
Recovery % RSD(n=6)				2%
Recovery 95% Confidence interval (n=6)				[96%,100.7%]

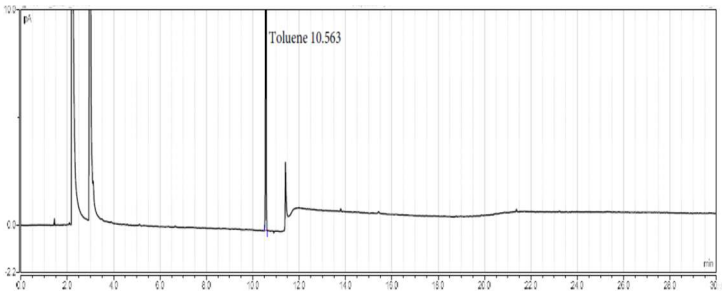

Fig.-6: Accuracy and Repeatability Solution Chromatogram

Limit of Quantification (LOQ)

The method should be able to quantify the analyte of interest with acceptable precision and accuracy at the lowest concentration of interest. The limit of quantitation was determined by injecting the LOQ solution six times and calculating the signal-to-noise ratio. The %RSD for the Toluene peak area in 6 LOQ injections is less than 15%. The signal-to-noise ratio (S/N) for the Toluene peak for six replicate LOQ solution injections is more than 10. LOQ concentration is below the limit concentration (0.5 µg/mL). LOQ Results are listed in Table-4.

Table-4: Results of LOQ

Name	LOQ-1	LOQ-2	LOQ-3	LOQ-4	LOQ-5	LOQ-6	%RSD
Area (pA*min）	0.0105	0.0100	0.0094	0.0092	0.0093	0.0092	5
s/n	32	28	31	47	34	42	NA
Toluene concentration	0.1058µg/mL						

Linearity

The method should be linear over the range of concentrations expected in the sample. Linearity can be assessed by analyzing samples with varying concentrations and evaluating the linearity of the calibration curve. Linearity and Range: The linearity and range were evaluated by determining five concentration levels in triplicate from LOQ to 120% level with respect to a standard solution. The correlation coefficient (R) should be NLT 0.99; % the y-intercept should be NMT ±25% of the standard response. Report the slope of the regression line and residual sum of the squares. The test method is linear within the range of LOQ~1.2µg/mL. In Fig.-7 the Linearity Graph of Toluene is mentioned. The linearity results are listed in Table-5.

Range

The range of the method should be appropriate for the intended use of the method. The range was evaluated by determining at 5 concentration levels in triplicate from the LOQ to 120% level with respect to a standard solution. The range results were listed in Table-6.

Table-5: Linearity Test Results

Name	Level	Concentration(µg/mL)	Peak Area
Linearity Solution 1	LOQ	0.0104	0.0105
			0.0097
			0.0095
Linearity Solution 2	40%	0.4180	0.3524
			0.3420
			0.3423
Linearity Solution 3	80%	0.8361	0.6790
			0.6842
			0.6938
Linearity Solution 4	100%	1.0451	0.8561
			0.8596
			0.8763
Linearity Solution 5	120%	1.2541	1.0243
			1.0369
			1.0231
Linearity equation : y=0.6711x + 0.0069			
The correlation coefficient (R)	1.00	% y-intercept	0%
Slope	0.6711	Q	0.000637

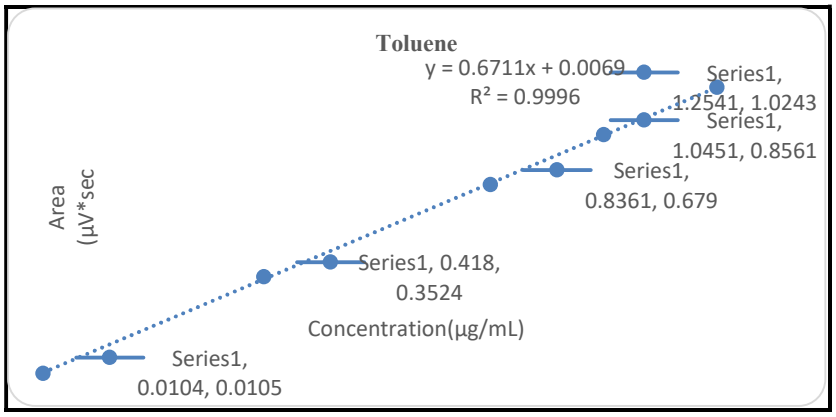

Fig.-7: Linearity Graph of Toluene

Table-6: Range Test Results

Name of Compound	LOQ~120%
Toluene	0.0104µg/mL~1.2541µg/mL

Solution Stability

The stability of the standard solution and sample solution was evaluated by using the standard solution and spike sample solution at room temperature at a suitable time interval. The %RSD of the peak area of Toluene is less than 15.0% for the Standard solution at each time point. The standard solution is stable up to 49h at room temperature. The sample solution is stable up to 19h at room temperature. The validation results are detailed in Table-7 and Table-8.

Table-7: Standard Solution Stability Test Results

Time Point	8h	13h	18h	20h	25h	30h	35h	39h	42h	48h	49h
%RSD	2.4	2.8	2.8	2.7	2.8	3.0	3.1	3.3	3.3	3.6	3.6

Table-8: Sample Solution Stability Test Results

Time Interval	0h	6h	19h
Toluene Amount(ug/mL)	1.0145	1.0019	0.9539
Time interval	0h	6h	19h
Percentage difference	NA	-1%	-6%

Systematic Method Assessment Approach

According to the dosing regimen of the product, the maximum daily dosage of Melphalan Hydrochloride is 28.8mg (1.6mg/m^2/Day×1.8m^2=28.8mg/Day) and the concentration of the constituted solution is 5mg/mL. Besides, as group 1 of carcinogens classified by the International Agency for Research on Cancer (IARC), Melphalan Hydrochloride for Injection is indicated for the palliative treatment of patients with multiple myeloma for whom oral therapy is not appropriate.[13-15] Referring to the ICH M7 guideline, the maximum daily intake is calculated as 1.5 µg. The validation results indicate that the HS-GC-FID method developed for the determination of migration of Toluene in Melphalan Hydrochloride for Injection and Sterile Diluent for Melphalan HCl for Injection is suitable for its intended use. System suitability was demonstrated before each experiment, and the system suitability requirement was met for all experiments. Specificity was evaluated and no interference was observed from diluent or other substances in the sample matrix. Accuracy and precision were also assessed and the results show that the method is accurate and precise for determining Toluene content. The limit of quantitation and limit of detection were determined and the technique was able to quantify and detect the analyte of interest at the lowest concentration of interest with acceptable precision and accuracy. The linearity and range were also evaluated and the method demonstrated linearity and range from LOQ to 120% level with respect to the standard solution. The solution stability of the standard solution was stable for 49 hours at room temperature, and the stability of the sample solution was stable for 19 hours at room temperature. The method has been validated successfully and all results met the respective acceptance criteria. In addition, samples of Melphalan Hydrochloride for Injection and Sterile Diluent for Melphalan HCl for Injection, no compound was detected with an amount exceeding the Analytical Evaluation Threshold (AET). This indicates that the stability of the drug product and diluent is acceptable for long-term storage.

CONCLUSION

In conclusion, this study of Headspace Gas chromatography using FID for the validation of volatile migrants amount in stoppers of oncology drug product, was very well validated. However, no HS-GC methods have until now been reported for measuring volatile migrants in the stoppers of oncology drug products. Hence, with this systematic approach, the gap in the literature is addressed by developing a reliable and systematic quantification approach for the detection of volatile migrants in stoppers. The method demonstrated precision, accuracy and sensitivity with no detected compounds exceeding the AET. Notably, it addressed a critical gap in the literature by establishing a reliable approach for assessing volatile migrants, specifically toluene content in stoppers used for the oncology drug product of Melphalan Hydrochloride for Injection. All validation parameters complied with regulatory standards, confirming the methods suitability for routine stability studies. The results consistently met predefined acceptance criteria, supporting its application for volatile migrant analysis. Given its robustness, this HS-GC-FID method provides a sustainable and efficient solution for routine quantification of volatile migrants in stoppers.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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:

Gorre Vijaya Chandra https://orcid.org/0009-0000-5203-6353
Lakshmi bavisetti https://orcid.org/0000-0001-9513-5415
D. Suryakala https://orcid.org/0000-0001-7964-1764
G. V. Padmakar Rao https://orcid.org/0009-0006-8606-900x

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