Method Development and Method Validation of Volatile Leachables Amount for Oncology Drug Product Injection by Headspace Gas Chromatography Mass Spectrometry Technique

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Abstract

An HS-GC-MS method for the analysis of volatile leachable amounts was develop and validated. The analytical method used to determine the volatile leachable amount of toluene in packaging materials for melphalan hydrochloride for injection and sterile diluent for melphalan for injection by HS-GC-MS method. The validation was performed on Agilent DB-624 30m×0.25mm×1.4µm chromatographic column and the flow mode is constant flow with inlet temperature 240℃ and set flow rate is 1.5 mL/min and the syringe temperature was set at 90℃. Incubation temperature is 80℃, incubation time is 20 minutes and split ratio is 50:1 and injection volume is 1000µL and carrier gas was helium.

The validation results of volatile leachables amount of toluene were linear over the concentration from 0.0101 µg/mL to 1.5156µg/mL (r = 0. 99) and the recoveries and precision ranged from 86% to 108% (RSD < 10%, n =6). This method has been developed and validated successfully and all the results met the acceptance criteria. Hence this method is suitable for testing of volatile leachable amounts of melphalan hydrochloride for injection and sterile diluent for melphalan for injection.

Keywords: Volatile, Leachables, HS-GC-MS, Packaging materials, Injection, Validation, Toluene and AET.

Introduction

Volatile leachables are chemical compounds that can migrate from a material such as a container or a component used in the manufacturing or packaging of a drug product into the product itself[11]. These chemical compounds can pose a risk to the quality, safety, or efficacy of the drug product[8]. Volatile leachables can come from a variety of sources including packaging materials, manufacturing equipment, or other components used in the drug manufacturing process[3]. They can include chemical compounds such as volatile compounds that are used in the manufacturing or packaging of the drug product. Volatile leachables are of particular concern in the pharmaceutical industry because they can affect the quality, safety, or efficacy of the drug product[4]. They can also pose a risk to patient health and safety, as well as to the reputation of the pharmaceutical company that produces the product[12].

As a result, volatile leachables are subject to rigorous testing and analysis during the development and manufacturing of drug products. This involves the use of specialized analytical techniques such as Headspace Gas Chromatography-Mass Spectrometry(GC-MS) hyphenated chromatography to identify and quantify the presence of volatile leachables in the drug product[13]. The goal of testing is to ensure that the drug product is safe and effective for use by patients.

The most critical volatile leachable is toluene, which is classified as a volatile organic compound[8]. In injectable pharmaceutical products, volatile leachable refers to unwanted chemical substances that can migrate from packaging materials or delivery systems into the drug product. Understanding the presence and impact of volatile leachable like toluene is crucial for ensuring the safety and efficacy of injectable formulations[9]. Toluene can leach into injectable products from various sources including plastic packaging components, rubber stoppers and other materials used in the manufacturing and storage of pharmaceuticals. Regulatory agencies like the FDA and EMA have guidelines in place for assessing the safety of volatile leachables in drug products[12]. The presence of toluene as a volatile leachable must be evaluated to determine its potential impact on patient safety. Toluene has been associated with certain toxicological concerns.

It has been identified as a potential carcinogen and may have other harmful effects depending on the level of exposure. Common method for detecting and quantifying toluene includes Headspace Gas Chromatography-Mass Spectrometry(GC-MS) hyphenated chromatography[10]. These techniques enable the identification of volatile leachables at very low concentrations. Conducting a risk assessment involves evaluating the concentration of toluene in the final product and comparing it to established safety thresholds. This assessment helps to determine whether the levels of leachables are acceptable for patient safety[10].

To minimize the risk of leachables, manufacturers can implement strategies such as using alternative materials that are less likely to leach harmful substances, conducting thorough compatibility testing and employing robust quality control measures[15]. Based on the literature survey and review for the volatile leachables for the melphalan

hydrochloride for injection, it was found that there was no sufficient information of analytical methods for the volatile leachables amount in the drug product. Most of the packing materials and the detection of volatile leachables related to orally inhaled nasal products have recommendations[6] to develop a new method for volatile leachables for melphalan hydrochloride for injection and sterile diluent for melphalan HCl for injection, by HS-GC-MS[15].

There is a need for the high sensitivity method and its method validation with a HS-GC-MS technique is essential requirement for oncology injectable products. So the developed HS-GC-MS method can very well detect and separate all the volatile leachables present in the rubber stoppers of the melphalan hydrochloride for injection and sterile diluent for melphalan hydrochloride for injection. Based on the complete understanding of the literature information, we have developed and validated a very simple Headspace Gas Chromatography-Mass Spectrometry(GC-MS) hyphenated chromatography method.

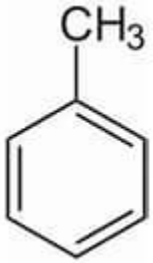
Melphalan hydrochloride for Injection is a chemotherapy drug which is used to treat certain types of cancers including multiple myeloma and ovarian cancer[9]. It is a potent alkylating agent that works by interfering with the DNA synthesis of cancer cells, leading to cell death. The drug is usually administered intravenously. Melphalan Hydrochloride for injection is typically supplied as a sterile powder in a vial containing 50mg of melphalan hydrochloride. The powder must be reconstituted with a sterile diluent before it can be administered. The sterile diluent is supplied in a separate vial, usually containing 10mL of the diluent[9].

According to PQRI and guidance of E and L study for chemical products and elastomeric container closure systems, analytical evaluation threshold (AET) is a threshold: compound with amount above this threshold need to be identified, quantified and reported by chemists to toxicologists for safety assessment when necessary[3,8,11]. AET is derived from safety concern threshold (SCT) or threshold of toxicological concern (TTC) referring to the dosing regimen and treatment duration. Compounds with amount below SCT or TTC are considered that have an amount so low and will not pose any safety concern to human regardless of carcinogenesis.

According to the dosing regimen of product, the maximum daily dosage of melphalan is 28.8mg (1.6mg/m2x1.8m2=28.8mg) and the concentration of constituted solution is 5mg/ml.

Besides, as group 1 of carcinogens classified by 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. Referring to the ICH M7 guideline[5], the maximum daily intake is calculated as 1.5 μg, the uncertainty factor of test method is calculated as 50%, hence the AET was derived as follows:

$$\text{AET} = 1.5\mu g \times 5mg/mL \div 28.8mg \div \text{Dilution factor}(5) \times \text{Uncertainty factor}\ (50\%) = 0.026\mu g/mL$$



Structure of Toluene

Molecular Weight	: 92.14
Molecular Formula	: $C_6H_5CH_3$
IUPAC Name	: Toluene

Material and Methods

Chemical and reagents: Toluene standard was purchased from Beijing BeiFang WeiYe Institute of Measurement Technology, China. (purity ≥ 99.99%); Sodium citrate dihydrate, ingredient grade was purchased from Avantor, US. Dehydrated alcohol was purchased from Merck, US, 1,2-Propanediol was purchased from Merck, China; Melphalan Hydrochloride for Injection, 50mg/vial and Sterile Diluent for Melphalan HCl for Injection, 10mL/vial in this study were provided by Kindos Pharma Ltd.

HS-GC-MS Apparatus and Conditions: The flow mode is constant flow with inlet temperature 240°C and set flow rate is 1.5 mL/min and the syringe temperature was set 90°C. Incubation temperature is 80°C, Incubation time is 20 minutes and split ratio is 50:1. Injection volume is 1000μL and carrier gas was helium.

Standard and Samples Preparations: Transfer 70 mL of purified water in to a beaker, add 50 mL of dehydrated alcohol, mix well, then weigh and transfer about 22.8 g of sodium citrate dihydrate in to the beaker, stir to dissolve, then add 600 mL of 1,2-Propanediol, mix well, then transfer above solution 1000-mL volumetric flask, dilute and make up to volume with purified water, mix well. Transfer 20 mL of above solution 100-mL volumetric flask, dilute and make up to volume with purified water, mix well.

Standard stock solution A: Weigh about 50 mg of toluene standard into a 50-mL volumetric flask containing about 20 mL dehydrated alcohol, dilute and make up to volume with dehydrated alcohol, mix well (About 1.0mg/mL).

Standard stock solution B: Transfer 2.5 mL of the standard stock solution A in to a 50-mL volumetric flask, dilute and make up to volume with dehydrated alcohol, mix well (About 50μg/mL).

HS-GC-MS Conditions

MS Ion Source Temperature	300℃	MS Scan Mode	Full Scan	Scan Time	0.2 second
Segment Start Time	0 minute	Start and End Mass	33~450amu	MS Transfer-line Temperature	240℃
Analysis time	35 minutes	Heating Program	40℃ hold 6min, 15℃/min heat to 150℃, hold 3min, 20℃/min heat to 240℃, hold 10min.		

Standard stock solution C: Transfer 1.0 mL of the standard stock solution B into a 100-mL volumetric flask, dilute and make up to volume with dehydrated alcohol, mix well (About 0.5µg/mL).

Blank solution: Transfer 2.0 mL of diluent into a 10-mL headspace vial and seal.

Standard solution: Transfer 2.0 mL of diluent into a 10-mL headspace vial, add 40 µL standard stock solution B, seal and mix well (About 1.0µg/mL).

System suitability solution: Transfer 2.0 mL of diluent into a 10-mL headspace vial, add 40 µL standard stock solution C, seal and mix well (About 0.01µg/mL).

Sample solution: Take 1 vial of melphalan hydrochloride for Injection, 50mg/Vial, use 1 vial of sterile diluent for melphalan hydrochloride for injection, 10mL/Vial to reconstitute, mix well. Transfer 2.0 mL of reconstituted solution into a 10-mL volumetric flask, dilute and make up to volume with purified water, mix well. Transfer 2.0 mL of above solution into a 10-mL headspace vial and seal.

Method Validation: Validation of the method was done according to the International Conference on Harmonization guideline [5]. The method was validated for system suitability, specificity, accuracy and precision, linearity and range and limit of quantification (LOQ) .

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 %RSD area of toluene peak from the six replicate injections of standard solution was determined. System suitability was evaluated by using blank solution, standard solution, system suitability solution and record the total ion chromatogram (TIC). The system suitability requirement was met for all experiments. The test results were summarized in table 1.

The %RSD of area of toluene peak from the six replicate injections of standard solution was NMT 15%. The signal-to-noise ratio (S/N) for toluene peak in system suitability solution was NLT 10.

Specificity: The method should be specific for the analyte of interest and should not respond to other substances in the sample matrix. To evaluate the specificity, blank solution, standard solution, sample spike solution and sample solution into the HS-GC-MS system, record the total ion chromatogram (TIC). There was no interference observed from blank at the retention time of toluene and other compound peaks from sample solution. The retention time of toluene peaks in the sample spike solution corresponds to that in the standard solution. The specificity requirement was met. The test results are summarized in table 2.

Injection: Inject blank solution, standard solution, sample spike solution and sample solution into the HS-GC-MS system, record the total ion chromatogram (TIC). Blank solution has not given any interfering peak at the retention time of toluene and other compound peak from sample solution. The recovery of toluene in sample spike solution was between 70%-130%.

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 quantification was determined by injecting the LOQ solution in six times. Calculate signal to noise ratio. LOQ results are listed in table 3.

LOQ stock solution: Weigh about 50 mg of toluene standard into a 50-mL volumetric flask containing about 20 mL dehydrated alcohol, dilute and make up to volume with dehydrated alcohol, mix well. Transfer 2.5 mL of above solution into a 50-mL volumetric flask, dilute and make up to volume with dehydrated alcohol, mix well. Transfer 1.0 mL of above solution into a 100-mL volumetric flask, dilute and make up to volume with dehydrated alcohol, mix well (About 0.5µg/mL).

LOQ Solution: Transfer 2.0 mL of diluent into a 10-mL headspace vial, add 40 µL of LOQ stock solution, seal and mix well (About 0.01µg/mL).

Injection: Inject LOQ standard solution into the chromatograph for six times, record the chromatograms, determine the signal to noise ratio and calculate the %RSD of area of toluene. The %RSD of toluene peak area in 6 LOQ injections was NMT 20%. The signal-to-noise ratio (S/N) of toluene peak in 6 LOQ injections was NLT 10.

Linearity and Range: 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. The linearity and range were evaluated by determining at 6 concentration levels in triplicate from LOQ to 150% level with respect to standard solution. The results are listed in table 4.

Table 1
System suitability Results

Inj. No.	Retention Time(min)	Toluene Peak Area (Counts*min)
1	9.309	3588912.7
2	9.309	3389370.3
3	9.316	3593245.1
4	9.312	3529084.7
5	9.316	3506968.0
6	9.316	3045482.1
Average	9.313	3442177.2
STDEV	0.003	207970.74
%RSD	0.0	6.0
S/N	N/A	55

Table 2
Specificity test results

Name	Spike concentration (µg/mL)	Measured concentration (µg/mL)	Recovery of Toluene
Sample solution	N/A	Not detected	N/A
Sample spike solution	0.9970	1.0844	109%

Table 3
LOQ test results

Name	LOQ-1	LOQ-2	LOQ-3	LOQ-4	LOQ-5	LOQ-6	%RSD
Area（Counts*min）	36922.1	38566.2	37313.7	37162.7	36533.1	35806.6	2%
Amount(µg/mL)	0.0102	0.0106	0.0103	0.0103	0.0101	0.0099	NA
S/N	41	43	42	34	50	52	NA
Toluene Concentration	0.0101 µg/mL						

Linearity stock solution A: Weigh accurately about 50 mg of toluene to a 50-mL volumetric flask, dissolve and dilute to volume with dehydrated alcohol, mix well (About 1mg/mL).

Linearity stock solution B: Transfer accurately 1.5 mL of linearity stock solution A to a 20-mL volumetric flask, dissolve and dilute to volume with dehydrated alcohol, mix well (About 75µg/mL).

Linearity stock solution C: Transfer accurately 2.5 mL of linearity stock solution A to a 50-mL volumetric flask, dissolve and dilute to volume with dehydrated alcohol, mix well (About 50µg/mL).

Linearity stock solution D: Transfer accurately 2.0 mL of linearity stock solution A to a 50-mL volumetric flask, dissolve and dilute to volume with dehydrated alcohol, mix well (About 40µg/mL).

Linearity stock solution E: Transfer accurately 2.5 mL of linearity stock solution A to a 100-mL volumetric flask, dissolve and dilute to volume with dehydrated alcohol, mix well (About 25µg/mL).

Linearity stock solution F: Transfer accurately 2.0 mL of linearity stock solution C to a 20-mL volumetric flask, dissolve and dilute to volume with dehydrated alcohol, mix well (About 5µg/mL).

Linearity solution 1: Transfer 2.0 mL of diluent to a 10-mL headspace vial, add 40 µL of linearity stock solution B, seal and mix well (About 1.5µg/mL) (In triplicate).

Linearity solution 2: Transfer 2.0 mL of diluent to a 10-mL headspace vial, add 40 µL of linearity stock solution C, seal and mix well (About 1.0µg/mL) (In triplicate).

Linearity solution 3: Transfer 2.0 mL of diluent to a 10-mL headspace vial, add 40 µL of linearity stock solution D, seal and mix well (About 0.8µg/mL) (In triplicate).
Linearity solution 4: Transfer 2.0 mL of diluent to a 10-mL headspace vial, add 40 µL of linearity stock solution E, seal and mix well (About 0.5µg/mL) (In triplicate).

Linearity solution 5: Transfer 2.0 mL of diluent to a 10-mL headspace vial, add 40 µL of linearity stock solution F, seal and mix well (About 0.1µg/mL) (In triplicate).

Linearity solution 6: Transfer 2.0 mL of diluent into a 10-mL headspace vial, add 40 µL of LOQ stock solution, seal and mix well (About 0.01µg/mL) (in six replicates).

Dilute the LOQ stock solution by a suitable factor to make the signal-to-noise ratio (S/N) of toluene peak to comply

with acceptance criteria. Inject LOQ solution for 6 times into the HS-GC-MS system respectively and record the total ion chromatogram (TIC).

Evaluation for Linearity: Linearity shall be evaluated by visual inspection of a plot of response as a function of analyte concentration (Toluene). The correlation coefficient, % y-intercept, slope of the regression line and residual sum of squares shall be submitted. A plot of the data shall be submitted.

Calculate the % y-intercept and residual sum of square (Q) as follows:

$$\%\, y_{intercept} = \frac{y_{intercept} \times 100}{\text{slope} \times \text{concentration at 100\% level} + y_{intercept}}$$

$$Q = \sum (y_{estimated\ value} - y_{actual\ value})^2$$

where $y_{intercept}$ is Y-axis intercept and Q is residual sum of deviation (The square sum of the difference between the estimated y value and the actual y value).

The correlation coefficient (R) should be NLT 0.99, %y intercept was NMT ±25% of standard response. Report slope of the regression line and residual sum of square.

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 LOQ to 150% level with respect to standard solution.

Accuracy: The method should be able to 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.

Table 4(a)
Linearity and Range Test Results

Name	Level	Concentration (µg/mL)	Peak Area (Counts*min)
Linearity solution 1	150%	1.5156	4857298.9
			4847619.5
			4953112.2
Linearity solution 2	100%	1.0104	2479001.8
			3276404.0
			3119568.5
Linearity solution 3	80%	0.8083	2583599.1
			2673474.3
			1807103.7
Linearity solution 4	50%	0.5052	1668185.3
			1696394.2
			1673187.6
Linearity solution 5	10%	0.1010	335906.8
			316623.0
			314195.0
Linearity solution 6	LOQ	0.0101	36922.1
			38566.2
			37313.7
			37162.7
			36533.1
			35806.6
Linearity equation: y=3123449.766x-13769.722			
The correlation coefficient (R)	0.99	% y-intercept	0%
Slope	3123449.766	Q	1.11867×10^{12}

Table 4(b)
Range Test Results

Name of compound	LOQ~150%
Toluene	00.0101µg/mL~1.5156µg/mL

The test method was linear within the range of LOQ~1.5µg/mL.

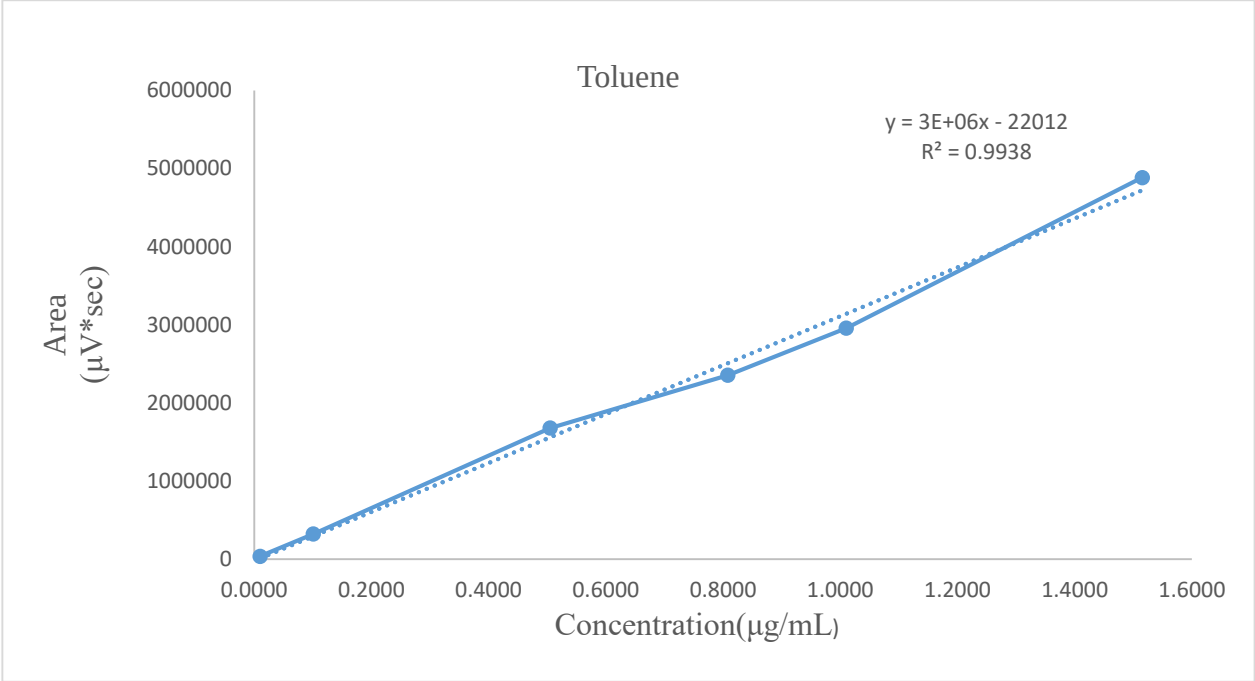


Figure 1: Linearity Graph Diagram of Toluene

Precision: The method should be reproducible and precise. Precision can be evaluated by analyzing multiple samples and determining the variation in the results obtained. Accuracy and precision were evaluated by determining the content of toluene from the six determinations of sample spike solution and the % RSD. Mean content, standard deviation, %RSD and confidence interval of toluene were reported. All test results met acceptance criteria and are summarized in table 5 and table 6.

Spike solution: Weigh about 50 mg of toluene standard into a 50-mL volumetric flask containing about 20 mL dehydrated alcohol, dilute and make up the volume with dehydrated alcohol, mix well. Transfer 2.5 mL of above solution into a 50-mL volumetric flask, dilute and make up to volume with dehydrated alcohol, mix well (About 50µg/mL).

Precision solution: Take 1 vial of melphalan hydrochloride for injection, 50mg/Vial, use 1 vial of sterile diluent for melphalan hydrochloride for injection, 10mL/vial to reconstitute, mix well. Transfer 2.0 mL of reconstituted solution into a 10-mL volumetric flask, dilute and make up to volume with purified water, mix well. Transfer 2.0 mL of above solution into a 10-mL headspace vial, add 40 µL spike solution, seal and mix well.

Inject sample solution, precision solution (in six replicate) into the HS-GC-MS system respectively and record the total ion chromatogram (TIC).

Calculate the %recovery as follows:

$$C'_{spike} = \frac{A_{spike} \times C_{std}}{A_{std}}$$

$$C_{\text{sample}} = \frac{A_{sample} \times C_{std}}{A_{std}}$$

$$C_{\text{spike}} = \frac{W_{spike}}{D_{Spike}} \times Purity$$

$$Recovery\,\% = \frac{C'_{spike} - C_{sample}}{C_{spike}} \times 100\%$$

where C'_{spike} is measured concentration of toluene in sample spike solution, µg/mL, C_{spike} is spiked concentration of toluene in sample spike solution, µg/mL, C_{sample} is measured concentration of toluene in sample solution, µg/mL, A_{spike} is area of toluene in the sample spike solution, Astd is average area of toluene in the standard solution (n=6), A_{sample} is area of toluene in sample solution, Cstd is concentration of toluene in the standard solution, µg/mL, Wspike is weight of toluene in the sample spike solution, µg, Dspike is dilution times of standard in the sample spike solution, $Purity$ is purity of standard.

Calculate the confidence interval as follows (the confidence is 0.95):

$$\mu = \bar{X} \pm z_{\alpha/2} \frac{\sigma}{\sqrt{n}}$$

where $\bar{X}$ is average of % assay (or recovery), $z_{\alpha/2}$ is bilateral standard normal distribution quantiles, σ is standard deviation of each component and n is the number of determinations.

The recovery of toluene should be 70%-130%, the %RSD (n=6) of recovery of six analyzed results for Toluene should be NMT 20%. Report mean, standard deviation, related

standard deviation and 95% confidence interval of the six recovery results. Hence the method is Accurate and Precise.

Results and Discussion

According to the dosing regimen of 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 constituted solution is 5mg/mL. Besides, as group 1 of carcinogens classified by 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. Referring to the ICH M7 guideline, the maximum daily intake is calculated as 1.5 μg and the uncertainty factor of test method is calculated as 50%, hence the AET was derived as follows:

$$\begin{aligned}\text{AET} = {} & 1.5\mu g \times 5mg/mL \div 28.8mg \div \text{Dilution factor}(5) \\ & \times \text{Uncertainty factor}\ (50\%) \\ & = 0.026\mu g/mL\end{aligned}$$

The validation results indicate that the HS-GC-MS method developed for the determination of volatile leachables amount from stopper in melphalan hydrochloride for injection and sterile diluent for melphalan HCl for injection is suitable for its intended use. System suitability was demonstrated prior to 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 evaluated and the results show that the method is accurate and precise for the determination of toluene content.

The limit of quantitation and limit of detection were determined and the method 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 150% level with respect to the standard solution. 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 AET. This indicates that the stability of the drug product and diluent is acceptable for long-term storage.

Table 5
Accuracy and Precision test results

Name	Spike concentration (μg/mL)	Measured concentration (μg/mL)	Peak Area (Counts*min)	%Recovery
Sample solution	NA	Not detected	Not detected	NA
1	0.9970	1.0388	3545215.4	104%
2		1.0751	3669174.5	108%
3		1.0497	3582286.4	105%
4		1.0388	3545382.5	104%
5		1.0467	3572190.6	105%
6		0.8566	2923389.4	86%
Recovery Mean（n=6）				102%
Recovery Standard Deviation（n=6）				8.02%
Recovery %RSD（n=6）				8%
Recovery 95% Confidence Interval （n=6）				93.65% - 110.49%

Table 6
Precision test results

Name	Measured concentration (μg/mL)		Peak Area (Counts*min)	%Recovery
Sample solution	Not detected		Not detected	NA
1	1.0093		3444637.4	100.9
2	1.0404		3550779.2	104.0
3	0.9523		3249996.1	95.2
4	1.0431		3559939	104.3
5	0.9040		3085183.5	90.4
6	0.5776		1971389.9	57.8
Recovery Mean（n=6）			NA	92%
Recovery Standard Deviation（n=6）			NA	17.6%
Recovery %RSD（n=6）			NA	19.2%

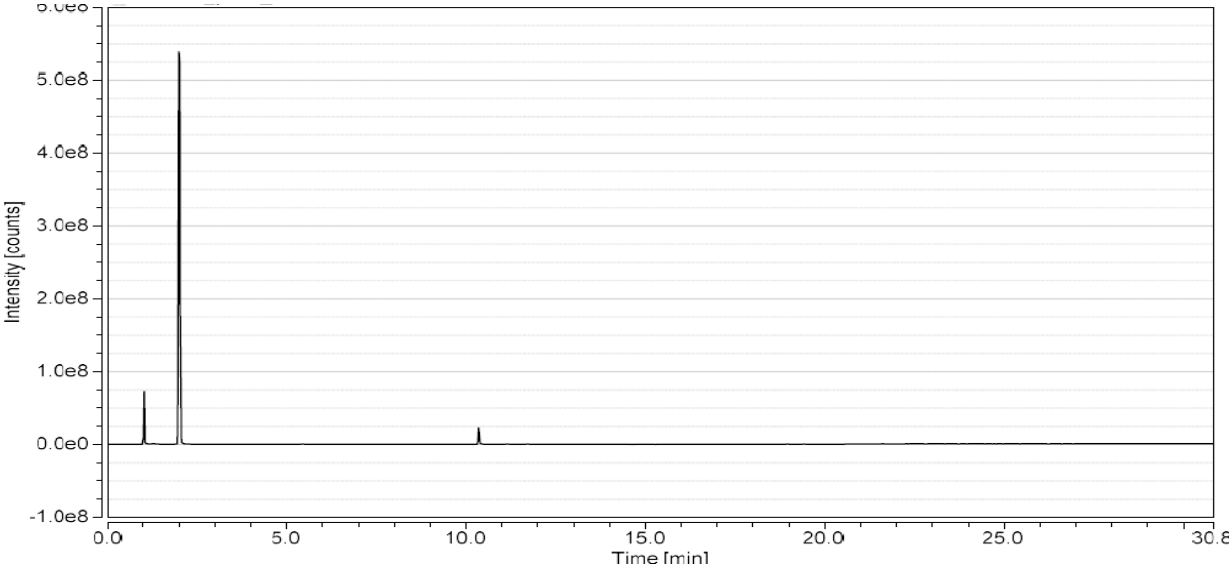


Figure 2: Blank Solution Chromatogram

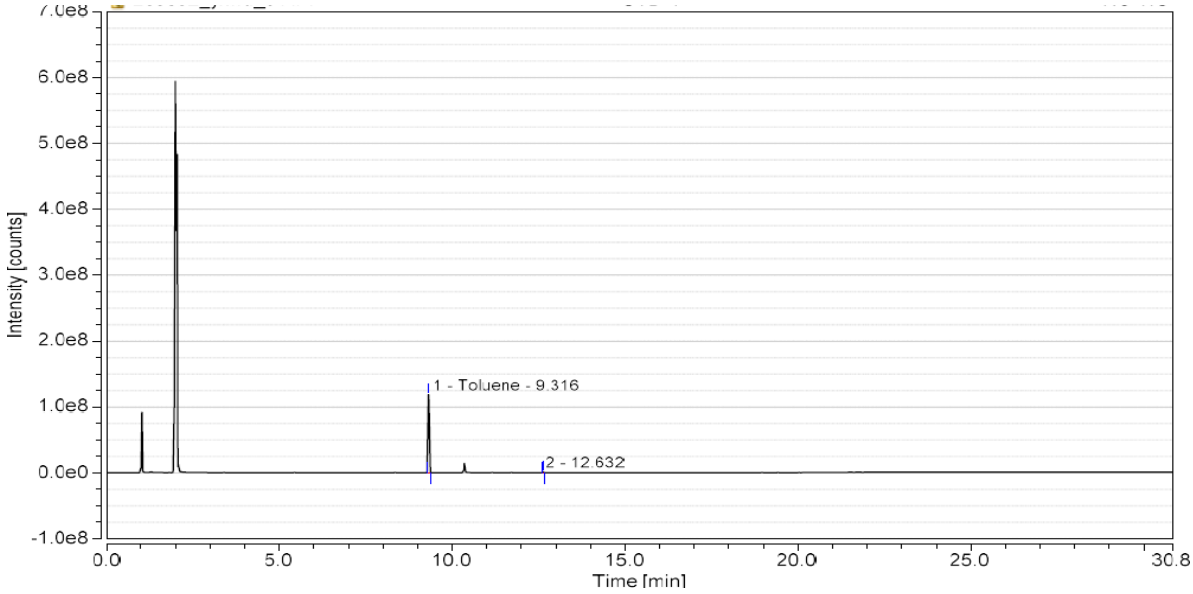


Figure 3: Standard Solution Chromatogram

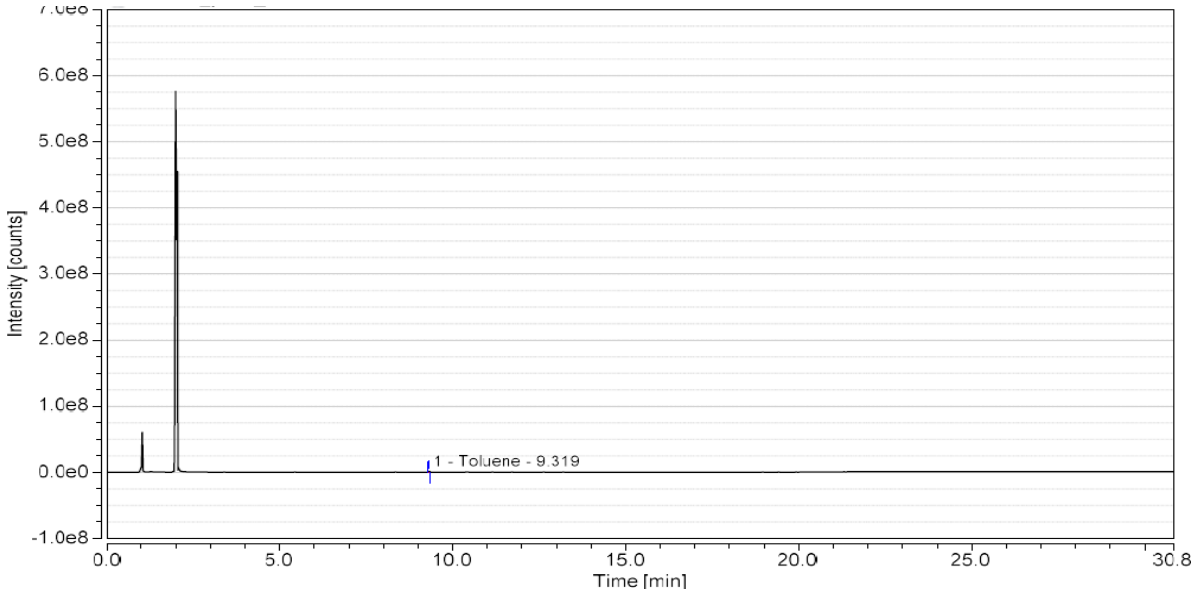


Figure 4: System Suitability Solution Chromatogram

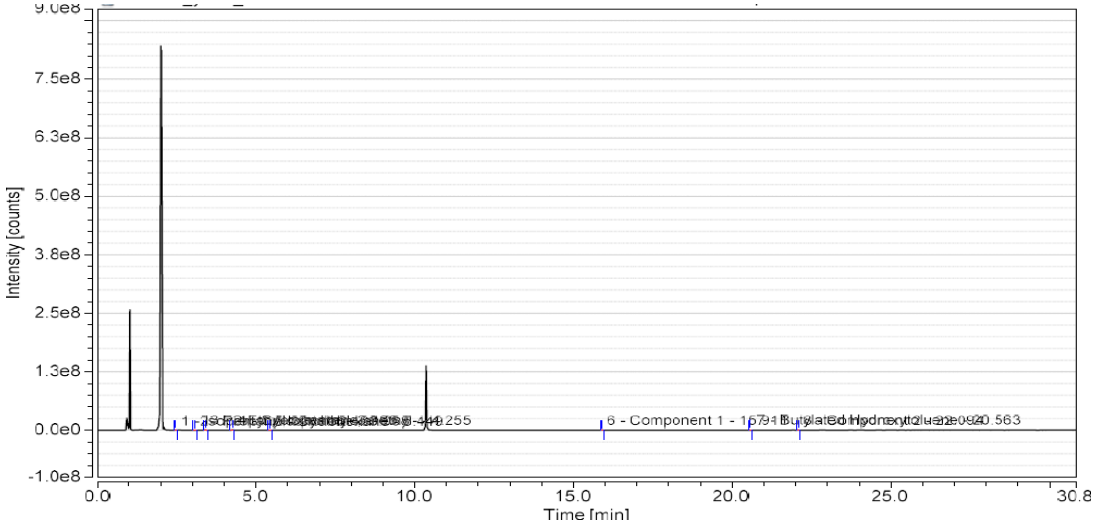


Figure 5: Melphalan Hydrochloride for Injection and Sterile Diluent for Melphalan HCl for Injection, Sample Solution Chromatogram

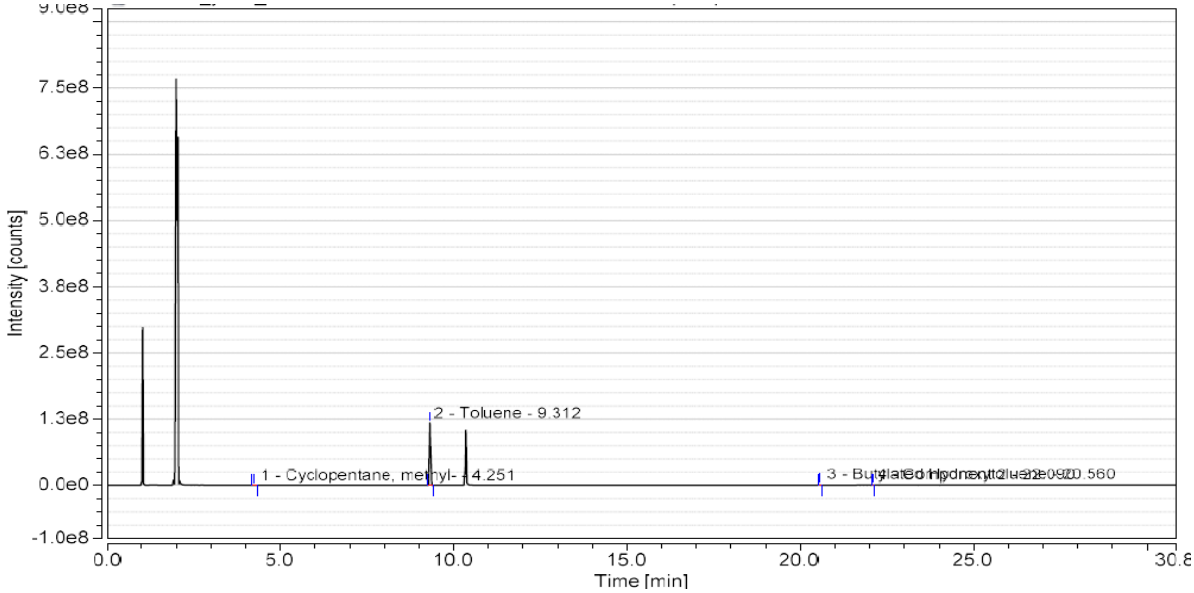


Figure 6: Sample Spike Solution of Melphalan Hydrochloride for Injection and Sterile Diluent for Melphalan HCl for Injection Chromatogram

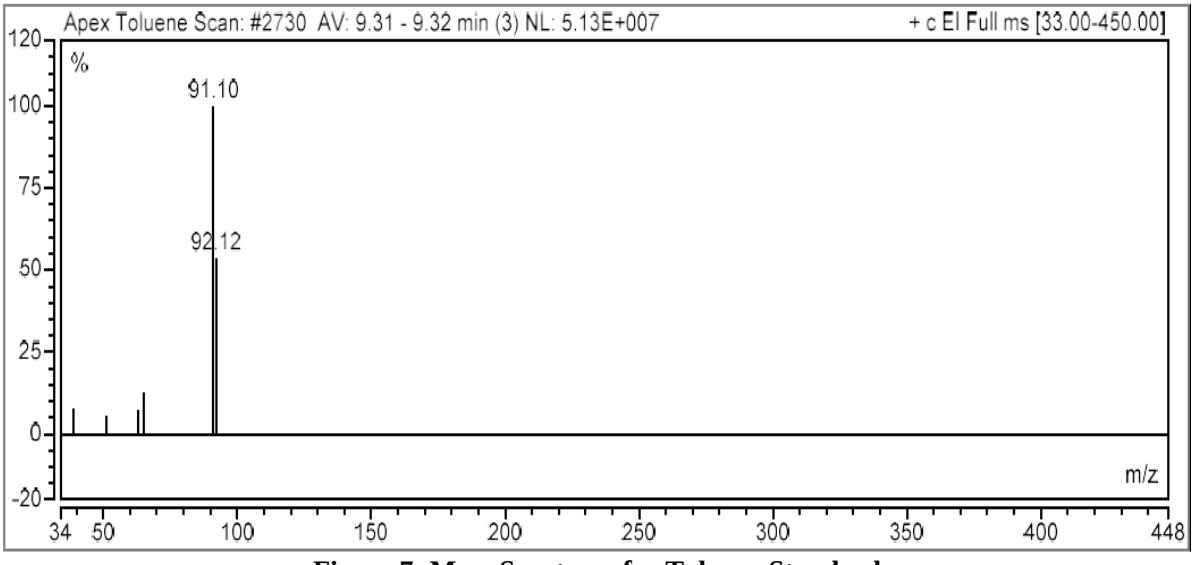


Figure 7: Mass Spectrum for Toluene Standard

Conclusion

The HS-GC-MS method developed for the determination of volatile leachables amount from stopper in melphalan hydrochloride for Injection and sterile diluent for melphalan HCl for injection is suitable for routine testing. The method demonstrated system suitability, specificity, LOQ, accuracy, precision, linearity and range. The samples of the drug product and diluent were also acceptable. The method has been validated successfully and all results met the respective acceptance criteria. Hence, this method is suitable for testing volatile leachables amount from stopper. No compound was detected with an amount exceeding the AET.

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