RESEARCH ARTICLE

Migration of Leachables Amount of Antioxidants and Vulcanizers: Analytical Method Development and Validation for Antineoplastic Drug Injection by HPLC

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ABSTRACT

An HPLC method for the analysis of leachables amounts of Antioxidants and vulcanizers was developed and validated. The method was developed and validated for quantitation on six targeted Antioxidants and vulcanizers in rubber stoppers of antineoplastic Drug Injection. *Methods:* The analytical method used to determine the leachableamount of antioxidants (BHT,Antioxidant 1010, Antioxidant 1330, Antioxidant 1076, Antioxidant 168) and vulcanizer(Sulfur) in packaging materials for Melphalan Hydrochloride as Injection 50mg/vial and sterile diluent to Melphalan as Injection 10.00mg/vial by HPLC method. The validation was performed on YMC-Pack ODS-AQ (4.6mm×100 mm, 3µm) chromatographic column at 30 °C, and wavelength is 225nm. Mobile phase A is methanol (100%) and Mobile Phase B is purified water at a rate of flow as 1.50mL/min, and the volume injected as 20.00µL. Gradient was set as 0min 25% and 75%, 2min 25% and 75%,4min 100% and 0%,7min 100% and 0%,9min 25% and 75%,12min 25% and 75%. *Results:* The validation results of Leachables amount of antioxidants(BHT, Antioxidant 1010, Antioxidant 1330, Antioxidant 1076, Antioxidant 168) and vulcanizer (Sulfur) were linear over the concentration from 0.99 µg/mL to 8.078µg/mL (r = 0. 99) and the recoveries and precision ranged from 96.8% to 100.8% (RSD <10%, n =6). *Conclusion*:This method has been developed and validated successfully and all the results met the acceptance criteria. Hence this method is suitable for testing of Migration of Antioxidants and vulcanizer Melphalan Hydrochloride as Injection 50.00mg/vial along with sterile diluent for Melphalan as Injection 10mg/vial.

Keywords: Leachables, Migration, HPLC, Antioxidants, Vulcanizer, BHT,Sulfur, Packaging materials,Injection, and AET

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INTRODUCTION

Leachables are generally represented as chemical compounds which can emigrate from an material, such as a container or a component utilized in manufacturing or packaging of product drug, into the product itself. These chemical compounds can pose a risk for quality, impregnability, or competence of drug. Leachables can come from various sources, including packaging materials, manufacturing equipment, or other components used in the drug manufacturing process. They can include chemical compounds such as plasticizers, antioxidants, stabilizers, lubricants, or other compounds used in fabrication or packaging of drug. Leachables were of particular concern in the pharmaceutical industry because they can affects quality of the drug, either safety, nor impregnability. They can also risk to patient safety as well as health also the reputation of the pharmaceutical company that produces the product. The drug product is stored on stability under a variety of controlled environmental circumstances also analyzed for leachables at multiple time points over anticipated shelf-life of the drug.[1] As a result, 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 HPLC chromatography for identification and quantification. Antioxidants and vulcanizers are two types of chemical compounds that can be found in leachables. In the context of leachables, antioxidants may be present in materials such as polymers, coatings, or other components that are utilized in manufacturing or packaging product of drug. If these antioxidants migrate from the material into the drug product, they could potentially affect its stability or efficacy. Vulcanizers are chemical compounds that are used to improve the strength and durability of rubber materials. Both antioxidants and vulcanizers are considered potential leachables that may be present in materials used in the pharmaceutical industry. As a result, they are subject to rigorous testing and analysis during the development and manufacturing of drug products. Raw materials with complex chemical compositions as well as assortment of potential leachables.[2] Migration of leachables refers to the process by which compounds that are present in a drug product container. This consideration is crucial to packaging entrails those may be indirect contact as dosage form, but it is also applicable to anycomponent from which substances may migrate into the dosage form.[3] It is

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Time(min)	Mobile Phase A% as MeOH	Mobile Phase B% as H_2O
0	25	75
2.0	25	75
4.0	100	0
7.0	100	0
9.0	25	75
12.0	25	75

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

Area RSD of Standard Solution (%)						
No./Name.	BHT	S	1010	1330	1076	168
n=6	0.5	0.5	0.5	0.4	0.7	0.5
n=7	1.1	1.1	1.1	1.0	1.3	1.0
n=8	1.6	1.6	1.4	1.4	1.7	1.5
n=9	1.9	1.9	1.6	1.9	1.7	2.0
n=10	2.3	2.3	1.9	2.1	1.7	2.4
n=11	4.1	4.1	3.7	3.9	3.8	3.9
n=12	4.0	4.0	3.7	3.9	3.7	4.0
n=13	3.8	3.9	3.5	3.8	3.6	3.8
n=14	3.7	3.7	3.4	3.7	3.5	3.7
n=15	3.6	3.6	3.3	3.6	3.4	3.6
n=16	3.5	3.5	3.2	3.5	3.2	3.5

important to conduct appropriate analytical tests to quantify the level of leachables are present in product of drug also for ensuring that levels were within acceptable limits.

HPLC process is developed as well as validated for detecting and quantifying specific antioxidants also vulcanizers which are available in Melphalan Hydrochloride for Injection drug product. To determine the migration of leachables, a study was conducted to simulate the storage and use conditions of the drug product.[4] This was include exposing the drug product to elevated temperatures and humidity, and conducting extraction studies using solvents that simulate the drug product environment. The results of the leachables study was used for determining appropriate controls as well as specifications to container, closure, also for delivery system to minimize risk for migration of leachables.[5] These controls can include the use of appropriate materials, surface treatments, and coatings to minimize the association among product of drug as well asw container, closure, or delivery structure. It is crucial to note that specific excigencies for leachables testing and control may vary susceptible over regulatory guidelines also specific drug product being analyzed.[6] Melphalan Hydrochloride for Injection is a chemotherapy drug that is used to treat certain types of cancers, including multiple myeloma and ovarian cancer. 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.[7] 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. And the packing details Melphalan hydrochloride for Injection,15mL vial with stopper (Lyo, Gray, Butyl rubber, with B2 coating on flat top and flurotec film on plug, RS) and Sterile diluent for Injection,10mL vial (USP Type I, Tubing, clear,20mm Finish) and 20mm Stopper.[7]

Table 2: System Suitability Results –Standard solution Peak Resolution

Standard Solution Peak Resolution						
No./Name.	BHT	S	1010	1330	1076	168
First Injection	7.6	2.6	3.5	7.4	3.1	N/A
Last Injection	7.5	2.6	3.4	7.2	3.1	N/A

Table 3: System Suitability Results –Check Standard solution

Check Standard Solution %Recovery(%)						
No./Name.	BHT	S	1010	1330	1076	168
Recovery	98.8	100.2	98.2	98.7	97.0	98.5

Table 4: System Suitability Results –S/N of LOQ solution

The S/N of LOQ solution						
No./Name.	BHT	S	1010	1330	1076	168
S/N	16	21	22	98	17	66

Table 5: Specificity Results

Sample Type	Name	Retention Time (min)	Peak Match	Resolution
Standard Solution	BHT	5.453	1000	7.6
	S	5.960	1000	2.6
	1010	6.147	1000	3.5
	1330	6.413	1000	7.4
	1076	7.150	1000	3.1
	168	7.560	1000	NA
Melphalan Hydrochloride for Injection Sample Spike Solution	BHT	5.457	1000	7.7
	S	5.963	1000	2.6
	1010	6.153	1000	3.5
	1330	6.420	1000	7.3
	1076	7.157	1000	3.1
	168	7.567	1000	NA

MATERIALS AND METHODS

Chemical and Reagents

Antioxidant BHT(2,6-Di-tert-butyl-4-methylphenol), standard was purchased from Aladdin, China. (purity ≥ 99.97%); Antioxidant 1010, standard was purchased from sigma, US. (purity ≥ 98%); Antioxidant 1330 standard was purchased from sigma, US. (purity ≥ 99.97%); Antioxidant 1076 standard was purchased from sigma, US. (purity ≥ 99.8%);Antioxidant 168 standard was purchased from sigma, US (purity ≥ 98%);Sulfur, was purchased from Aladdin, China,(purity ≥ 99.99%);Methanol, was purchased from Fisher,UK; Methylene Chloride, was procured by Fisher,UK,All reagents were analytical grade, it they were not stated otherwise; Water purified by utilizing Millipore Ultra Clear™ system (Millipore Technologies Corp);Melphalan Hydrochloride for Injection, 50mg/vial as well as Sterile Diluent for Melphalan HCl as Injection, 10mL/Vial in this study were obtained gift samples from Kindos pharmaceuticals Co., Ltd.

Table 6: Results of Accuracy and Precision

Name		BHT	S	1010	1330	1076	168
Theoretical Spike concentration（μg/mL）		4.9910	5.1520	5.2023	5.2023	5.1277	5.3856
Sample		-	-	-	-	-	-
Measured concentration （μg/mL）	1	4.8883	5.2181	5.0569	5.0896	5.1760	5.3400
	2	4.8924	5.2405	5.1075	5.0977	5.2566	5.3334
	3	4.7725	5.1345	4.9874	5.0027	5.1214	5.2771
	4	4.8687	5.2073	5.0727	5.0530	5.2011	5.2465
	5	4.7835	5.1285	4.9306	4.9630	5.0541	5.1143
	6	4.7941	5.2031	5.0351	5.0574	5.1881	5.3834
Recovery	1	98%	101%	97%	98%	101%	99%
	2	98%	102%	98%	98%	103%	99%
	3	96%	100%	96%	96%	100%	98%
	4	98%	101%	98%	97%	101%	97%
	5	96%	100%	95%	95%	99%	95%
	6	96%	101%	97%	97%	101%	100%
Mean (n=6)		96.8%	100.7%	96.7%	97.0%	100.8%	98.1%
SD (n=6)		1.1%	0.9%	1.2%	1.0%	1.4%	1.8%
%RSD (n=6)		0%	0%	0%	0%	0%	0%
95% Confidence interval (n=6)		95.7% 98.0%	99.8% 101.7%	95.4% 98.0%	95.9% 98.0%	99.3% 102.2%	96.2% 99.9%

Table 7: Results of LOQ

Component	BHT		S		1010		1330		1076		168	
Strength in μg/mL	0.0998		0.1030		0.1040		0.1040		0.1026		0.1077	
Inj.#	Area	S/N	Area	S/N	Area	S/N	Area	S/N	Area	S/N	Area	S/N
1	0.0328	13	0.0586	59	0.0263	19	0.0764	34	0.0179	14	0.0659	24
2	0.0331	16	0.0555	32	0.0261	28	0.0760	38	0.0162	14	0.0666	23
3	0.0323	15	0.0551	41	0.0273	42	0.0775	33	0.0180	16	0.0682	35
4	0.0332	21	0.0553	41	0.0263	41	0.0745	32	0.0168	14	0.0693	35
5	0.0334	20	0.0556	28	0.0267	28	0.0766	33	0.0166	16	0.0646	20
6	0.0319	15	0.0547	55	0.0259	40	0.0753	65	0.0167	15	0.0687	26
%RSD (n=6)	1.8%	NA	2.5%	NA	1.8%	NA	1.4%	NA	4.3%	NA	2.7%	NA

Apparatus and Circumstances

HPLC is achieved over Thermo Ultimate 3000 Series (Thermo Technologies). The separation was done on a YMC-Pack ODS-AQ 3μm, size 100 × 3.0 mm i.d. (YMC, USA). The elution was performed on Gradient program. The rate of flow is identified at 1.50mL/min at column temperature as 30°C also Sample tray-controlled room temperature. 225nm is the wavelength and volume injected is 20.00μL to each and every samples as well as solution of standard.

Standard and Samples Preparations

For this analysis used methanol as a diluents. Different solutions are prepared for this analysis like Antioxidants standard stock solution, Sulfur standard stock solution, Standard solution, solution for LOD, LOQ solution, Sample solution, Antioxidant Stock solution, Sulfur Stock solution, Sample Spike Solution of Melphalan Hydrochloride for Injection, Linearity Solution 1(150% Level), Linearity Solution 2(100% Level), Linearity Solution 3(40% Level), Linearity Solution 4(20% Level), Linearity Solution 5(L5, LOQ), Stock solution of Melphalan Hydrochloride for Injection, and Precision solution (in six replicate)

Method Validation

Validation of the method was done according to the International Conference on Harmonization guideline. This process is subjected to validation by considering the ICH rules and regulations.[8]

Table 8: Results of LOD

Component	BHT	S	1010	1330	1076	168
Strength in μg/mL	0.02 99	0.03 09	0.031 2	0.031 2	0.030 8	0.032 3
Inj.#	S/N	S/N	S/N	S/N	S/N	S/N
1	8	16	15	29	6	13
2	6	39	8	20	5	13

System Suitability

The method should be highly suitable to its predetermined utilization and is evaluated during routine use to ensure continued performance. System suitability was demonstrated prior to each experiment. The LOQ solution, standard solution, the check standard solution and bracketing standard solution were used to evaluate the system suitability. The S/N of LOQ solution was determined. The %RSD of peak areas of all components from 6 standard injections and bracketing standard solutions were determined. The recovery of peak areas of all components from check standard solution with respect to mean peak areas of each component from 6 consecutive injections of standard solution was determined. The resolution of each peak from first injection of standard

Table 9: Results of Linearity for Butylated Hydroxy Toluene (BHT)

Linearity and Range for Butylated Hydroxy TolueneBHT				
Level	Strength in µg/mL	Area for the Peak		
LOQ	0.0998	0.0330	0.0330	0.0328
20%	0.9982	0.3412	0.3414	0.3412
40 %	1.9964	0.6748	0.6551	0.6600
100%	4.9910	1.6772	1.6791	1.6874
150 %	7.4865	2.4678	2.5226	2.4950
Linearity Equation	Y=0.3338x+0.0033			
Correlation Coefficient R	1.00			
Slope	0.3338			
%y-intercept	0%			
Residual Sum of Square	0.0026			

solution and last injection of bracketing standard solution were determined. The system suitability requirement was met for all experiments. The %RSD of area of Antioxidants and Sulfur peak from the total six replicate injections of standard solution is not greater to that of the value 5.0%.

The %RSD of area of Antioxidants and Sulfur peak for all injections of Standard solution (including Bracketing) should be not more than 5.0%. The resolution between antioxidants and Sulfur peak from the first injection of standard solution and for the last bracketing standard solution is not less than to that of 1.5. The %recoveries of area of Antioxidants and Sulfur peak for the Check standard solution should be between 95.0%～105.0%. The S/N of antioxidants and sulfur in LOQ solution should not be less than 10. The test results were summarized in Table 1to Table 4.

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, diluent, standard solution, sample solution and sample spike solution are passed into system of HPLC, also various chromatograms are taped. There was no any interference observed from

Table 10: Results of Linearity for Sulfur (S)

Linearity and Range for Sulfur (S)				
Level	Strength in µg/mL	Area for the Peak Area		
LOQ	0.1030	0.0563	0.0542	0.0567
20%	1.0304	0.5626	0.5622	0.5610
40 %	2.0608	1.1303	1.1000	1.1077
100%	5.1520	2.8553	2.8544	2.8686
150 %	7.7280	4.2443	4.3380	4.2909
Linearity Equation	Y=0.33x-0.0109			
Correlation Coefficient R	1.00			
Slope	0.33			
%y-intercept	0%			
Residual Sum of Square	0.0069			

Table 11: Results of Linearity for Antioxidant 1010

Linearity and Range for Antioxidant1010				
Level	Strength in µg/mL	Area for the Peak Area		
LOQ	0.1040	0.0275	0.0255	0.0266
20%	1.0405	0.2756	0.2791	0.2760
40 %	2.0809	0.5586	0.5442	0.5483
100%	5.2023	1.4392	1.4418	1.4457
150 %	7.8035	2.0796	2.1330	2.1167
Linearity Equation	Y=0.269x-0.0028			
Correlation Coefficient R	1.00			
Slope	0.269			
%y-intercept	0%			
Residual Sum of Square	0.0051			

diluent at the retention time of each component (Antioxidants and Sulfur). The resolutions between antioxidants peaks and sulfur peak in sample solution, standard solution and sample Spike solution were checked. No antioxidants and sulfur were detected in the sample. The Rt for antioxidants and peaks of sulfur in taken sample spike,

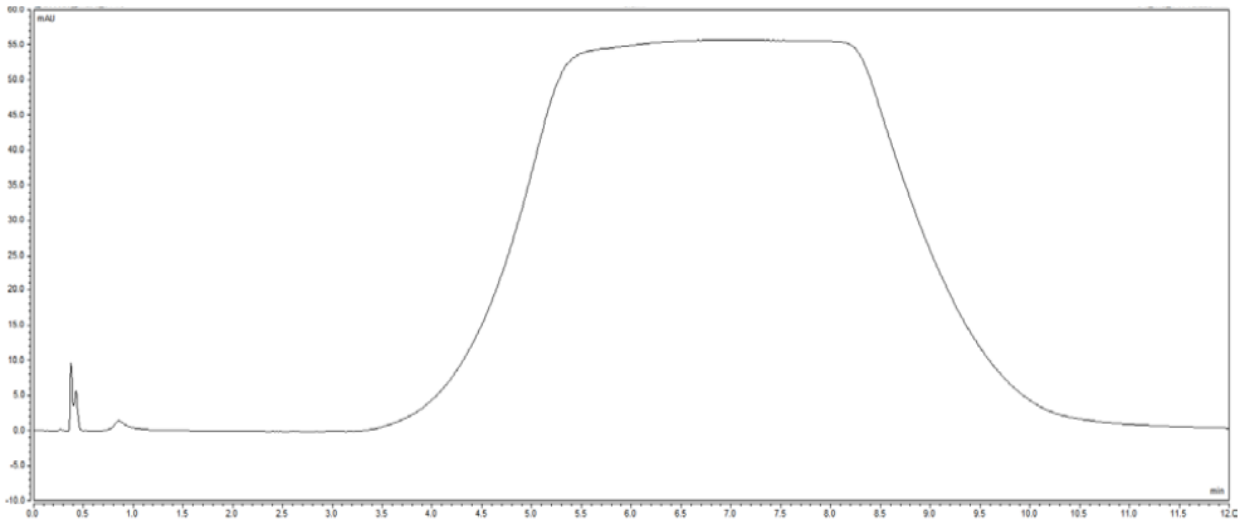

Figure 1: Blank Solution Chromatogram

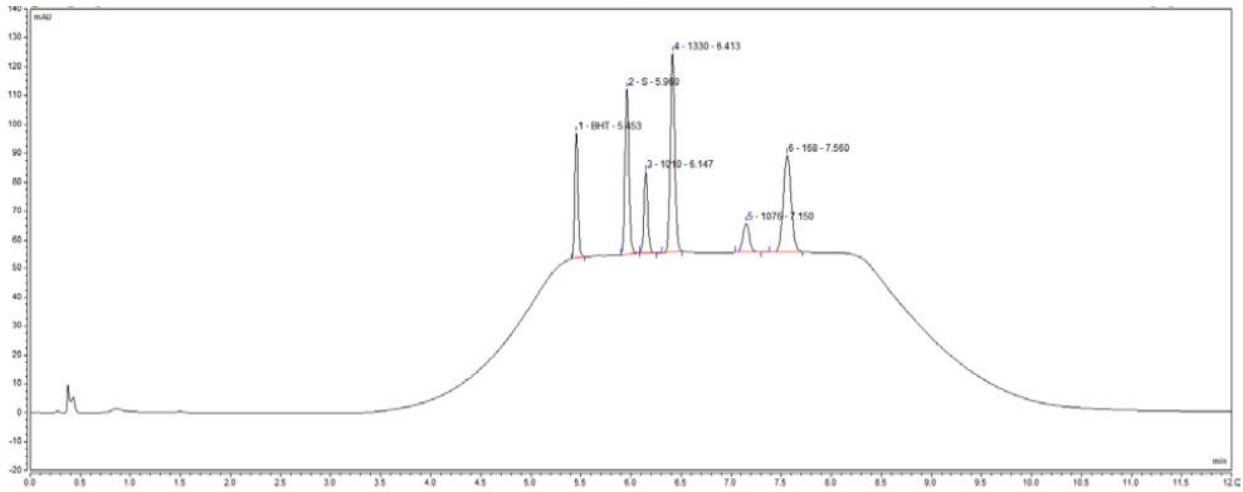

Figure 2: Standard Solution Chromatogram; HPLC Chromatograms of Leachables. Peak Identity: (1) Butylated hydroxytoluene-(BHT) (2) Sulfur-(S) (3) Antioxidant-1010 (4) Antioxidant-1330 (5) Antioxidant-1076 (6) Antioxidant-168

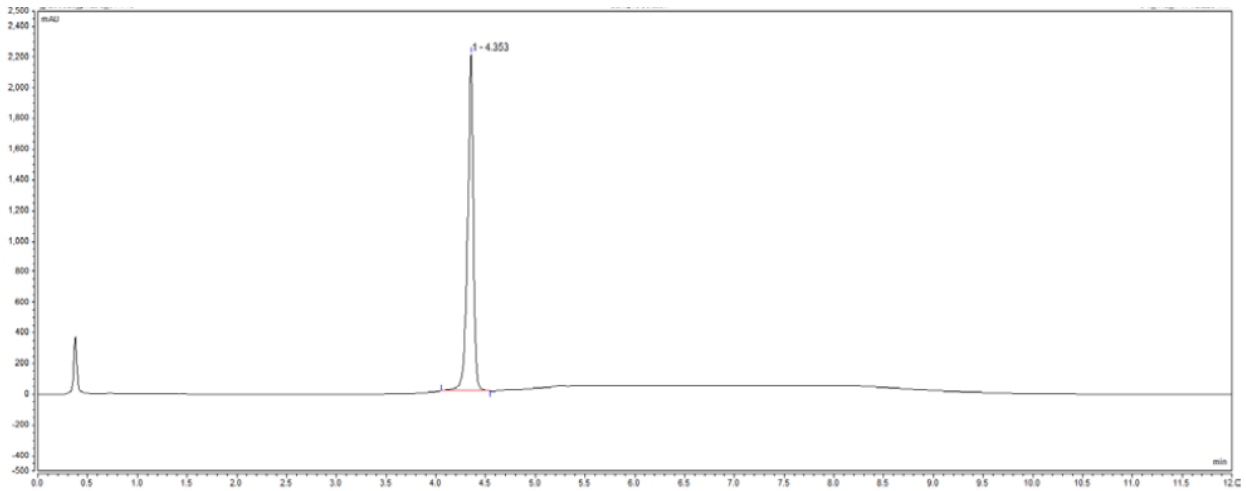

Figure 3: Melphalan Hydrochloride for Injection, 50.00mg/vial also sterile diluent for Melphalan HCl toInjection, 10mL/Vial Sample Solution Chromatogram (10mg/mL); HPLC Chromatograms of Leachables. Peak Identity: (1) Melphalan

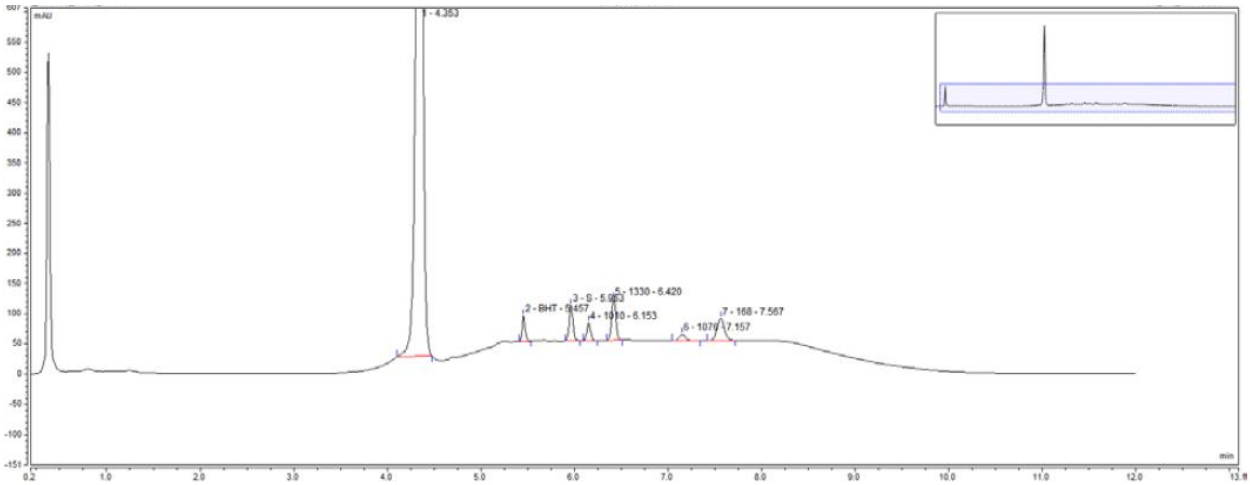

Figure 4: Melphalan Hydrochloride for Injection, 50mg/vial and Sterile Diluent for Melphalan HCl for Injection, 10mL/Vial Sample Spike Solution Chromatogram (10mg/mL). HPLC Chromatograms of Leachables. Peak Identity: (2) Butylated hydroxytoluene-(BHT) (3) Sulfur-(S) (4) Antioxidant-1010 (5) Antioxidant-1330 (6) Antioxidant-1076 (7) Antioxidant-168

Table 12: Results of Linearity for Antioxidant 1330

Linearity and Range for Antioxidant1330				
Level	Strength in µg/mL	Area for the Peak Area		
LOQ	0.1040	0.0758	0.0773	0.0766
20%	1.0405	0.7659	0.7687	0.7656
40 %	2.0809	1.5428	1.5036	1.5114
100%	5.2023	3.9599	3.9653	3.9792
150 %	7.8035	5.7451	5.8725	5.8162
Linearity Equation	Y=0.7422x-0.0085			
Correlation Coefficient R	1.00			
Slope	0.7422			
%y-intercept	0%			
Residual Sum of Square	0.0326			

Table 13: Results of Linearity for Antioxidant 1076

Linearity and Range for Antioxidant1076				
Level	Strength in µg/mL	Area for the Peak Area		
LOQ	0.1026	0.0162	0.0146	0.0197
20%	1.0255	0.1577	0.1597	0.1568
40 %	2.0511	0.3154	0.3122	0.3117
100%	5.1277	0.8042	0.8012	0.8003
150 %	7.6916	1.1538	1.1840	1.1688
Linearity Equation	Y=0.1528x+0.0028			
Correlation Coefficient R	1.00			
Slope	0.1528			
%y-intercept	0%			
Residual Sum of Square	0.0015			

correspond to that in of standard. The peak match of antioxidants and sulfur peaks were determined in standard and sample spike solution. The specificity requirement was met. Diluent has not given any interfering peak at the retention time of Antioxidants and sulfur peak. The resolution between antioxidants and sulfur was not less than 2.0 for sample spike, sample and standard. The retention time of antioxidants and sulfur in the sample spike and sample correspond to that in the standard. The peak match of antioxidants and sulfur peak was more than 980 in standard solution and sample spike spiking with impurities. Hence the method is found to be specific. The test results are summarized in Table 5.

Accuracy

The method should be able to measure the analyte of interest with acceptable accuracy. The the outcome values are compared with the results by 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 obtained. Accuracy and Precision: Accuracy and Precision were evaluated by determining the content of antioxidants and sulfur from the six determinations of sample spike solution and the %RSD.

Table 14: Results of Linearity for Antioxidant 168

Linearity and Range for Antioxidant168				
Level	Strength in µg/mL	Area for the Peak Area		
LOQ	0.1077	0.0674	0.0682	0.0709
20%	1.0771	0.7123	0.7180	0.7146
40 %	2.1542	1.4257	1.3985	1.4057
100%	5.3856	3.6518	3.6605	3.6784
150 %	8.0784	5.3152	5.4420	5.3984
Linearity Equation	Y=0.6711x-0.0069			
Correlation Coefficient R	1.00			
Slope	0.6711			
%y-intercept	0%			
Residual Sum of Square	0.0237			

Table 15: Results of Range

Name of compound	LOQ~150%
Butylated hydroxytoluene (BHT)	0.0998µg/mL~7.4865µg/mL
Sulfur(S)	0.1030µg/mL~7.7280µg/mL
Antioxidant1010	0.1040µg/mL~7.8035µg/mL
Antioxidant1330	0.1040µg/mL~7.8035µg/mL
Antioxidant1076	0.1026µg/mL~7.6916µg/mL
Antioxidant168	0.1077µg/mL~8.0784µg/mL

Reports mean content, standard deviation, %RSD & confidence interval of each component. The %recovery of six recovery results of antioxidants and sulfur was between 80%~120%, The %RSD of six determination of recovery for Antioxidants and sulfur was not more than 10% (n=6). The mean, standard deviation and confidence interval of recovery for Antioxidants and Sulfur (n=6).Hence the method is Accurate and Precise.

All test results are met acceptance criteria and summarized in Table 6.

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 in six copies and calculated ratio as signal to noise. The %RSD for area to peak of antioxidants and sulfur from LOQ Solution wasnot more than 15.0% (n=6).The Signal to noise(S/N) of the Antioxidants and Sulfur peak in the LOQ Solution was not less than 10.

LOQ Results were listed in Table 7.

Limit of Detection (LOD)

The method should be able to detect the lowest concentration of the analyte of interest with acceptable sensitivity. The limit of detection was determined by injecting the LOD solution in twice and calculated signal to noise ratio for antioxidants and sulfur in LOD solution was not less than 3.

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: Both linearity as well as range are checked by determining at 5 concentration levels in triplicate from LOQ to 150%level with respect to standard solution. The correlation coefficient (R) wasnot less than 0.99.%y intercept was not more than ±25% of standard response. The linearity results were listed in Table 9 to Table 14. The linearity curves were listed in Figure 5 to Figure 10.

Range

The range of the method should be appropriate for the intended use for the process. The range is evaluated by determining at 5 strength levels in triplicate from LOQ to 150%level with respect to standard solution. The range results were listed in Table 15.

Solution Stability

Stability of standard solution as well as sample spike was assessed by storing solution at room temperature and injecting at suitable time interval. The %RSD of peak area of Antioxidants and Sulfur was not more than 5.0% for Standard Solution at each time point. The solution stability of standard solution was stable to 28 hours in room temperature. The Stability of Standard Solution results of Antioxidants and sulfur were listed in Table 15. The stability of sample solution is evaluated after storing spike at room temperature also injecting at suitable time interval. The %difference in thecontent of antioxidants and sulfur should be not more than 20% for Sample Spike Solution at each time point. At room temperature, the stability of sample solution was stable to 5.5h. Results were listed in Table 16 to Table 18.

RESULTS AND DISCUSSION

From a genetic toxicology perspective, for non-genotoxic leachables that exceeds 5µg/day, a toxicological risk assessment should be provided. Antioxidants and vulcanizer are non-genotoxic compounds, the limit is

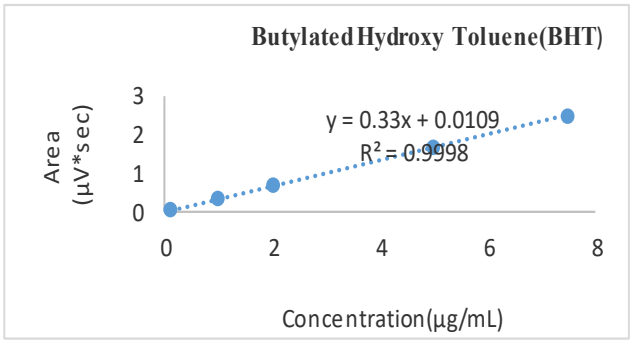

Figure 5: Linearity Diagram of Antioxidant Butylated Hydroxy Toluene(BHT)

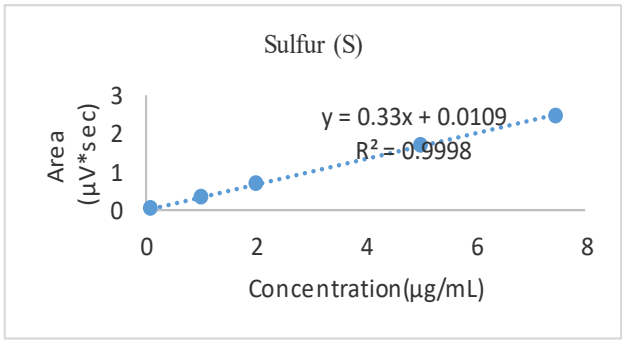

Figure 6: Linearity Diagram of Sulfur (S)

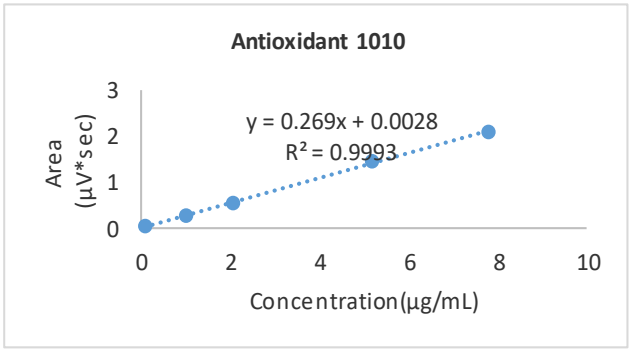

Figure 7: Linearity Diagram of Antioxidant 1010

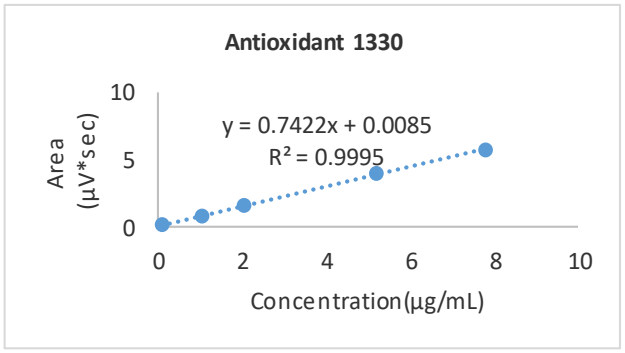

Figure 8: Linearity Diagram of Antioxidant 1330

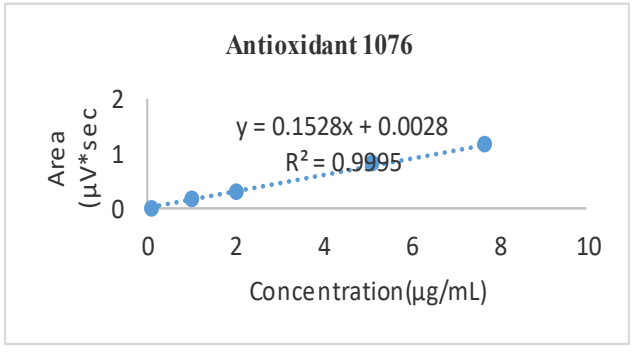

Figure 9: Linearity Diagram of Antioxidant 1076

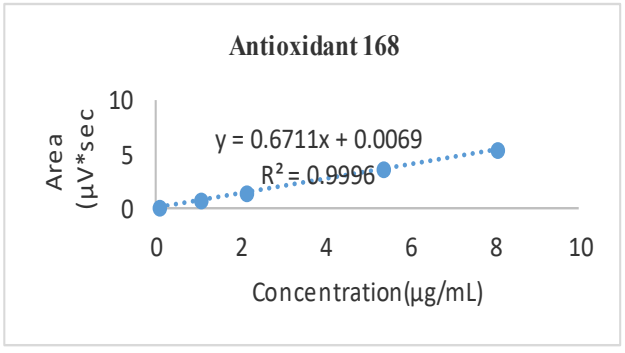

Figure 10: Linearity Diagram of Antioxidant 168

Table 16: Results of Solution Stability- Standard Solution

Sample Name	Component Time Point(hours)	BHT %RSD	S %RSD	1010 %RSD	1330 %RSD	1076 %RSD	168 %RSD
B-STD-1	3.5	1.1	1.1	1.1	1.0	1.3	1.0
B-STD-2	5.0	1.6	1.6	1.4	1.4	1.7	1.5
B-STD-3	6.5	1.9	1.9	1.6	1.9	1.7	2.0
B-STD-4	8.0	2.3	2.3	1.9	2.1	1.7	2.4
B-STD-5	20.0	4.1	4.1	3.7	3.9	3.8	3.9
B-STD-6	21.0	4.0	4.0	3.7	3.9	3.7	4.0
B-STD-7	22.5	3.8	3.9	3.5	3.8	3.6	3.8
B-STD-8	24.5	3.7	3.7	3.4	3.7	3.5	3.7
B-STD-9	27.0	3.6	3.6	3.3	3.6	3.4	3.6
B-STD-10	28.0	3.5	3.5	3.2	3.5	3.2	3.5

Table 17: Results of Solution Stability- Sample Spike Solution

Component	Time	BHT	S	1010	1330	1076	168
Concentration (µg/mL)	0h	4.7079	5.0334	4.8758	4.7606	5.0037	5.0694
	2.5h	4.8450	5.0798	4.9559	4.8622	5.1021	5.1380
	5.5h	4.9124	5.1395	5.0062	4.9276	5.1322	5.1063
%Difference	2.5h	3%	1%	2%	2%	2%	1%
	5.5h	4%	2%	3%	4%	3%	1%

calculated as 5µg/day. The maximum daily dose of Melphalan Hydrochloride for Injection is 16mg/m^2, and the strength is 50mg/vial, diluent is 10 mL/vial, so the concentration is 5mg/mL. So the maximum daily dose converted into volume is 5.76mL, calculate the (Analytical Evaluation Threshold)AET of antioxidants and sulfur from the maximum daily dose.[9-11]

The validation results indicate that the HPLC process which is developed to determine migration of antioxidants as well as vulcanizer 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 highly accurate and precise to determine antioxidants and sulfur content. Both LOD as well as LOQ are 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 solution stability of standard solution is stable for 28 hours at room temperature, and the stability of the sample solution was stable for 5.5 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 exceed the AET. This indicates that the stability of the drug product and diluent is acceptable for long-term storage.

CONCLUSION

The HPLC method developed to determine migration of leachables amount of antioxidants and vulcanizer in Melphalan Hydrochloride for Injection and Sterile Diluent for Melphalan HCl for Injection is suitable for routine testing. The method demonstrated specificity, accuracy, precision, linearity, range, and stability. 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 of migration of antioxidants and vulcanizer for melphalan hydrochloride for injection, 50mg/vial and sterile diluent for melphalan HCl for Injection, 10mL/Vial and hence No compound was detected with an amount exceed the AET.

Table 18: Results of Test Sample Solution

Name	AET (µg/mL)	LOQ (µg/mL)	Content/(µg/ mL) Control samples	
			Sample-1	Sample-2
BHT	0.2	0.0998	N.D	N.D
S	0.2	0.1030	N.D	N.D
1010	0.2	0.1040	N.D	N.D
1330	0.2	0.1040	N.D	N.D
1076	0.2	0.1026	N.D	N.D
168	0.2	0.1077	N.D	N.D
Acceptance Criteria		The Antioxidants and Sulfur test results are not more than(Analytical Evaluation Threshold) AET.		

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