LABORATORY INFORMATION BULLETIN

Identification and Quantitation of Benzene Impurity in Sunscreen Product by Headspace Gas Chromatography-Mass Spectrometry Detection (HSGC-MS)

(CARTS Project No. IR01923)

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ABSTRACT

Based on a recent Citizen Petition claiming the discovery of benzene impurity at levels exceeding FDA guidance limit in non-prescription sunscreen drug products and after-sun care products, CDER issued a mission critical, high priority, For-Cause sample collection assignment. The sunscreens require identification and quantitation of benzene impurity if present. The analytical procedure described here uses HSGC-MSD for quantitation of benzene in sunscreens from levels as low as 0.2 ppm up to 12 ppm. This method was successfully validated using a lotion and spray sunscreen sample, and the validation data is here included.

INTRODUCTION

A recent Citizen Petition claiming the discovery of benzene at levels exceeding 2 ppm in non-prescription sunscreen drug products and after-sun care products, has become an FDA priority and concern. The manufacture of drug substances, excipients, and drug products should not employ benzene, a "Class 1 solvent," due to its unacceptable toxicity and possible carcinogenic effects. Nevertheless, if its use is unavoidable in the manufacture of a drug product, the level of benzene is restricted to under 2 ppm unless otherwise justified[1].

The For-Cause sample collection assignment is intended to confirm the presence of and amounts of benzene impurity in sunscreen drug products. Initial testing of non-prescription sunscreen drug products and after-sun care products by Valisure LLC (FEI: 3012063246) detected benzene levels exceeding 2 ppm in specific batches of sunscreen products containing the ingredients avobenzone, octisalate, homosalate, octocrylene, oxybenzone, zinc oxide, or a combination.[2] Samples collected were tested by the Office of Regulatory Affairs (ORA), Office of Regulatory Science (ORS), Office of Medical Products and Specialty Laboratory Operations (OMPSLO) Pacific Southwest Medical Products Laboratory (PSMPL). The method in this bulletin is able to quantify benzene down to a level of 0.2 ppm in sunscreen drug products. The validation of this method for analysis of benzene in a sunscreen lotion and spray sample is here described.

METHODS AND MATERIALS

Equipment

a. Shimadzu GC-2010 Plus with Shimadzu AOC-5000 Plus autosampler and Shimadzu GCMS-TQ8040 (Mass Spectrometry Detector), or equivalent
b. R J&W VF-624ms GC Column, Agilent P/N: CP9102, Nominal Dimensions: ID: 0.25 mm; Film Thickness: 1.4 microns; Length 30 m
c. Positive Displacement Pipette – Eppendorf Repeater E3x, 1 µL – 50 mL, or equivalent
d. Class A volumetric flasks

Sample

Sunscreen Lotion / Sunscreen Spray
Active pharmaceutical ingredient: oxybenzone, 6%; octocrylene, 10%; avobenzone 3%; octisalate, 5%; homosalate, 15%

Reagents and Standards

a. Residual Solvent Class 1 Benzene, USP reference standard, concentration 10.2 mg/mL, catalog number: 1601146
b. Benzene-*d6* 99.9%, Sigma Aldrich reference standard, catalog number: 175870
c. Dimethyl Sulfoxide (DMSO), Sigma Aldrich, catalog number 472301

Solutions

All procedures were conducted at laboratory temperature which was 72 ^{o}C ±2

a. Internal Stock Standard Solution (950 µg/mL)
Pipette 100.0 µL of benzene-*d6* to a 100 mL volumetric flask and dilute to volume with dimethyl sulfoxide.

b. Internal Working Standard Solution (ISTD) (2 µg/mL)
Pipette 210.0 µL of benzene-*d6* Internal Stock Standard Solution to a 100.0 mL volumetric flask and dilute to volume with DMSO.

c. Benzene Stock Standard Solution (100 µg/mL)
Pipette 0.980 mL of USP Benzene Standard (10.2 mg/mL) to a 100.0 mL volumetric flask and dilute to volume with DMSO.

d. Benzene Spike Standard Solution (2 µg/mL)
Pipette 1.000 mL of 100 ppm Benzene Stock Standard to a 50.0 mL volumetric flask and dilute to volume with DMSO.

e. Sample solution preparation
Option 1

Prepare and store all sample solutions in amber glassware.

Weigh 0.5 g of sample into a tared 20.0 mL amber headspace vial and record the weight of the sample. Dilute with 3.500 mL of DMSO and add 1.000 mL of ISTD Solution to the vial.

Option 2

Pipette 0.5 mL of sample into a tared 20.0 mL amber headspace vial and record the weight of the sample. Dilute with 3.500 mL of DMSO and add 1.000 mL of ISTD to the vial. The total sample solution volume is 5.000 mL in each Headspace vial.

Mix all samples gently for 10 seconds using the vortex. While vortex mixing, make sure none of the sample touches the septa at the top of the cap.

f. Sample solution and spike sample solutions — see Table 1 below.

Table 1: Preparation of Sample and Spike Sample (prepare in triplicate)

Solution Name	Volume of Each Spike Standard Solution Added (mL)	Volume of ISTD Added (mL)	Volume of Sample Stock Added (mL)	Volume of DMSO Added (mL)	Spike Concentration Level (%v/v)	Amount of Standard Added to Headspace Vial (µg)
Sample Solution	0	1.0	0.5	3.500	0	0
10% Spike Sample Solution	0.050	1.0	0.5	3.450	10	0.1
50% Spike Sample Solution	0.250	1.0	0.5	3.250	50	0.5
100% Spike Sample Solution	0.500	1.0	0.5	3.000	100	1.0
200% Spike Sample Solution	1.000	1.0	0.5	2.500	200	2.0

Validation Solutions for Linearity, LOD and LOQ Determination

a. Linearity 1 Solution
Pipette 0.050 mL of Benzene Spike Standard, 1.000 mL of Working Internal Standard, and 3.950 mL of DMSO to a headspace vial; vortex mix.

b. Benzene LOD Solution

Pipette 0.030 mL of Benzene Spike Standard to a 20.0 mL amber headspace vial, add 1.000 mL of Working Internal Standard and 3.970 mL DMSO – see Table 3 below.

c. Other linearity, and LOQ solutions – see Table 2 below.

Table 2: Preparation of Linearity and LOQ Solutions for Validation

Solution Name	Volume of Benzene Spike Standard Added (mL)	Volume of ISTD Added (mL)	Volume of DMSO Added (mL)	Concentration Level of Limit (%)	Amount of Standard in Headspace Vial (µg)
Linearity 1 (LOQ)	0.050	1.0	3.950	10	0.10
Linearity 2	0.100	1.0	3.900	20	0.20
Linearity 3	0.250	1.0	3.750	50	0.50
Linearity 4 (Work.Std.)	0.500	1.0	3.500	100	1.0
Linearity 5	1.000	1.0	3.000	200	2.0
Linearity 6	2.000	1.0	2.000	400	4.0
Linearity 7	3.000	1.0	1.000	600	6.0

Table 3: Preparation of LOD Solutions for Validation

Solution Name	Volume of Benzene Spike Standard Added (mL)	Volume of ISTD Added (mL)	Volume of DMSO Added (mL)	Concentration Level of Limit (%)	Amount of Standard in Headspace Vial (µg)
LOD 1	0.030	1.0	3.970	0.1	0.06
LOD 2	0.030	1.0	3.970	0.1	0.06
LOD 3	0.030	1.0	3.970	0.1	0.06

Instrumentation

A Shimadzu GC-2010 Plus gas chromatography system with mass spectrometry detector, and a J&W VF-624ms GC Column was used for the method validation and sample analysis. Table 4 on the next page describes the HSGC-MSD conditions used.

Table 4: HSGC-MSD Parameters

Instrument	Shimadzu GC-2010 Plus
Detector	Shimadzu GCMS-TQ8040
Column	J&W VF-624ms Agilent P/N: CP9102 Nominal Dimensions: ID: 0.25 mm; Film Thickness: 1.4 microns; Length 30 m
Chromatographic Conditions	
Carrier Gas	Helium
Flow Rate	1.0 mL/min
Average Linear Velocity	36 cm/sec
Nominal Pressure	8.8 psi
Mode	Constant flow
Headspace Parameters	Shimadzu AOC-5000 Plus
Injection Volume	1000 µL
Incubation Temperature	90 °C
Incubation Time	1200 sec
Agitator Speed	250 rpm

Syringe Temperature	90 °C
Injection Speed	500 µL/sec
GC Parameters	
Inlet temperature	220 °C
Mode	Split 5:1
Oven initial temperature	60 °C
Initial hold time	5.0 min
Ramp rate #1	40 °C/min
Final temperature after ramp	240 °C
Hold time after ramp #1	2.0 min
Run time	11.5 min
MSD	Shimadzu GCMS-TQ8040
Mass range (m/z)	45 – 150
Acquisition type	Q3-SIM/Scan
Ionization	EI 70 eV
Solvent delay	3.0 minutes
MS Interface	250 °C
MS Source	230 °C
Quant ions	m/z 78 for benzene, m/z 84 for benzene-*d6*
Qual ions	m/z 77 and 51 for benzene

Quantitation of benzene is performed by single point calibration using the peak area ratio of benzene analyte found in sample solution to internal standard (benzene-d_6). The following formula is used:

$$\text{Result (ppm)} = \text{Amount of benzene in Standard vial (µg)} \times \left(\frac{Ru}{Rs}\right) \times \left(\frac{1}{\text{Sample Wt (g)}}\right)$$

Amount of benzene in Standard vial = 1 µg (System suitability standard)

R_U = Peak response ratio obtained from the Sample Solution preparation
R_S = Peak response ratio obtained from the Standard Solution preparation (average from 6 system suitability injections)

RESULTS and DISCUSSION

Sample Analysis

Six preparations of sunscreen spray and lotion samples were performed and analyzed according to the method. The results are in Table 5. An m/z 78 peak was detected in all of the unspiked samples, but levels were well below the LOQ, therefore exact amounts were not determined. It is expected that a major fraction or all of this signal came from the benzene-d_6 internal standard. Figures 1–3 show the chromatograms of standard solution, lotion and spray samples solution.

Table 5. Unspiked sample

Validation Test Series	Peak Area Ratio with ISTD, % of Working Standard			
	Prep 1	Prep 2	Prep 3	Average
Analyst 1 Lotion	2.2	1.9	2.5	2.2
Analyst 2 Lotion	1.4	1.9	1.6	1.6
Analyst 2 Spray	3.0	2.3	4.0	3.1
Analyst 3 Spray	2.9	3.8	4.1	3.6
Sample Criteria	≤ 100			
LOQ Level	10% of working standard level (~0.2 ppm in sample)			
Pass/Fail	All below LOQ level – Pass			

Figure 1: HSGC-MS Benzene Quant Ion m/z 78 and Qual Ions m/z 77 and 51 for System Suitability 1 (100% limit level)

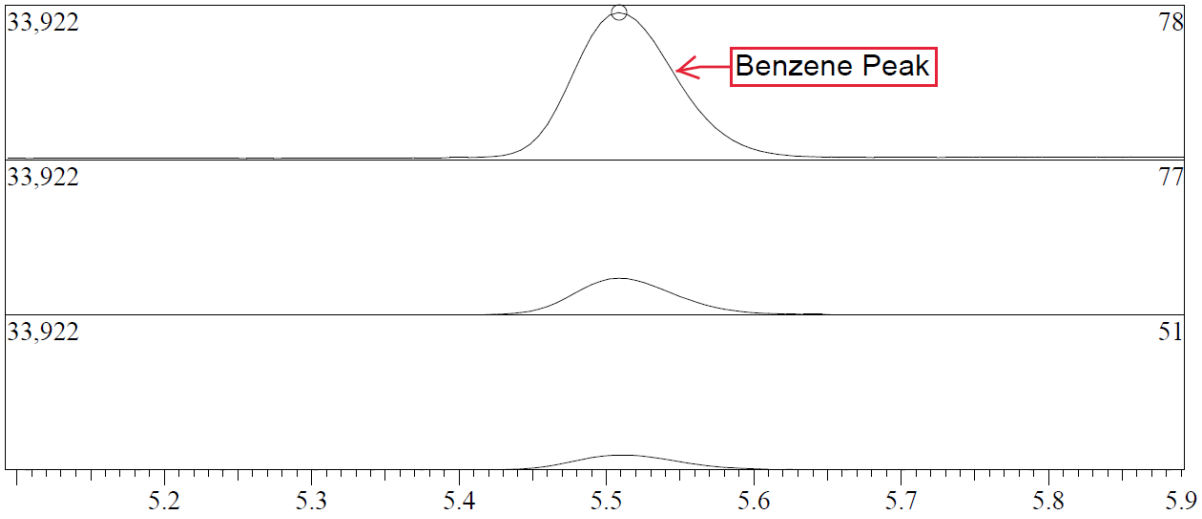


Figure 2: HSGC-MS Benzene Quant Ion m/z 78 and Qual Ions m/z 77 and 51 for sunscreen lotion sample

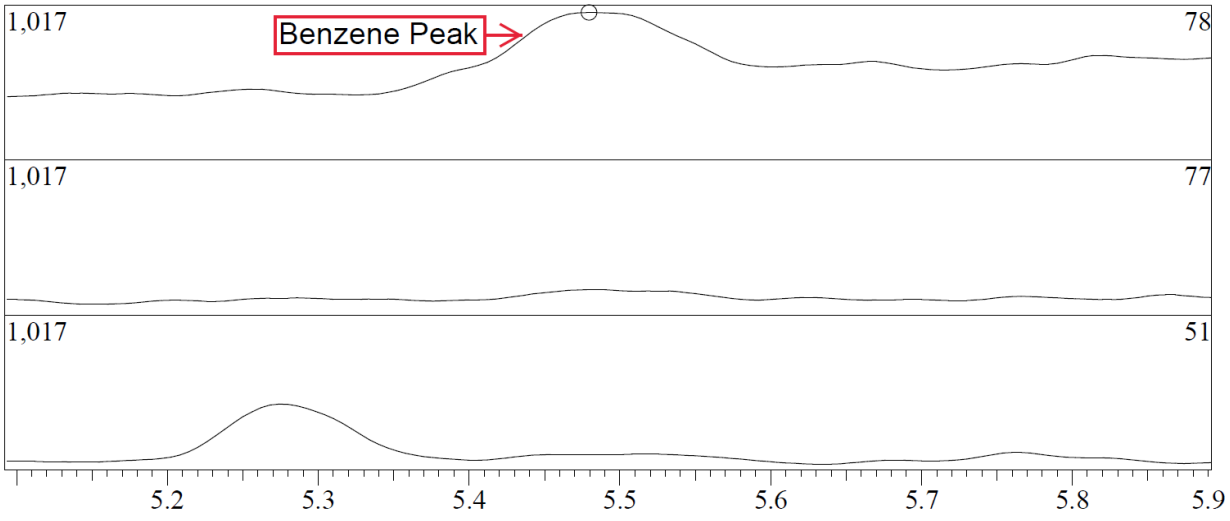
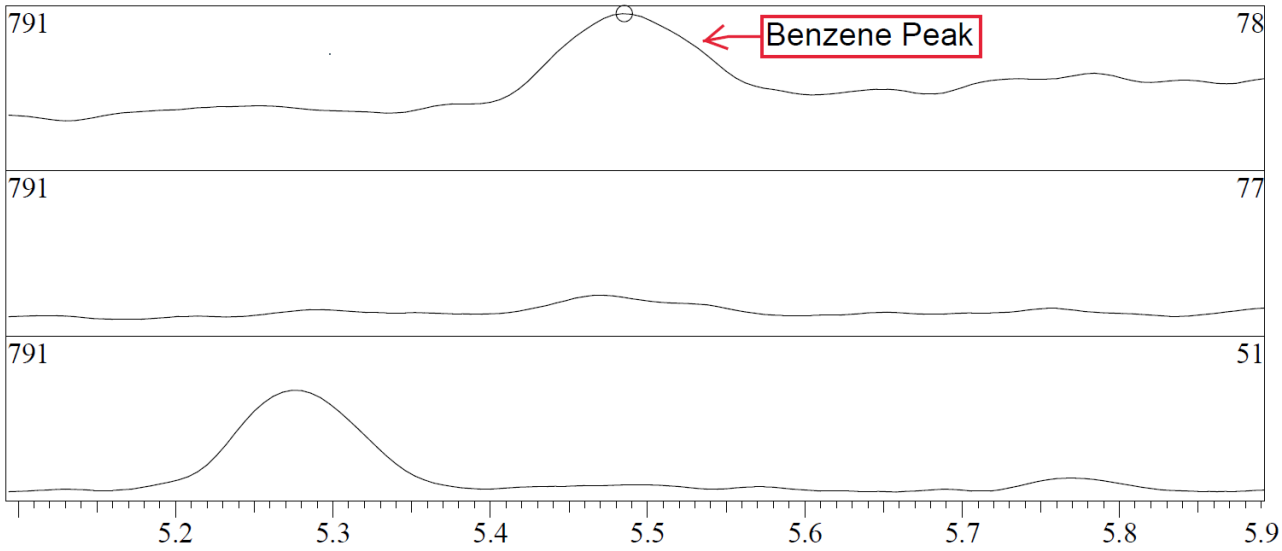


Figure 3: HSGC-MS Benzene Quant Ion m/z 78 and Qual Ions m/z 77 and 51 for sunscreen spray sample



Method Validation

The method was validated using a similar sunscreen lotion and spray sample used for sample analysis. The results and acceptance criteria are given in Tables 6-11. System suitability was determined using 6 preparations of Standard Solution (Linearity 4). System suitability criteria is %RSD ≤ 10% for the 6 injections of the 100% limit standards based on peak area ratios. Linearity was determined by injecting standard solutions at seven concentration levels, from 10% to 600% of the limit concentration of benzene. LOD and LOQ were determined using 3 preparations of 6% and 10% of the limit concentration of benzene, respectively. Due to the presence of a small m/z 78 peak in the internal standard solution, contributed from the benzene-d_6, signal-to-noise ratios for the LOD solution were calculated as the ratio of m/z 78 peak area in LOD solution to that of the internal standard solution. Accuracy and precision were determined using 3 preparations of un-spiked sunscreen lotion and spray sample and 3 preparations each at 3 different spike concentration levels. Recoveries were determined by the calculated concentration relative to the known spike amount. Each preparation was injected once. No interfering peaks were observed on the sample chromatograms. All acceptance criteria were met. Figure 4 shows the linear calibration curve of benzene in DMSO.

Table 6. System suitability results

Validation Test Series	Benzene %RSD Peak Area Ratios (n = 6)
Analyst 1 Lotion run sequence	0.94
Analyst 2 Lotion run sequence	1.35
Analyst 2 Spray run sequence	0.86
Analyst 3 Spray run sequence	0.64
Criteria	≤ 10
Pass/Fail	Pass

Table 7. Linearity results; coefficient of determination (R^2)

Validation Test Series	Standard Amount Range (µg)	Equiv. Conc. Range in Lotion (ppm)	Equiv. Conc. Range in Spray (ppm)	r^2
Analyst 1	0.1 - 6	0.2 – 12	0.2 – 14	0.998
Analyst 2	0.1 - 6	0.2 – 12	0.2 – 14	1.000
Criteria				≥ 0.995
Pass/Fail				Pass

Note: Lotion Density = 1.028 g/mL; Spray Density = 0.8341 g/ml. Densities were determined by independently weighing three 0.500 mL aliquots of each sample and calculating the average weight.

Table 8. Signal-to-Noise (S/N) of LOD solutions

Validation Test Series	LOD amount in headspace vial (µg)	Equivalent LOD amount in sample (ppm)	m/z 78 Quant Ion in ISTD Blank N=6	m/z 78 Quant Ion in LOD solution		
				Prep 1	Prep 2	Prep 3
Analyst 3 – Peak Area	0.06	0.12	1337	5465	4759	5466
S/N (LOD ÷ ISTD)			N/A	4.1	3.6	4.1
Criteria	S/N ≥ 3					
Pass/Fail	Pass					

Table 9. %Recovery and %RSD for LOQ solution (0.10 µg in headspace vial, 0.20 ppm in sample)

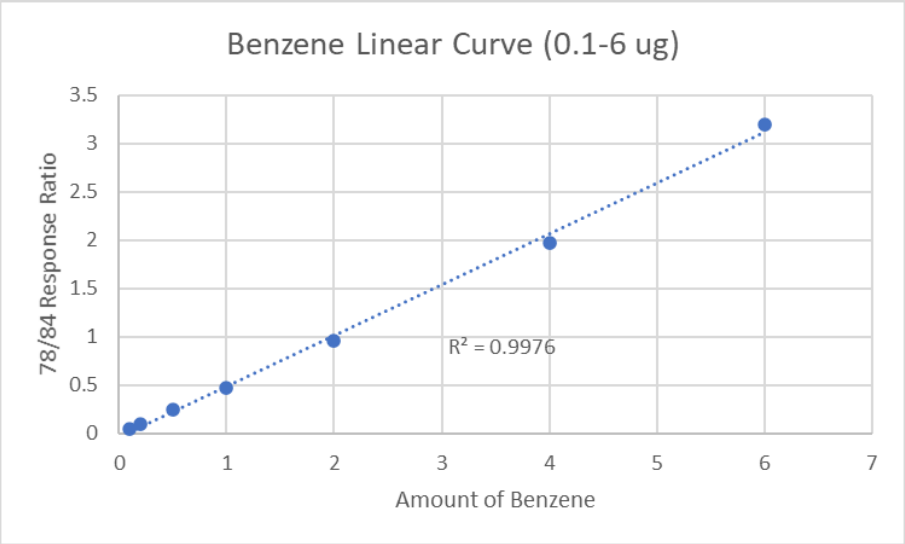
Validation Test Series	%Recovery				%RSD
	Prep 1	Prep 2	Prep 3	Average	
Analyst 1 Lotion	105	102	105	104	2.0
Analyst 2 Spray	101	103	94	99	4.6
Criteria				80–120	≤ 10
Pass/Fail	Pass				

Table 10. Accuracy and Precision, Spike %Recovery

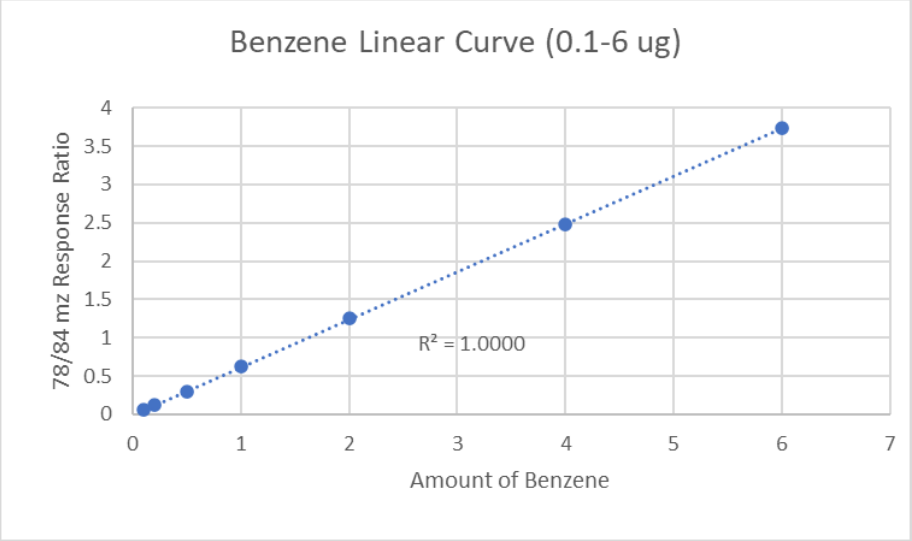
Limit Level	Validation Test Series	%Recovery				%RSD	%RSD Intermediate Precision
		Prep 1	Prep 2	Prep 3	Average		
50%	Analyst 1 Lotion	110	110	111	110	0.4	5.8
	Analyst 2 Lotion	98	104	98	100	3.5	
	Analyst 2 Spray	101	102	99	101	1.3	5.0
	Analyst 3 Spray	113	107	108	110	3.1	
	Criteria				80–120	≤ 10	≤ 15
	Pass/Fail	Pass					
	Analyst 1 Lotion	111	111	111	111	0.1	5.6
	Analyst 2 Lotion	99	101	101	100	0.9	

Limit Level	Validation Test Series	%Recovery				%RSD	%RSD Intermediate Precision
		Prep 1	Prep 2	Prep 3	Average		
100%	Analyst 2 Spray	102	103	102	102	0.8	2.1
	Analyst 3 Spray	99	98	99	99	0.6	
	Criteria				80–120	≤ 10	≤ 15
	Pass/Fail	Pass					
200%	Analyst 1 Lotion	110	110	110	110	0.2	5.3
	Analyst 2 Lotion	100	101	99	100	0.7	
	Analyst 2 Spray	103	104	101	103	1.6	2.8
	Analyst 3 Spray	98	98	98	98	0.4	
	Criteria				80–120	≤ 10	≤ 15
	Pass/Fail	Pass					

Figure 4: Linear Calibration Curve: Benzene in DMSO



Analyst 1 lotion sample



Analyst 2 spray sample

CONCLUSION

The VAL-CHEM-061 Identification and Quantitation of Benzene in Sunscreen Products method was successfully validated. The method validation study showed that the HSGC-MS method for the analysis of benzene in sunscreen lotion and spray sample is specific, accurate, precise, and linear within a range of 10–600% of the limit concentration of 2 ppm benzene in sample. LOD values were calculated at 0.12 ppm in test sample. LOQ was established at 0.2 ppm in test sample.

REFERENCES

1. Food and Drug Administration, Q3C – Tables and List Guidance for Industry (2017) (https://www.fda.gov/media/71737/download)
2. Valisure Citizen Petition on Hand Sanitizer Products Containing Benzene Contamination and Other Significant Issues, https://www.valisure.com/wp-content/uploads/Valisure-FDA-Citizen-Petition-on-Hand-Sanitizer-v4.14.pdf