Identification and Quantification of Residual Benzene in Sunscreen Products by HS-GCMSMS

This method describes the identification (≥ 0.040 µg/g) and quantification (0.10 – 9.0 µg/g) of residual benzene in sunscreen products (aerosol, mist and lotion) by Headspace-GC-MS/MS using Shimadzu GCMS-TQ8050NX.

Contents

Acquisition method

Headspace analysis conditions

In order to prevent potential formation of artefactual benzene, this method utilises low-temperature headspace analysis using the conditions below:

Setup		Analysis		Advanced	
Gas Chromatograph	GC1	**Syringe Tool**	HS 2	**Enable Pre Filling**	True
Sync Before Incubation End Time	0min	**Incubation Temperature**	37°C	**Filling Strokes Count**	5
Agitator	Agitator 1	**Incubation Time**	20min	**Filling Strokes Volume**	1.2mL
Do Agitation	True	**Syringe Temperature**	80°C	**Filling Strokes Aspirate Flow Rate**	6mL/min
Heat Agitator	True	**Agitator Speed**	700rpm	**Delay After Filling Strokes**	30s
Wait For Readiness Agitator	True	**Pre Purge Time**	0s	**Sample Aspirate Flow Rate**	6mL/min
Sample Vial Depth	15mm	**Injector**	Injector 1	**Sample Post Aspirate Delay**	0s
Heat Syringe	True	**Injection Flow Rate**	25mL/min	**Sample Vial Penetration Speed**	25mm/s
Wait For Readiness Syringe	True	**Post Purge Time**	120s	**Injector Penetration Speed**	25mm/s

Injection Signal Mode	PlungerUp	**Analysis Time**	11min	**Pre Injection Dwell Time**	3s
Injector Penetration Depth	40mm			**Post Injection Dwell Time**	10s
Continuous Purge	False			**Agitator On Time**	5s
				Agitator Off Time	2s

GC separation conditions

GC column: Bruker BR-624MS 30.0 mx 0.25 mm ID x 1.40 µm or equivalent
Glass liner/ insert: Restek Topaz 3.5 mm ID (3.5 x 5.0 x 95 mm) Straight Split Inlet Liner w/ Wool, Catalog#23319 or equivalent
Injection volume: 1000 µL

Column Oven Temp.	50.0 °C	**Oven Temp. Program:**		
Injection Temp.	250.00 °C	Rate	Temperature (°C)	Hold Time (min)
Injection Mode	Split	**-**	50.0	1.50
Flow Control Mode	Column Flow	40.00	90.0	0.00
Pressure	171.8 kPa	120.00	240.0	1.25
Total Flow	21.0 mL/min	**Oven Cooling Rate**	Fast	
Column Flow	3.00 mL/min	**< Ready Check Heat Unit >**		
Linear Velocity	62.8 cm/sec	**Column Oven**	Yes	
Purge Flow	3.0 mL/min	**SPL1**	Yes	
Split Ratio	5	**MS**	Yes	
High Pressure Injection	OFF	**< Ready Check Detector(FTD/BID) >**		
Carrier Gas Saver	OFF	**< Ready Check Baseline Drift >**		
Splitter Hold	OFF	**< Ready Check Injection Flow >**		
		SPL1 Carrier	Yes	
		SPL1 Purge	Yes	
		< Ready Check APC Flow >		
		< Ready Check Detector APC Flow >		
		External Wait	No	
		Equilibrium Time	0.5 min	
		PrepRun Start	Auto	

MS/MS conditions

IonSourceTemp	220.00 °C
Interface Temp.	250.00 °C
Solvent Cut Time	2.30 min
Detector Gain Mode	Relative to the Tuning Result
Detector Gain	+0.40 kV
Threshold	0
Acquire Data without Using CID Gas(Q3Scan)	OFF
[MS Table]	
--Group 1 - Event 1--	
Compound Name	Benzene
Start Time	2.40min
End Time	5.00min
Acq. Mode	MRM

Event Time	0.100sec
Q1 Resolution	Unit
Q3 Resolution	Unit
Ch1-m/z	(Precursor)78.10>(Product)52.00 (CE)20.00V
Ch2-m/z	(Precursor)77.00>(Product)51.00 (CE)20.00V
Ch3-m/z	(Precursor)78.10>(Product)39.00 (CE)20.00V
--Group 1 - Event 2--	
Compound Name	Benzene-d6
Start Time	2.40min
End Time	5.00min
Acq. Mode	MRM
Event Time	0.100sec
Q1 Resolution	Unit
Q3 Resolution	Unit
Ch1-m/z	(Precursor)84.10>(Product)82.10 (CE)20.00V
Ch2-m/z	(Precursor)84.10>(Product)56.10 (CE)20.00V
Sample Inlet Unit	GC

Preparation of diluent and standard solutions

Stock standard solutions

a. Benzene stock standard solution

Working under the fumehood, carefully open the 2000 µg/mL certified benzene standard solution by an ampule opener. Using a 1-mL glass pipette transfer 1 mL of this solution to a 20 mL volumetric flask containing approximately 10 mL GCMS grade (or equivalent) methanol. Add methanol to volume and shake well. This solution contains approximately 100 µg/mL benzene and is to be stored at -20 ºC freezer. When stored under these conditions, this stock solution was assessed to be stable for a minimum of 15 days. Using this solution for quantitative purposes beyond 15 days after preparation is allowed provided that its stability is proven through comparison of instrument response factor for this solution against a freshly prepared stock standard solution.

b. Internal standard (Benzene-D6) stock standard solution

Following the procedure outlined above, transfer 1 mL of 2000 µg/mL certified benzene standard solution to a 20 mL volumetric flask containing approximately 10 mL GCMS grade (or equivalent) methanol. Add methanol to volume and shake well. This solution contains approximately 100 µg/mL ISTD and is to be stored at -20 ºC freezer.

Diluent

Due to insolubility of sunscreen products in water, Headspace-GC grade dimethyl sulfoxide (DMSO) is used as headspace solvent in this method. Prepare 0.2 µg/mL solution of ISTD in DMSO using appropriate volume of the ISTD stock standard solution (b). This solution is to be used for preparation of calibrant standard solutions, system sutiability and control standard solutions as well as for sunscreen samples.

Intermediate standard solutions, prepared daily

c. Using stock standard solution (a), prepare a 10 µg/mL solution of benzene in diluent.
In order to maintain a constant concentration of the ISTD for all solutions, add an appropriate volume of solution (b) or an intermediate ISTD solution (in DMSO) to adjust the final ISTD concentration to 0.2 µg/mL. Prepare a second aliquot of solution (c) as described above and dilute this solution to obtain a 1 µg/mL solution of benzene in diluent.

Calibration standards and system suitability solution, prepared daily

Using solutions (c) and (d), prepare solutions e-m in 25 mL volumetric flasks adjusting to volume using the diluent:

d. Solution (e) serves as system suitability solution and its inclusion in the calibration curve is optional. A minimum S/N ratio of 10 should be obtained for this solution at the beginning of the batch and prior to analysis of samples.

Calibration Standard	Concentration (µg/mL)	Amount in 5 mL (µg)	Equiv. to sample concentration (µg/g)	Volume (mL) added from (c)	Volume (mL) added from (d)
e (system suitability)	0.006	0.030	0.06		0.150
f	0.010	0.050	0.10		0.250
g	0.020	0.100	0.20		0.500
h	0.100	0.500	1.00	0.250	
i	0.180	0.900	1.80	0.450	
j	0.260	1.300	2.60	0.650	
k	0.340	1.700	3.40	0.850	
l	0.480	2.400	4.80	1.200	
m	0.620	3.100	6.20	1.550	

Control standard solution, prepared daily

n. Prepare a 0.2 µg/mL solution of benzene in diluent using a separate intermediate standard solution, this is to verify the calibration and should NOT be prepared using solution (c) (if available, a secondary batch of stock standard solution can be used for preparation of this solution). 5 mL of this solution contains an amount equivalent to 2.0 µg/g benzene (current regulatory limit). This solution is to be injected throughout the sample batch every 26 injections, as shown in the example below.

Spiking standard solution, prepared daily

Recovery samples are to be prepared for all analysed samples and solution (n) can also be used as the spiking solution.

NOTE: Depending on the existing levels of benzene in the sample, it may be necessary to spike the sample at a higher level following the initial measurements.

Preparation of samples

Reagent blank

Using a glass pipette or multipette, transfer 5.0 mL of the diluent to a 10 mL screw cap headspace vial.

Sunscreen samples

For aerosol and spray samples, shake the container as per the instructions on the container and prime the product by aiming at an empty conical flask under the fume hood holding down the nozzle for approximately 10-20 seconds. Once primed, spray approximately 500 mg of the sample in a 10 mL headspace vial, record the weight (to 4 significant figures i.e. 501.3 mg), immediatley add 5 mL of the diluent using a glass pipette or multipette and place the cap on the vial. Gently vortex this solution (for approximately 10 seconds at 1000 rpm to mix) prior to transferring to the instrument rack. Repeat the process for a second preparation, all spiked and unspiked samples are to be prepared in duplicate.

Following a similar procedure for lotion and mist samples, ensure that the product pump is primed, if applicable.

For spiked samples, add 5.0 mL of solution (n) (or the appropriate spiking solution) following the same steps as above, vortex the solution and place on the instrument rack.

Example instrument batch

- 3x solvent blank (diluent)
- Standard solutions e-m, two vials of each standard
- Solvent blank
- 2x control standard (n), reinject every 26 injections
- Solvent blank
- Sample 1, two preparations
- Sample 1 spiked at 2.0 µg/g, two preparations
- Solvent blank
- Sample 2, two preparations
- Sample 2 spiked at 2.0 µg/g, two preparations
- Solvent blank
- Sample 3, two preparations
- Sample 3 spiked at 2.0 µg/g, two preparations
- Solvent blank
- Sample 4, two preparations
- Sample 4 spiked at 2.0 µg/g, two preparations
- Solvent blank
- Sample 5, two preparations
- Sample 5 spiked at 2.0 µg/g, two preparations
- Solvent blank
- 2x control standard (n), reinject every 26 injections
- Solvent blank
- Sample 6, two preparations
- Sample 6 spiked at 2.0 µg/g, two preparations
- Solvent blank
- Sample 7, two preparations
- Sample 7 spiked at 2.0 µg/g, two preparations
- …
- Standard solutions f-m, two vials of each standard
- Solvent blank

Calculations and reporting of results

Following the in-house validation requirements, detection of a minimum of two product ions attributable to analyte structure and matching the target ion retention time is required for confirmation of benzene identity. Additionally, ion ratios from positive samples should be within ±30% (relative) of average of calibration standards from the same instrument batch. Provided that all of the above requirements are met, a sample can be deemed positive.

The current method uses 78.1 → 52.0 m/z ($[C_6H_6]^+ \rightarrow ([C_4H_4]^+$) as the target ion while 77.0 → 51.0 m/z ($[C_6H_5]^+ \rightarrow ([C_4H_3]^+$) and 78.1 → 39.0 m/z ($[C_6H_6]^+ \rightarrow ([C_3H_3]^+$) are used as confirming ions.

Calibration curve is ploted based on the area ratio of benzene against amount (µg) in calibration standards, therefore for each sample:

$$Benzene\ concentration\ in\ sample\ \left({}^{\mu g}/_{g}\right) = \frac{{}^{(sample\ area\ ratio - intercept)}/_{slope}}{sample\ weight\ (g)}$$

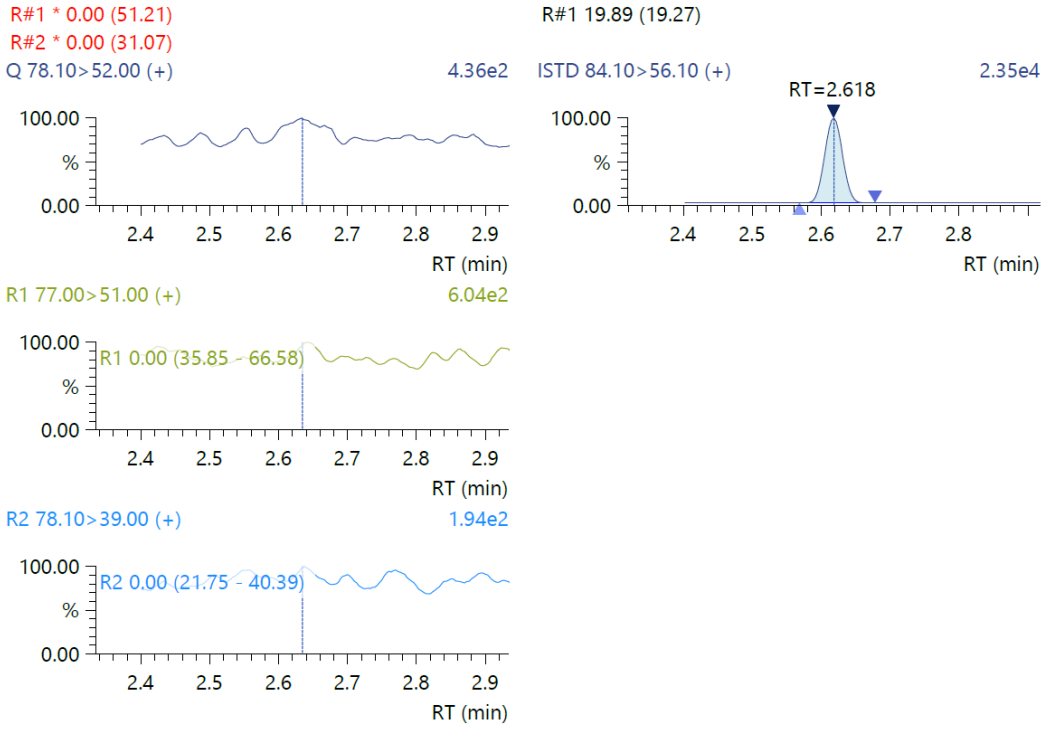
For assessment of calibration curve linearity refer to Chem-SOP-9. Additionally, signal drift between the bracketing injections for the calibration standards injected at the beginning of the batch vs those

injected at the end of the batch should be within ±15% (taking the response at the beginning of the batch as 100 %).

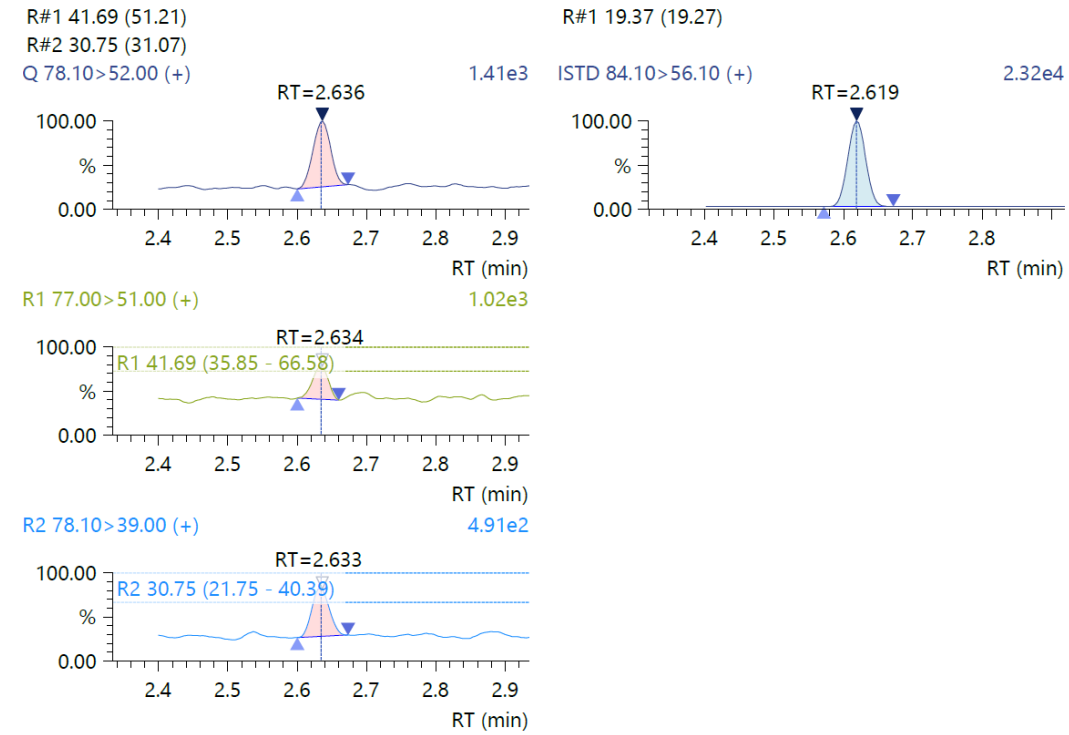
All samples are to be prepared and analysed in duplicate alongside duplicate spiked preparations. Average concentration shall be reported as the final result to 2 significant figures provided that average %recovery obtained for spiked samples falls within the specifications outlined in-house validation SOP and, identification criteria outlined above are met for both unspiked and spiked samples.

Example Chromatograms

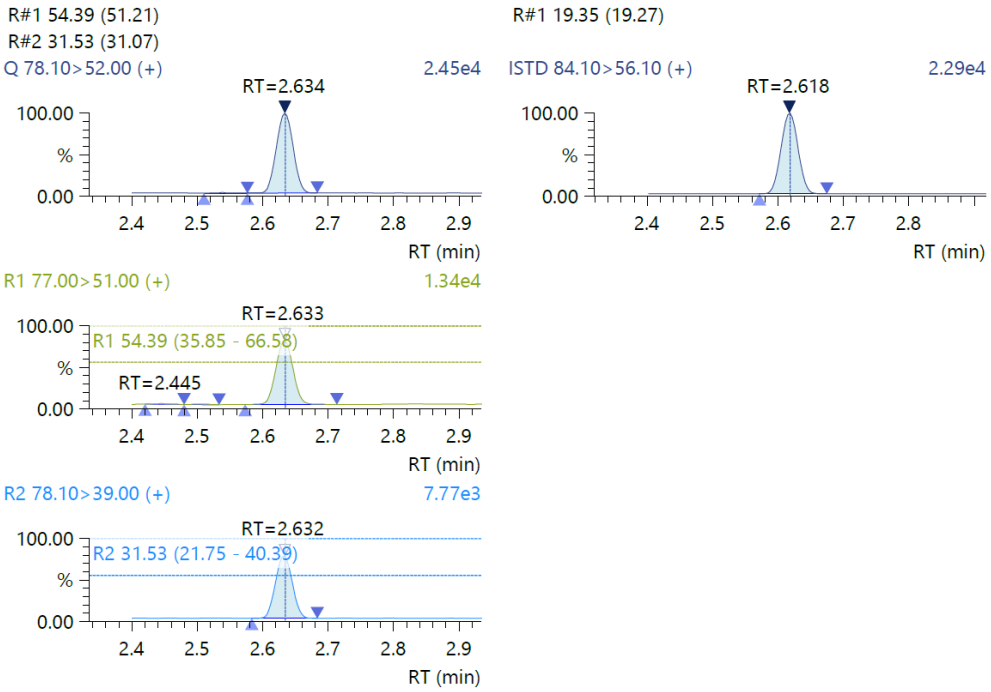
Solvent blank:



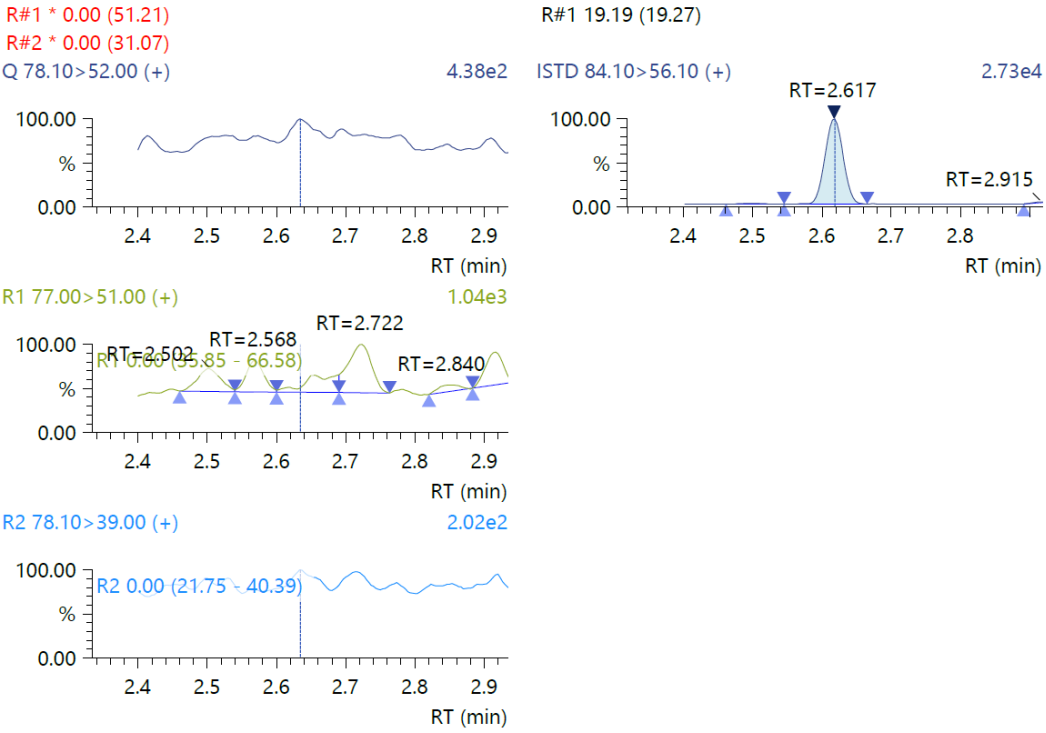
Standard equivalent to 0.040 µg/g in diluent:



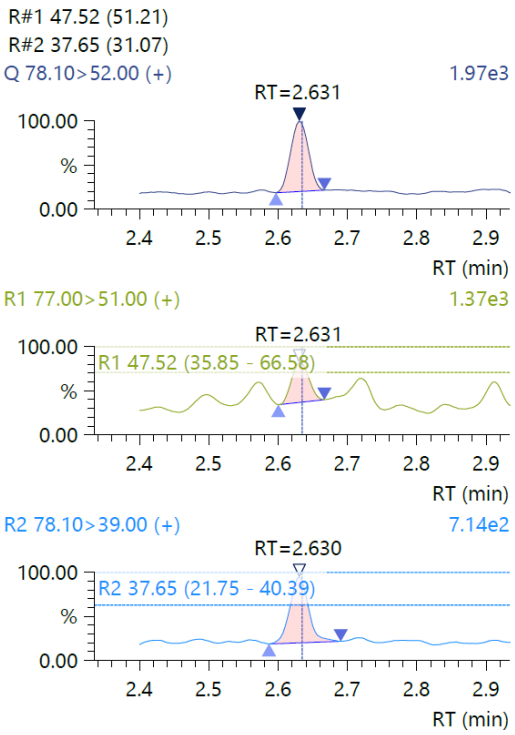
Standard equivalent to 1.0 µg/g standard solution (control) in diluent:

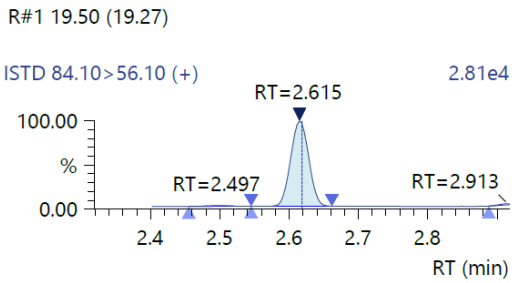


Negative sunscreen sample:



Sunscreen sample spiked at 0.10 µg/g:





Sunscreen sample spiked at 2.0 µg/g: