	Title	Revision No.	Date	Document No.
	DETERMINATION OF 1,4-DIOXANE IN COSMETIC PRODUCTS BY GAS CHROMATOGRAPHY MASS SPECTROMETRY HEAD SPACE SAMPLER (GC-MS/HSS)	0	23/11/2023	ACM 011

1. SCOPE AND FIELD OF APPLICATION

This method specifies a procedure for determination of 1,4-Dioxane in cosmetic products by Gas Chromatography Mass Spectrometry Head Space Sampler.

2. PRINCIPLE

1,4-Dioxane could separated from the sample matrices according to its natural properties of volatility and polarity and analyzed by GC-MS Head Space Sampler using 1,4 – Dioxane d8 as the internal standard (m/z 1,4 – Dioxane : 88, 58, 43; m/z 1,4 – Dioxane d8 : 96, 64, 46)

3. REAGENTS

All reagents must be of analytical purity and suitable for GC MS where appropriate, except for Reference Standard (RS).

Water shall be distilled water, or water of at least equal purity.

3.1 Sodium sulfate anhydrous

3.2 Solvent: 20 % sodium sulphate solution

Preparation 20 % sodium sulphate solution:
Weigh 100 g sodium sulphate anhydrous into a 500-ml volumetric flask, dissolve and make up the volume with water

3.3 1,4-Dioxane Reference Standard

3.4 1,4-Dioxane d8 Reference Standard

4. APPARATUS

Normal laboratory equipment, and:

4.1. Gas chromatography mass spectrometry with head space sampler

4.2. Analytical column:
Capillary column contain 6% cyanopropylphenyl, 94% dimethyl polisiloxane, 30 m, 0.25 mm, 1.4 µm (VF-624 ms or equivalent)

4.3. 20 mL Head Space Sampler (HSS) vial

4.4. Pipette, 1 – 10 µL; 100 – 1000 µL; 1000 – 10000 µL

4.5. Volumetric flask with specification of 10, 100, and 500 mL

4.6. Analytical balance

4.7. Vortex mixer

5. PROCEDURE

5.1. Internal standard solution

5.1.1. Pipette 5.0 µl 1,4-Dioxane-d8 into a 10-ml volumetric flask, make up to volume with water (**a1**)

5.1.2. Pipette 1.0 ml **a1** solution into a 100-ml volumetric flask, make up to volume with water (**a2**)

5.2. Standard stock 1,4-*Dioxane* solution

5.2.1. Pipette 6.25 µl 1,4-Dioxane (density 1.03 mg/µl) into a 10-ml volumetric flask, make up to volume with water (**b1**)

5.2.2. Pipette 1 ml **b1** solution into a 100-ml volumetric flask, make up to volume with water (**b2** concentration 6.4375 µg/ml)

5.3. Standard calibration solutions

5.3.1. Pipette 0.1; 0.2; 0.4; 0.6; 0.8; and 1.0 ml, respectively, of **b2** solution into different 20.0 mL HSS vials

5.3.2. Add 1.0 ml of **a2** solution into each vial
5.3.3. Add 3.9; 3.8; 3.6; 3.4; 3.2; and 3.0 ml of solvent, respectively
5.3.4. Vortex the solution until homogenous (C1, C2, C3, C4, C5 and C6)

Or as follows:

Concentration (µg/ml)	**C1 (0.1287)**	**C2 (0.2575)**	**C3 (0.5150)**	**C4 (0.7725)**	**C5 (1.0300)**	**C6 (1.2875)**
Standard stock 1,4-*Dioxane* solution (b2) (ml)	0.1	0.2	0.4	0.6	0.8	1.0
Internal standard solution (a2) (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Solvent (ml)	3.9	3.8	3.6	3.4	3.2	3.0
Total volume (ml)	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**	**5.0**

5.4. Sample solution preparation
5.4.1 Weigh accurately 0.5 g of sample into a 20 mL HSS vial
5.4.2 Add 1.0 mL of **a2** solution
5.4.3 Add 4.0 ml of solvent
5.4.4 Vortex the solution until homogenous
Note: vortex speed should be maintained to avoid foam formation

5.5. Gas chromatography mass spectrometry (GCMS)
5.5.1. Gas chromatographic conditions
5.5.1.1 Column temperature: 40 ^{o}C
5.5.1.2 Injector temperature: 185 ^{o}C
5.5.1.3 Analysis technique: Temperature programme
5.5.1.4 Temperature programme:

(^{O}C /min)	Temp. ^{O}C	Time (min)
-	40	3
5	75	0
50	230	3

5.5.1.5 Carrier gas: helium
5.5.1.6 Injection volume: 1000uL
5.5.1.7 Flow control mode: linear velocity
5.5.1.8 Injection mode: split (5:1)
5.5.1.9 Flow rate: 1.0 ml/minute

5.5.2. Mass spectrometry conditions
5.5.2.1. Ionization source: EI
5.5.2.2. Ionization energy: 70 eV
5.5.2.3. Ion source temperature: 230 ^{O}C
5.5.2.4. Interface temperature: 230 ^{O}C
5.5.2.5. Fragment scan range: 40 m/z-150 m/z
5.5.2.6. SIM (m/z): 88, 58, 43 (1,4-Dioxane)
96, 64, 46 (1,4-Dioxane-d8)
5.5.2.7. Solvent cut time: 6 min

5.5.3 Head space sampler conditions

5.5.3.1. Incubation temperature: 90 ^{O}C

5.5.3.2. Incubation time: 45 min

5.5.3.3. Injector temperature: 90 ^{O}C

5.6. Sequence of injection to the GC-MS system

Sequentially inject the prepared solution to GC-MS and record peak area as follows:

5.6.1. System suitability: Inject standard solution, C5 (5.3) to examine the retention time and replicate six injections to determine standard deviation of peak area. The acceptance criteria is as follows

Name	%RSD of peak area (n=6)
1,4-Dioxane	11

5.6.2. Inject C1, C2, C3, C4, C5 and C6 (5.3) respectively for construction of the calibration curve

5.6.3. Inject sample solution

6. CALCULATION

6.1. Plotting calibration curve between concentration and ratio of the peak area of each standard 1.4-Dioxane solutions to the peak area of the internal standard from linear regression equation:

$$y = bx + c$$

When

b = slope

c = intercept

x = concentration of 1,4-Dioxane (µg/mL)

y = ratio peak area

6.2. Calculation 1,4-Dioxane in µg/g by mass, using the formula:

$$\text{1,4-Dioxane (µg/g)} = \frac{\text{concentration of 1,4−Dioxane } \left(\frac{\mu g}{mL}\right) x \ dilution\ factor}{sample\ weight\ (g)}$$

dilution factor = total volume of solution per aliquot volume (ml)

7. REMARKS

7.1. Method validation information

7.1.1. Specificity

PARAMETER	Retention Time (minutes)	m/z
1,4-Dioxane	8.816	88, 58, 43
1,4-Dioxane d8	8.704	96, 64, 46

7.1.2. Repeatability

Concentration	RSD (%)	Requirement
12.5 % or 1.21 µg/g	0.3%	< 7.3
100 % or 10.28 µg/g	1.1%	< 7.3
125 % or 13.09 µg/g	0.2%	< 7.3

7.1.3. Accuracy

Concentration	Recovery (%)	Requirement
12.5 % or 1.21 µg/g	94.6% - 95.7%	80% - 110%
100 % or 10.28 µg/g	99.4% - 101.0%	
125 % or 13.09 µg/g	101.7% - 102.1%	

7.1.4. Linearity

Correlation coefficient (r) = 1.000 (requirement $r \geq 0.995$);

V_{x0} = 1.4% (requirement $V_{x0} \leq 5\%$)

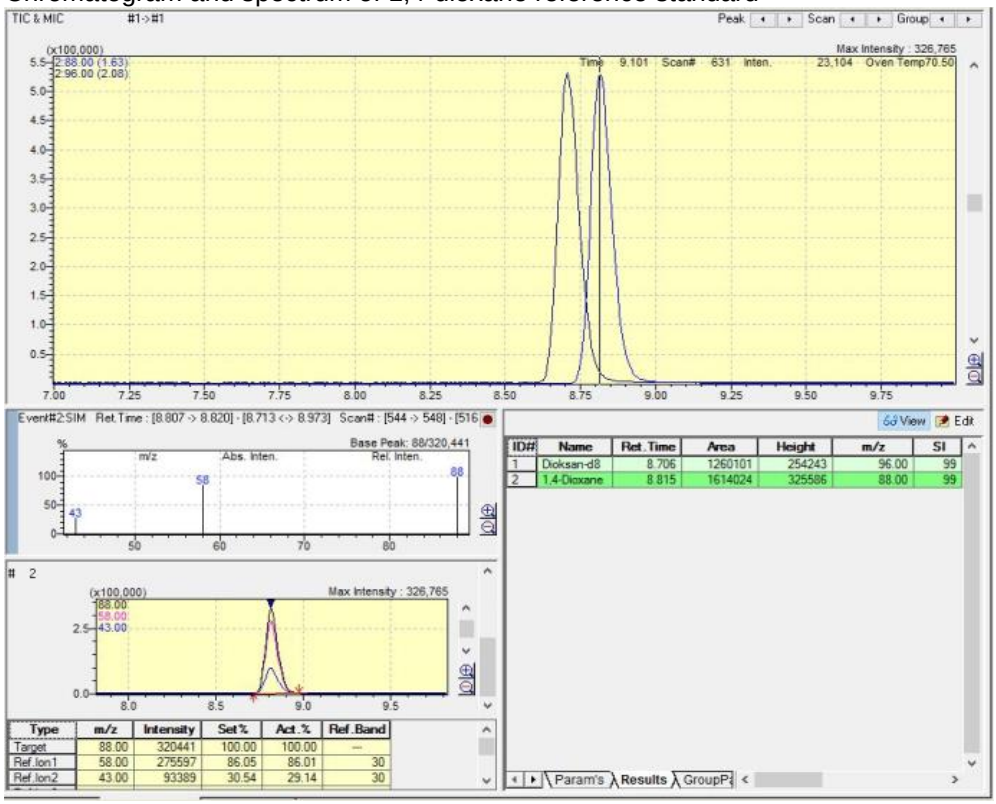
7.1.5. Limit of Quantitation (LOQ) and Limit of Detection(LOD)

LOQ = 0.27 µg/g

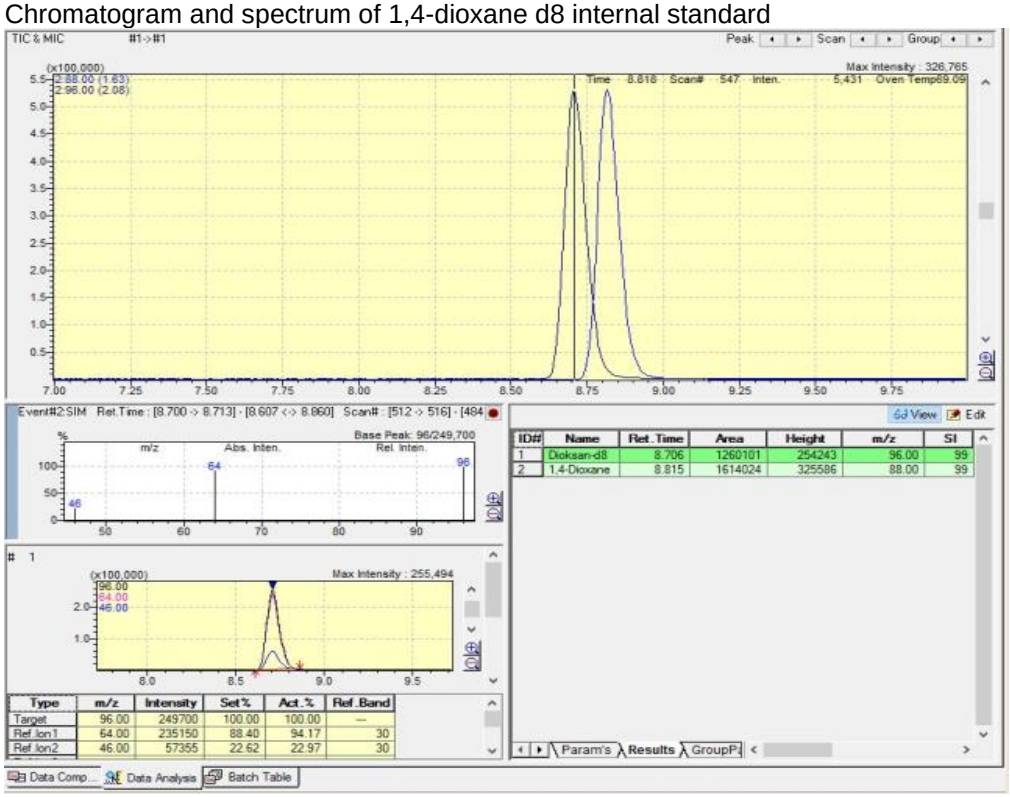
LOD = 0.08 µg/g

7.2. Profile of chromatograms

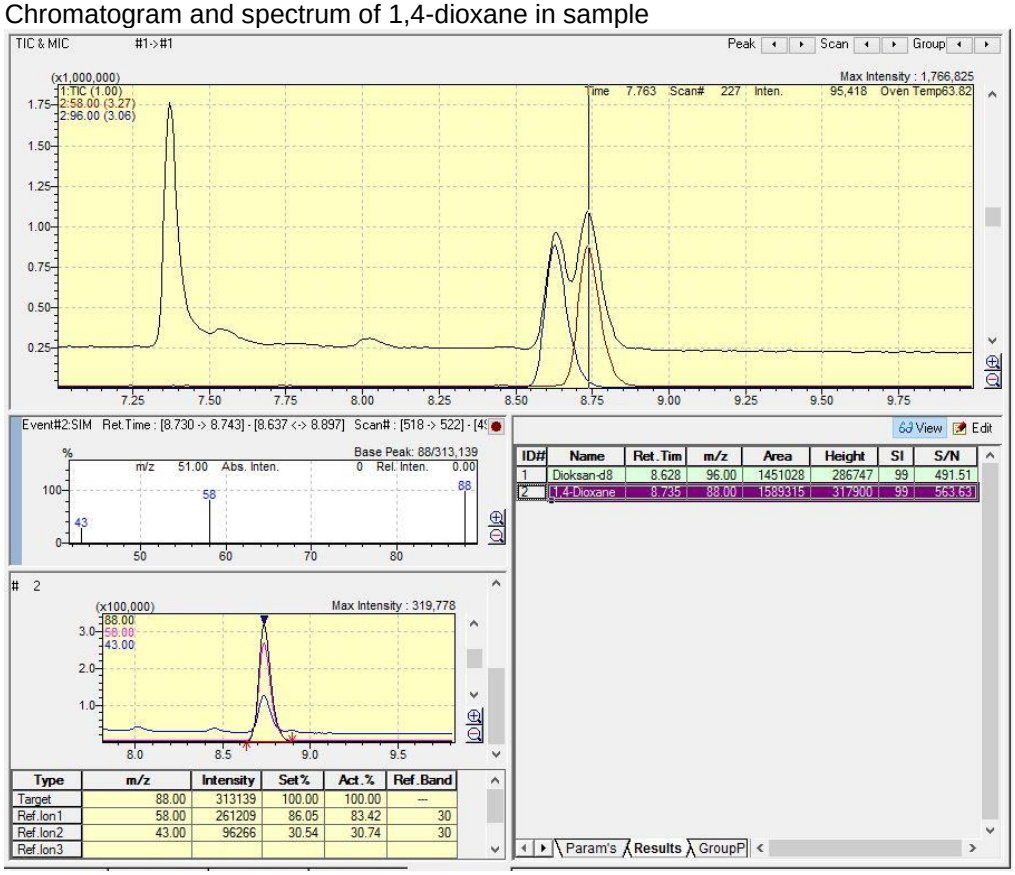
7.2.1. Chromatogram and spectrum of 1,4-dioxane reference standard



7.2.2. Chromatogram and spectrum of 1,4-dioxane d8 internal standard



7.2.3. Chromatogram and spectrum of 1,4-dioxane in sample



Note: The chromatograms were obtained by Shimadzu GCMS-QP 2010 Plus HSS AOC 6000

Disclaimer: The chromatogram images are for illustrative and reference purposes only.

Harmonised method:

Approved by AMS delegates on the 21st ACTLC Meeting, 21 November 2023