



Reference Manual Book 5: Laboratory Policies and Procedures

Part B: Test Method Section

**Method C62.1: Determination of methyl eugenol in cosmetic products by
gas chromatography triple quadrupole mass spectrometry (GC-TQ)**

1 SCOPE

- 1.1 This method describes a general procedure for the determination of methyl eugenol in cosmetic products by gas chromatography triple quadrupole mass spectrometry (GC-TQ).

2 SAFETY

- 2.1 Adequate precautions to avoid undue exposure. Wherever possible, conduct all steps of the analytical process in a well-ventilated fume hood. The analyst must wear protective gloves when handling all working solutions or concentrates.

3 APPLICABLE DOCUMENTS

- 3.1 SOPC-12, Standard Operating Procedures for Balances
- 3.2 Log book LBC-12.2, 12.3, 12.6, 12.7, 12.8, 12.9 or 12.10, Balance Mettler Toledo XS204 / XSR204, or XP205 / XPR205
- 3.3 Log book LBC-111.3, Eppendorf 5810 R Centrifuge
- 3.4 Log book LBC-115, Triple Quadrupole Gas Chromatography Mass Spectrometry (GC-TQ)
- 3.5 SOPC-115, Standard Operating Procedure For Agilent 8890/7010B Triple Quadrupole Gas Chromatography Mass Spectrometry (GC-TQ)

4 DEFINITIONS

- 4.1 N/A

5 REAGENTS AND APPARATUS

5.1 Reagents

- 5.1.1 Ethyl acetate ($C_4H_8O_2$), GCMS grade or equivalent¹, CAS 141-78-6
- 5.1.2 Magnesium Sulfate ($MgSO_4$), anhydrous, $\geq 99\%$ or equivalent¹, CAS 7487-88-9
- 5.1.3 Sodium chloride (NaCl) crystalline or equivalent¹, CAS 7647-14-5
- 5.1.4 Primary second amine (PSA) Agilent or equivalent¹

¹ Where applicable, suitable equivalent reagents and materials may be used



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- 5.1.5 Methyl eugenol 99.9%, AccuStandard or equivalent¹, CAS 93-15-2
- 5.1.6 Methyl eugenol-13C,d3, MedChemExpress or equivalent¹
- 5.1.7 C62-RM-1, cosmetic product free of methyl eugenol

5.2 Labware

- 5.2.1 Graduated centrifuge tubes, 30 mL polypropylene with screw caps
- 5.2.2 Volumetric flasks, 10 and 25 mL
- 5.2.3 GC Vials, 2 mL
- 5.2.4 Pasteur pipettes

5.3 Equipment

- 5.3.1 Glass syringe, Hamilton or equivalent (10 µL, 50 µL, 250 µL, 500 µL & 1000 µL)
- 5.3.2 Vortex shaker, VWR or equivalent¹
- 5.3.3 Centrifuge, Eppendorf 5810R Centrifuge or equivalent¹
- 5.3.4 Solid phase extraction tube, Agilent Bond Elut C18, 500mg/6mL or equivalent¹
- 5.3.5 Fridge below 5°C or equivalent¹

5.4 Analytical Instruments

- 5.4.1 Agilent 8890/7010b GC/TQ, equipped with a capillary column, Agilent HP-5ms, (2) 15 m × 0.25 mm or equivalent¹
- 5.4.2 CombiPAL Autosampler or equivalent¹
- 5.4.3 Analytical balance with a readability of 0.1 mg

6 EXPERIMENTAL PROCEDURE

6.1 Preparation of Standards

6.1.1 Methyl eugenol, stock standard (1000 mg/L)

Weigh approximately 10 mg of analyte to the nearest 0.1 mg, in a 10 mL volumetric flask. Make up to volume with ethyl acetate. Record the concentration taking into account the reference standard purity. Store in the fridge, maintained below 5 °C. Prepare an index card (6.1.6).

6.1.2 Methyl eugenol-C13,d3, stock internal standard (400 mg/L)



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Weigh approximately 10 mg of analyte to the nearest 0.1 mg, in a 25 mL volumetric flask. Make up to volume with ethyl acetate. Record the concentration taking into account the reference standard purity. Store in the fridge, maintained below 5 °C. Prepare an index card (6.1.6).

6.1.3 Methyl eugenol-C13,d3, intermediate internal standard (10 mg/L)

Add the appropriate amount of stock standard to a 25 mL volumetric flask to obtain a final concentration of 10 mg/L. Make up to volume with ethyl acetate. Store in the fridge, maintained below 5 °C. Prepare an index card (6.1.6).

6.1.4 Methyl eugenol Intermediate standard (10 mg/L)

Add the appropriate amount of the stock solution to a 25 mL volumetric flask to obtain a final concentration of 10 mg/L. Make up to volume with ethyl acetate. Store in the fridge, maintained below 5 °C. Prepare an index card (6.1.6).

6.1.5 Working standards

Prepare a series of at least four working standard solutions ranging from 0.005 mg/L to 1.0 mg/L. Make the appropriate dilutions of the intermediate standard in 10 mL volumetric flasks. Add 100 µL of the intermediate internal standard to obtain a concentration of 0.1 mg/L and make up to volume with ethyl acetate. Store in the fridge, maintained below 5 °C. Transfer 1 mL to a 2 mL vial.

6.1.6 Index cards

6.1.6.1 To compensate for any solvent loss due to evaporation, the solutions are weight calibrated. Weigh the solution, record the weight on the index card, date and initial.

6.1.6.2 Store the standard solution in the fridge.

6.1.6.3 Before each use, remove the standard solution from the fridge. Allow the standard solution to reach ambient temperature (~20 °C). Bring the solution up to the last recorded weight using the appropriate fresh solvent.

6.2 Sample Preparation

6.2.1 Accurately weigh a 0.5 g portion to the nearest 0.1 mg. Record the mass. Prepare the sample in triplicates.

6.2.2 Add 100 µL of the methyl eugenol-C13,d3, intermediate internal standard (10 mg/L) for a concentration of 0.10 mg/L.

6.2.3 Add 10 mL of ethyl acetate.



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6.2.4 Cap the flask tightly with a stopper and vortex for about 15-30 sec to homogenize.

6.2.5 Add 2.0 g of MgSO_4 , 0.5 g NaCl , and 0.150 g of PSA. Cap the flask tightly with a stopper and vortex for about 15 sec to homogenize.

6.2.6 Centrifuge for 10 minutes at 3500 rpm.

6.2.7 Elute 6-8 mL onto the SPE C18 by gravity.

6.2.8 Transfer approximately 1 mL of the eluant to a GC vial and inject on the GC-TQ.

6.3 Blank Preparation

6.3.1 Instrument blank: Prepare an instrument blank, by transferring 1 mL of ethyl acetate to a vial. Add 10 μL of the Intermediate internal standard.

6.3.2 Matrix blank: Prepare a matrix blank solution by completing steps 6.2.1 to 6.2.8 with C62.1-RM-1

6.4 Control Preparation

6.4.1 Method control: Prepare one control sample by following the steps in section 6.2 using C62.1-RM-1 spiked with 250 μL of the methyl eugenol intermediate standard (10 mg/L) (6.1.4)

6.4.2 Sample control: Prepare a second control sample by following the steps in section 6.2 using the test sample spiked with 250 μL of the methyl eugenol intermediate standard (10 mg/L) (6.1.4).

6.5 Matrix Standard

6.5.1 Transfer approximately 1 mL of the eluant (6.2.8) to a GC vial and prepare a standard in matrix of 0.25 mg/L equivalent to the spike level.

7 CALIBRATION

7.1 Inject a 1 μL aliquot of each working standard solution into the GC-TQ using the analytical conditions specified in section 8.1.

7.2 Prepare a linear calibration curve of response ratio versus concentration ratio. A correlation coefficient of at least 0.98 must be achieved.



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8 DETERMINATION

8.1 Conduct the analysis on the GC-TQ with the following conditions (C62.1_ME_MRM.m):

Injection Parameters (SIM)		
Injection Mode	Splitless	
Injection Port temperature	250 °C	
Purge Flow & Time	25.0 mL /min	1.0 min
Gas Saver Flow & Time	20 mL/min	2.00 min
Oven Parameters		
Temperature Program	80 °C	2.00 min
25 °C/min	220 °C	1.00 min
30 °C/min	280 °C	5.00 min
MSD Transfer Line Temp	280 °C	
Column Parameters		
GC column	HP-5ms UI	2 x 15 m, 0.25 mm I.D., 0.25 µm film
Column 1 Initial Flow & Mode	0.8 mL /min	Constant Flow
Column 2 Initial Flow & Mode	1.0 mL /min	Constant Flow
PSD Purge	0.5 mL /min	
Detector & Outlet Pressure	MSD	Vacuum
Collision Cell EPC		
Quench Gas He	4 mL /min	
Collision Gas N2	1.5 mL /min	
Mass Spectrometer Parameters		
Tune Parameters	eihs tune	
Electron Multiplier Voltage	Gain factor, 1.0	
Solvent Delay	4.5 min	
Quadrupole Temperature	150 °C	
source Temperature	250 °C	

8.2 Selected ions for each analytes

Compound	Precursor Ion (m/z) MS1 Wide	Product Ion (m/z) MS1 Wide	Dwell time (ms)	Collision Energy
Methyl eugenol	178.0	107.1	50	15
		163.1	50	5
		91.1	50	35
		77.0	50	35



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ISTD Methyl eugenol-13C,d3	182.1	107.1	50	25
		91.1	50	35
		77.0	50	35

- 8.3 Determine the methyl eugenol concentration, in mg/kg, of the instrument blank before and after the analysis of a batch (6.3.2).
- 8.4 Determine the methyl eugenol concentration, in mg/kg, of the control samples (6.4). If the measurements are above of the range of calibration, dilute the control sample with ethyl acetate by the appropriate dilution factor.
- 8.5 Determine the methyl eugenol concentration, in mg/kg, of the samples (6.2.8). If the measurements are above of the range of calibration, dilute the sample with ethyl acetate by the appropriate dilution factor.

9 CALCULATIONS AND REPORTING

- 9.1 Calculate the concentration, in mg/kg, of methyl eugenol in the test sample according to the following equation:

$$\text{Methyl eugenol, mg/kg} = \frac{C_f \times V_f \times D_f}{W_t}$$

Where

C_f = Concentration of methyl eugenol measured by the instrument in the sample solution (mg/L),

V_f = Final volume of sample solution (mL),

D_f = Dilution factor (if required),

W_t = Weight of test sample used (g)

To convert to % (w/w):

$$\text{Methyl eugenol \% (w/w)} = \frac{\text{Methyl eugenol mg/kg}}{10000}$$

- 9.2 Where there is sufficient quantity of a sample and where practical, report the result of analysis as the average of a minimum of three replicate preparations. The precision should meet or exceed the specifications defined in section 11.1.
- 9.3 Where applicable, the average (x.xx) of replicate determinations plus or minus Student's t (for a confidence level of 0.975) times the standard deviation of the mean ($s/\sqrt{n}$) of replicate determinations (s for $n > 2$) will be calculated. Report the result of analysis in the following format.



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Sample no.	Specimen no.	Colour / Description	[Methyl eugenol] _{total}	
			(mg/kg)	% (w/w)
S20xx-0xxxx	A		$x.xx \pm \frac{t_{0.975[95\%]} \times s}{\sqrt{n}}$	$x.xx \pm \frac{t_{0.975[95\%]} \times s}{\sqrt{n}}$

Where:

$t_{0.975(95\%)}$ is the value for t, obtained from Student's T Values Table

$s / \sqrt{n}$ = standard deviation of the mean for the sample

n = number of values in the data set

- 9.4 When no signal is detected, report the result of analysis as less than the limit of quantification of the method, as per section 12.2.

10 QUALITY CONTROL PROCEDURE

Conduct the following quality control procedure concurrently with the analysis of the test sample. This ensures the proper operation of the analytical instrument. It also ensures that the precision and accuracy of the analytical measurements meet the specifications of the method.

- 10.1 If a correlation coefficient of 0.98 (7.2) is not obtained, recalibrate and/or repair the instrument to meet the prescribed operating conditions prior to proceeding with the analysis.
- 10.2 If the method blank concentration is greater than the limit of quantitation (LOQ) of the method for any analyte, repeat the analysis.
- 10.3 Record the concentrations of the control sample in the electronic control chart. Verify that the measurements are within the control limits ($\pm 3 s$). If the control sample results are outside of the control limits, run the calibration standards a second time and re-calculate the results. If the control sample results are still outside of the control limits, repeat the entire analytical procedure. Do not continue testing until the source of the problem is identified and resolved.
- 10.4 If the replicate test results for a given sample are within the repeatability limit of the method, the test result is considered within the limitations of the method.

If any replicate test result for a given sample is not within the repeatability limit of the method, the analyst must consult with the section head before proceeding. It may be necessary to repeat the test method in triplicate, where the quantity of sample is sufficient and where practical. Unless an error in the original test is documented or a result is determined to be an outlier, use all replicates to determine the mean and the standard deviation of the mean for a given sample for a confidence level of 95 %. Records of discussion with the section head must be documented.



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- 10.5 To confirm the presence of an analyte, the sample retention time must be within ± 0.1 min of the standard retention time². Acceptable chromatography (symmetry, peak shape) must also be observed. In addition, compare the sample mass ion ratios to the standard mass ion ratios to confirm they are in the acceptable range. If any of these conditions are not met, the compound is not confirmed to be present in the sample. If there are interferences observed with the compound peak (ex. peak splitting, peak fronting, peak tailing) and/or its quantifier/qualifier ion ratios deviate slightly from the standard ion ratios, consult the result with the section head. It may be necessary to report the compound as inconclusive on the test report and record its concentration as determined by the instrument on the result datasheet. All records of discussion with the section head must be documented.

11 PRECISION AND BIAS

11.1 Repeatability

The repeatability of the method was determined by conducting seven successive analyses on nine different matrices. The repeatability limit at 95% confidence level is calculated as follows:

$$\text{Repeatability limit} = 1.960 \times \sqrt{2} \times \text{CV} = 27 \%$$

Where: CV = Coefficient of variation (%)

11.2 Reproducibility

The reproducibility will be presented in a revised copy of the method.

11.3 Bias

There is no appreciable bias for this method.

12 LIMITS OF DETECTION AND QUANTIFICATION

- 12.1 The limit of detection (LOD) and limit of quantification (LOQ) of the instrument are presented in the following table.

Compound	Instrument LOD (mg/L)	Instrument LOQ (mg/L)
Methyl eugenol	0.002	0.008

- 12.2 The LOD and LOQ of the method are presented in the following table.

² A retention time shift of ± 0.1 min was observed in one sample during method validation, including the internal standard. To confirm the presence of methyl eugenol, a known amount was added into the sample extract to confirm



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Compound	Method LOD (mg/kg)	Method LOQ (mg/kg)
Methyl eugenol	0.02	0.05

13 REFERENCES

- 13.1 Ritchot, N., Determination of a series of fragrance allergens in cosmetics by gas chromatography mass spectrometry. PSL Report P2021-00027 (2023). Health Canada.
- 13.2 Changeliang Ke, Qi Liu, Liudong Li, Jiewen Chen, Xunuo Wang, Ke Huang. Simultaneous determination of eugenol, isoeugenol and methyl eugenol in fish fillet using gas chromatography coupled to tandem mass spectrometry. Journal of Chromatography B, 1031 (2016) 189-194
- 13.3 Ritchot, N., Method Development: C62.1 Determination of Methyl Eugenol in Cosmetic Products by GC-TQ. PSL Report P2025-00034 (September 2025). Health Canada.