The International Fragrance Association

21 January 2025

IFRA Analytical Method for Parabens

By the IFRA Analytical Working Group (AWG)

1. Introduction

1.1. Method principle

This protocol describes the method for the analysis of 9 parabens in fragrance raw materials using LCMS.

Detection is performed by MS-MS in MRM (Multiple Reaction Monitoring) mode. For each paraben, two transitions are scanned. Retention time and MRM-ratios are used for identification. Quantification is performed using internal standard calibration. The limit of quantification (LOQ) is in the range of 0.5 – 100 mg/kg in sample (with 20x dilution).

Appendix 1 displays the analytes included in this analysis with expected retention times

1.2. Scope

This protocol is applicable to samples including fragrance raw materials.

1.3. Performance

All analytes included in this analysis method and reporting limits are displayed in Appendix 1.

2. Materials and supplies

Table 1: Reagents and Supplies

Reagents	CAS #	Potential Suppliers / supplier codes
Acetone		Biosolve / 0001038402BS
MilliQ water (or equivalent)		
Methanol		Biosolve / 1368 7802
Ammonium acetate		Biosolve / 01244156
Methyl paraben	99-76-3	Sigma-Aldrich / 47889
Ethyl paraben	120-47-8	Sigma-Aldrich / 111988-5G
Propyl paraben	94-13-3	Sigma-Aldrich / P53357-5G
Isopropyl paraben	4191-73-5	Sigma-Aldrich / 05828-25MG
Butyl paraben	94-26-8	Sigma-Aldrich / 54680-50G-F
iso-Butyl paraben	4247-02-3	Sigma-Aldrich / 715077-25G
Pentyl paraben	6521-29-5	Sigma-Aldrich / 90744-25MG
Benzyl paraben	94-18-8	Sigma-Aldrich / 380709-100G
Phenyl paraben	17696-62-7	Sigma-Aldrich / 90668-25MG

D4-Ethyl paraben	1219795-53-5	Ritmeester / D-7237
D4- iso-Butyl paraben	1219805-33-0	Ritmeester / D-7236
D4- Benzyl paraben	1219805-81-8	Ritmeester / D-7234
D4-Methyl paraben	362049-51-2	Ritmeester / D-5457
D4-Propyl paraben	1219802-67-1	Ritmeester / D-7114
D4-Pentyl paraben	1219798-66-9	Ritmeester / D-7238

Note: The Suppliers and the associated codes are purely optional sources; other sources of these analytes are available but aspects such as material purity etc. should be checked before use.

Table 2: Overview of general equipment

General equipment
Pipette, 1-10 ml (green)
Pipette, 100-1000 µl (blue)
Pipette, 10-100 µl (yellow)
Pipette, 5-20 µl (yellow)
Pipette, 500-5000 µl (purple)
Balance, readability 0.1 mg

Table 3: Analysis equipment required

Analysis equipment
Triple quadrupole mass-spectrometer, e.g. Agilent 6470 Triple Quad LC/MS (G6470A)
LC system, e.g. Agilent Technologies 1290 Infinity II existing of :
G7120A 1290 High Speed Pump
G7167B 1290 Multisampler
G7116B 1290 MCT

Note: The analytical system and associated conditions refer to an Agilent based LCMS system. Other vendors systems may also be used but care should be taken when transferring parameters as some of these may not directly translate to other manufacturers' platforms.

3. Reagents

3.1. Reagents

3.1.1. Mobile phase A

Mobile phase A: 5mM Ammonium Acetate in water\MeOH (90/10)

- Add between 0.3758 g and 0.3950 g Ammonium Acetate to a 1L flask
- Add 500 ml MilliQ water (or equivalent)
- Dissolve the Ammonium Acetate well
- Add 100 ml MeOH
- Add MilliQ water (or equivalent) to 1L mark
- Homogenize and label with date of preparation and/or expiry date

3.1.2. Mobile phase B

Mobile phase B: 5mM Ammonium Acetate in MeOH

- Add between 0.3758 g and 0.3950 g Ammonium Acetate to a 1L flask
- Add 500 ml methanol
- Dissolve Ammonium Acetate well
- Add methanol to 1L mark
- Homogenize and label with date of preparation and/or expiry date

3.1.3. Needle wash solution

Needle wash solution is a mix of methanol, water and iso-propanol (50/40/10):

- Add 500 ml of methanol to a 1L bottle
- Add 400 ml iso-propanol
- Add 100 ml water
- Homogenize before use

3.2. Standards

3.2.1. Stock Standard solutions (5000 mg/l)

The individual parabens shown in table 4 below are prepared separately to be added to stock standard solution 1 (100 mg/l).

Accurately weigh 50 mg ± 1 mg of each target material into a 10 ml volumetric flask and add methanol to volume.

Table 4: Individual parabens covered by method

Name
Methyl paraben
Ethyl paraben
Propyl paraben
Isopropyl paraben
Butyl paraben
iso-Butylparaben
Pentyl paraben
Benzyl paraben
Phenyl paraben

3.2.2. Stock Standard solution 1: 100 mg/l

This solution is prepared from the individual stock standard solutions (see paragraph 3.2.1.).

- Add 200 µl of each stock standard solution from 3.2.1 above to a 10 ml measuring flask
- Dilute to volume with MeOH and homogenize

3.2.3. Intermediate Standard Solution 1: 20.0 mg/l

From Stock Standard Solution 1, prepare the stock solution used to produce the 4 upper calibration samples as follows:

- Add 2.0 mL from 100 mg/l Stock Standard Solution 1 to a 10ml volumetric flask
- Dilute to volume with MeOH

3.2.4. Dilution Standard Solution 2: 1.0 mg/L

From Stock Standard Solution 1, prepare the stock solution used to produce the 3 lower calibration samples as follows:

- Add 0.1 mL from 100 mg/l Stock Standard Solution 1 to a 10ml volumetric flask
- Dilute to volume with MeOH

3.2.5. Internal standard solution (Stock A)

Separate Internal stock solutions are prepared as follows to provide the concentrations listed in table 5 below for the Internal Standard Stock solutions.

- Weigh exactly 20 mg ± 1 mg in a 10 ml volumetric flask
- Add MeOH to volume
- For the Mix I.S. solution, pipette 1 ml of each of these and dilute to 10 ml with methanol to derive the concentrations as shown in table 6
- For the final working I.S. solution, pipette 0.25 ml of Mix I.S. solution (200 mg/l) and dilute to 10 ml with methanol to derive the individual concentrations of 5 mg/l as shown in table 7

Table 5: Parabens Internal Stock Solutions

#		Weight (mg)	Total volume in MeOH (ml)	Final concentration (mg/l)
1	D4-Ethylparaben	20	10	2000
2	D4- iso-Butylparaben	20	10	2000
3	D4-Benzylparaben	20	10	2000
4	D4-Methylparaben	20	10	2000
5	D4-Propylparaben	20	10	2000
6	D4-Pentylparaben	20	10	2000

Table 6: Parabens Internal Standards – MIX Solution concentrations

#		Conc. Stock A (mg/l)	Volume Stock solution A (ml)	Total dilution volume (ml)	Final concentration (mg/l)
1	D4-Ethylparaben	2000	1	10	200
2	D4- iso-Butylparaben	2000	1		200
3	D4- Benzylparaben	2000	1		200
4	D4-Methylparaben	2000	1		200
5	D4-Propylparaben	2000	1		200
6	D4-Pentylparaben	2000	1		200

Table 7: Parabens Internal Standards – Working Solution concentrations

CID		Conc. MIX solution (mg/l)	Volume MIX solution (ml)	Total dilution volume (ml)	Final concentration (mg/l)
1586	D4-Ethylparaben	200	0.25	10	5
1587	D4- iso-Butylparaben	200			5
1588	D4- Benzylparaben	200			5
1615	D4-Methylparaben	200			5
1616	D4-Propylparaben	200			5
1617	D4-Pentylparaben	200			5

3.3. Calibration preparation

Standards are made directly in 1 ml autosampler vials once a week following the dilution regime shown in table 8 and, after homogenization, are ready for injection.

Table 8: Autosampler vials with calibration solution and Internal Standard (IS) volumes, plus MeOH volume applied to normalize total volume

Vial number	Conc. (mg/l)	Vol. st @ 1 mg/l (µl)	Vol. st @ 20 mg/l (µl)	Vol. IS 5 mg/l (µl)	Vol. MeOH (µl)
1	0	-	-	100	900
2	0.025	25	-	100	875
3	0.1	100	-	100	800
4	0.5	-	25	100	875
5	1	-	50	100	850
6	2.5	-	125	100	775
7	5	-	250	100	650

4. Samples

4.1. Samples

All samples are stored in a cold room at 10°C before and after analysis.

4.2. Procedural blank

Methanol is used as a procedural blank. If the sample needs to be filtered to remove solid matter, the procedural blank needs to be filtered as well according to the same procedure.

5. Experimental

5.1. Sample preparation

This chapter describes the procedures for sample preparation. Samples are by default analysed following a 20x dilution in methanol:

- Weigh 50 mg of sample in 1 ml gc-vial
- Add 100 µl internal standard solution (5 mg/l)
- Add 850 µl MeOH
- Homogenize
- Sample is ready for injection

For concentrations found exceeding 100 mg/kg in sample, the sample must be further diluted prior to reanalysis.

5.2. Analytical method

The settings of the analytical equipment are described in the method file which is displayed in Appendix 2 Analytical method.
Note: When starting the analysis equipment, use the latest version of the acquisition method.

The method file is saved on the computer of the analysis equipment in the appropriate folder (e.g., for an Agilent based system this would be: C:\Masshunter\methods\TQMS3_PARABEN_Prot162_2142A.m).

5.3. Sample series and data storage

Samples are analysed in a bracket arrangement consisting of a maximum of 10 samples and a procedural blank.

After each series of 10 samples, calibration standard level 5 is reanalysed as a quality control standard.

The recommended sequence for the analysis of samples using the method described in Section 5.2 above is shown in table 9 below.

Table 9: Bracket Sample Analytical Sequence overview

Sample	Identification
Standard 5	Equilibrating column
Standard 5	Equilibrating column
Standard 5	Equilibrating column
Standard 0	
Standard 1	
Standard 2	Cal level 2
Standard 3	Cal level 3
Standard 4	Cal level 4

Sample	Identification
Standard 5	Cal level 5
Standard 6	Cal level 6
Standard 7	Cal level 7
Blank	
Sample 1	
Sample 2	
Sample 3	
Sample 4	
Sample 5	
Sample 6	
Sample 7	
Sample 8	
Sample 9	
Sample 10	
Standard 5	'QC Standard'

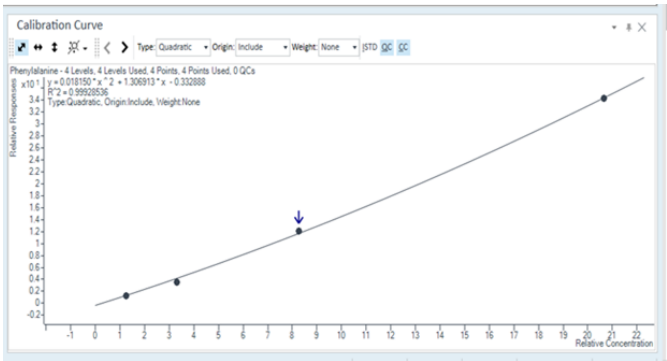
6. Quantification and quality control

6.1. Data interpretation

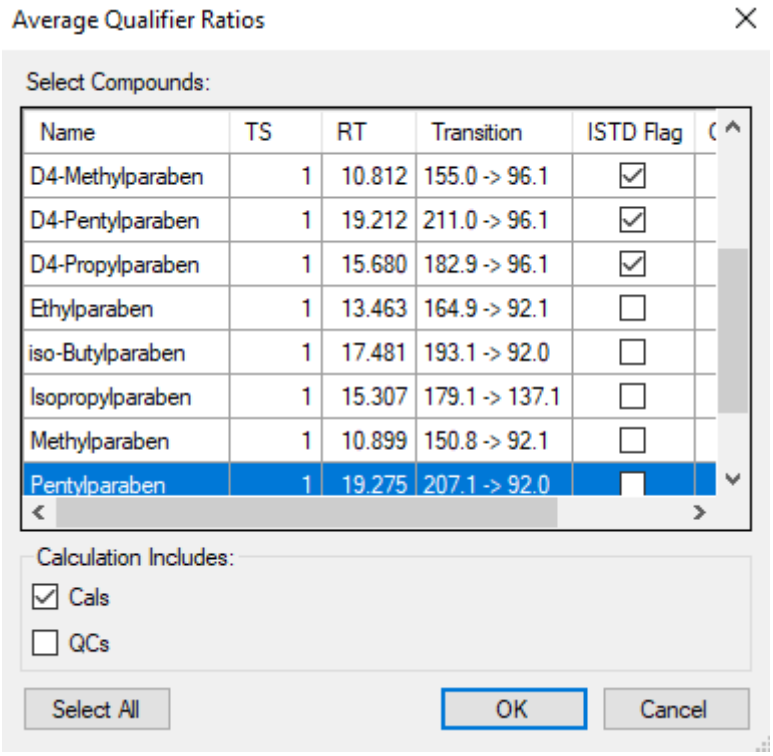
Follow the instructions below to create a result report.

Note: This is specific to Agilent Masshunter processing software; other vendors systems will be different, but this adds an illustrative sequence.

- Open Masshunter Quantitative analysis software "QQQ Quantification"
- Create a new batch: Home > New Batch
- Add all samples to the created batch
- Change under "SampleType" from "Sample" to "Cal" for Standards 2 to 7
- Open quantitation method "PARABEN": Method > Open > Open Method from Existing Batch
- Update retention times using Standard level 6: Update Retention Times
- Perform calibration by selecting under calibration button "Replace calibration"
    - Calibration settings: Type: Quadratic, Origin: Include, Weight: 1/X
    - Calibration line should have minimum R^2 = 0.995:



- Update qualifier ratio's using Standard level 2 – 7: Method > Edit > Update > Average Qualifier Ratios > select "Cals":

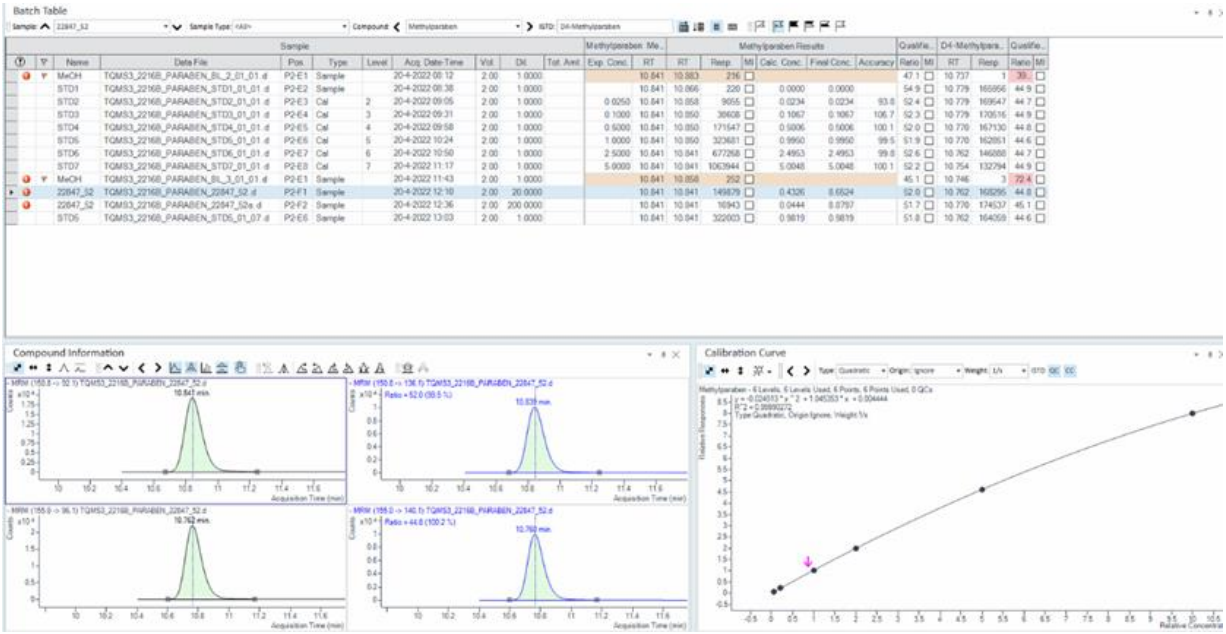


- Exit Method and apply to batch (Analyse)
- Adjust Dilution factor for samples: Default dilution factor = 20
- Quantitate batch under button "Quantitate"
- Check for correct integration by View > Compounds-at-a-Glance:
- Display "Flat table" and "Display Single Compound /Sample in batch table"

6.2. Sample quantification

The first, most sensitive MS/MS transition (SRM1) should be used for quantitation or that transition which offers the optimum signal/noise ratio at the lower level of the calibration curve.

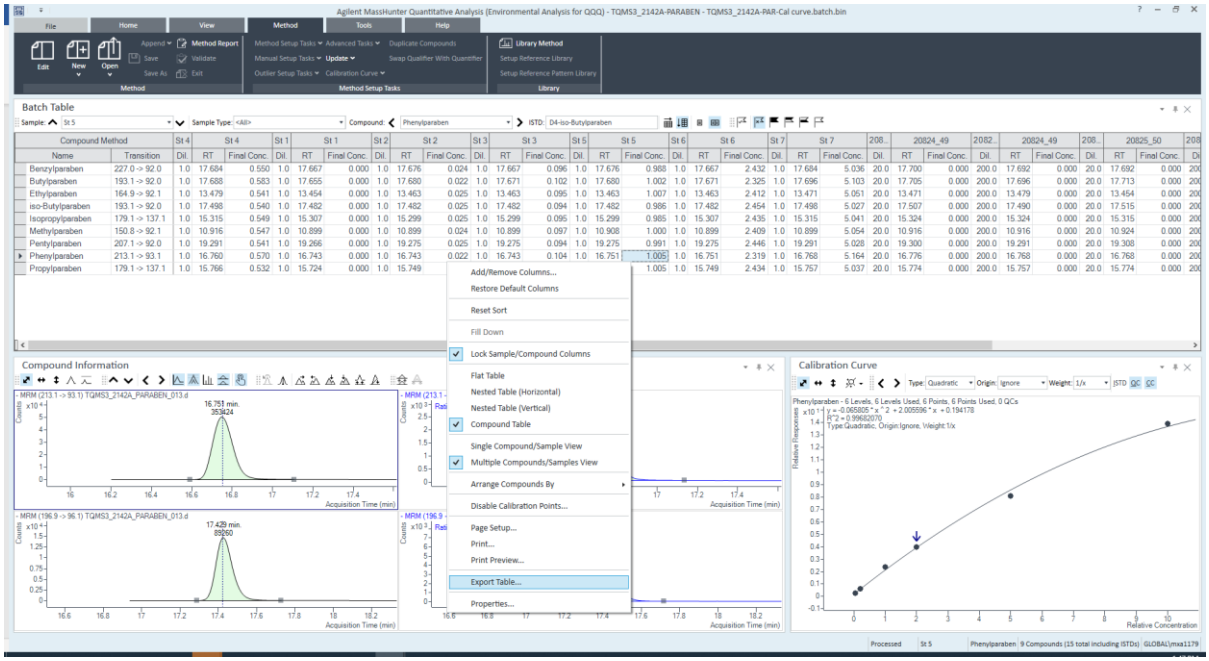
The "Final concentration" is automatically calculated when the dilution factor is included in the batch table:



Note: If one or more levels are above the higher reporting limit the sample should be prepared again according to Section 5.1

6.3. Reporting

Display “Compound table” and Display “Multiple Compounds /Samples in batch table”:



Appendix 1: List of analytes

#	Name	RT (min)	CAS	Lower RL (mg/kg)	Higher RL (mg/kg)
1	Methylparaben	10.64	99-76-3	0.5	100
2	Ethylparaben	13.17	120-47-8	0.5	100
3	Isopropylparaben	15.03	4191-73-5	0.5	100
4	Propylparaben	15.48	94-13-3	0.5	100
5	Phenylparaben	16.56	17696-62-7	0.5	100
6	iso-Butylparaben	17.28	4247-02-3	0.5	100
7	Butylparaben	17.48	94-26-8	0.5	100
8	Benzylparaben	17.46	94-18-8	0.5	100
9	Pentylparaben	19.11	6521-29-5	0.5	100
10	D4-Methylparaben	10.57	362049-51-2		
11	D4-Ethylparaben	13.12	1219795-53-5		
12	D4-Propylparaben	15.43	1219802-67-1		
13	D4-Iso-butylparaben	17.21	1219805-33-0		
14	D4-Benzylparaben	17.41	1219805-81-8		
15	D4-Pentylparaben	19.03	1219798-66-9		

Appendix 2: Analytical method

Pump	
Flow (ml/min)	0.200
Mobile phase A	Water
Mobile phase B	Acetonitrile
Gradient	Time (min) / A% / B%: 1 / 90 / 10; 20 / 15 / 85; 21 / 15 / 85; 22 / 90 / 10; 26 / 90 / 10
Column temp. (°C)	30
Autosampler	
Draw speed (µl/min)	100
Eject speed (µl/min)	400
Injection volume (µl)	2
MS Source	
Gas temp (°C)	340
Gas Flow (l/min)	10
Nebulizer (psi)	50
Sheath Gas heater (°C)	375
Sheath gas flow (l/min)	10
Capillary (V)	3000
Polarity	Negative (-)

MS parameters							
Compound	**ISTD**	**Prec Ion**	**Prod ion**	**Frac (V)**	**CE (V)**	**Ret Time (min)**	**Polarity**
Benzylparaben	No	227.016	136.03	110	16	17.47	Negative
Benzylparaben	No	227.016	92.04	110	28	17.47	Negative
Butylparaben	No	193.071	136.03	110	16	17.44	Negative
Butylparaben	No	193.071	92.04	110	28	17.44	Negative
D4-Benzylparaben	Yes	230.949	140.05	110	16	17.4	Negative
D4-Benzylparaben	Yes	230.949	96.05	110	28	17.4	Negative
D4-Ethylparaben	Yes	168.927	141.11	100	12	13.12	Negative
D4-Ethylparaben	Yes	168.927	96.06	100	24	13.12	Negative
D4-iso-Butylparaben	Yes	196.929	140.05	110	16	17.19	Negative
D4-iso-Butylparaben	Yes	196.929	96.06	110	28	17.19	Negative

D4-Methylparaben	Yes	154.96	140.09	90	12	10.59	Negative
D4-Methylparaben	Yes	154.96	96.08	90	24	10.59	Negative
D4-Pentylparaben	Yes	211.01	141.07	150	16	19.03	Negative
D4-Pentylparaben	Yes	211.01	96.07	150	28	19.03	Negative
D4-Propylparaben	Yes	182.94	140.06	100	16	15.42	Negative
D4-Propylparaben	Yes	182.94	96.08	100	24	15.42	Negative
Ethylparaben	No	164.915	137.06	100	12	13.19	Negative
Ethylparaben	No	164.915	92.05	100	24	13.19	Negative
iso-Butylparaben	No	193.071	136.03	110	16	17.26	Negative
iso-Butylparaben	No	193.071	92.04	110	28	17.26	Negative
Isopropylparaben	No	179.056	137.05	100	12	15	Negative
Isopropylparaben	No	179.056	92.05	100	28	15	Negative
Methylparaben	No	150.828	136.05	90	12	10.67	Negative
Methylparaben	No	150.828	92.05	90	24	10.67	Negative
Pentylparaben	No	207.103	137.03	150	16	19.09	Negative
Pentylparaben	No	207.103	92.04	150	28	19.09	Negative
Phenylparaben	No	213.103	93.05	90	20	16.56	Negative
Phenylparaben	No	213.103	65.05	90	64	16.56	Negative
Propylparaben	No	179.056	137.05	100	16	15.49	Negative
Propylparaben	No	179.056	92.04	100	24	15.49	Negative

V1: published on 21 January 2025