1. SCOPE AND FIELD OF APPLICATION

This method specifies a procedure for identification and determination of 2-phenoxyethanol, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, and butyl 4-hydroxybenzoate in cosmetic products.

2. PRINCIPLE

The sample is acidified by adding sulfuric acid and then suspended in a mixture of ethanol and water. After gently heating the mixture to melt the lipid phase to promote quantitative extraction, the mixture is filtered. The preservatives in the filtrate are determined by reversed phase HPLC using benzophenone (or isopropyl 4-hydoxybenzoate or ethylparaben) as the internal standard.

3. REAGENTS

All reagents must be of analytical purity and suitable for HPLC where appropriate, except for RS.

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

3.1 Ethanol, absolute
3.2 2-Phenoxyethanol Reference Standard (RS)
3.3 Methyl 4-hydroxybenzoate (methylparaben) RS
3.4 Ethyl 4-hydroxybenzoate (ethylparaben) RS
3.5 n-Propyl 4-hydroxybenzoate (propylparaben) RS
3.6 Isopropyl 4-hydroxybenzoate (isopropylparaben) RS
3.7 n-Butyl 4-hydroxybenzoate (butylparaben) RS
3.8 Benzophenone
3.9 Tetrahydrofuran
3.10 Methanol
3.11 Acetonitrile
3.12 Sulfuric acid solution (H2SO4) 2 M
3.13 Ethanol/water mixture, 9:1 (v/v)
3.14 Internal standard solution:
  3.14.1 Accurately weigh 0.125 g benzophenone or isopropylparaben or ethylparaben.
  3.14.2 Transfer to a 250 mL volumetric flask, dissolve and make up to volume with ethanol/water mixture.
3.15 Mobile phase:
  Tetrahydrofuran/water/methanol/acetonitrile mixture, 5:60:10:25 (v/v)
3.16 Preservative stock solution
  3.16.1 Weigh accurately 0.05 g of methyl, ethyl, propyl, butyl 4-hydroxybenzoate RS and 0.2 g of 2-phenoxyethanol RS and mix them into a 100 mL volumetric flask, respectively.
  3.16.2 Add 50 mL of ethanol/water mixture 9 : 1 (v/v), shake to dissolve it.
  3.16.3 Add ethanol/water mixture 9 : 1 (v/v) to volume, and mix.
  Kept in a refrigerator, they are stable for one week.
3.17 Standard preservative solutions
  3.17.1 Pipette 20.0 mL, 10.0 mL, 5.0 mL, 2.0 mL and 1.0 mL of preservative stock solution into each a 50 mL volumetric flask, respectively.
  3.17.2 Add 10.0 mL of internal standard solution into every flask,
  3.17.3 Add 1.0 mL of H_2SO_4 2 M and shake to homogenize it,

3.17.4 Add ethanol/water mixture 9 : 1 (v/v) to volume, and mix.

3.17.5 Filter through 0.45 µm membrane filter into HPLC injection vials.

These solutions should be freshly prepared.

4. APPARATUS

Normal laboratory equipment, and:

4.1 Water bath, capable of maintaining a temperature of 60 °C

4.2 High performance liquid chromatograph with a UV-detector, wavelength 280 nm

4.3 Analytical column:

Stainless steel, 25 cm x 4.6 mm i.d., packed with Nucleosil C18 (particle size: 5 µm) or equivalent (see 7.1)

4.4 100 mL glass tubes with screw cap or equivalent

4.5 Boiling chips, carborendum, size 2 to 4 mm, or equivalent

4.6 PVDF with glass filter or HVLP membrane filter pore size 0.45 µm, or equivalent

4.7 Centrifuge

5. PROCEDURE

5.1 Sample preparation (Make duplicate solutions)

5.1.1 Weigh accurately 1 g of sample into a 125-mL Erlenmeyer flask (with a screw cap when available).

5.1.2 Add 1.0 mL of H_2SO_4 2 M, 40.0 mL of ethanol/water mixture 9 : 1 (v/v), 1 g of boiling chips and 10.0 mL of internal standard solution.

5.1.3 Shake vigorously for 1 minute until homogeneous.

5.1.4 Place in water bath at (60 ± 1)°C for 5 minutes, cool the Erlenmeyer flask under running cold water and store in refrigerator for 1 hour, and let it cool to room temperature (about 10 -15 mins), when necessary.

5.1.5 Filter the solution through a membrane filter (0,45 µm) - after centrifugation when necessary - into a 125-mL Erlenmeyer flask (with a screw cap when available).

5.1.6 Transfer about 2 mL filtrate into a 5 mL sample vial.

5.1.7 Perform determination of the filtrate by HPLC within less than 24 hours.

5.2 High-performance liquid chromatography (HPLC)

5.2.1 Chromatographic conditions

5.2.1.1 Mobile phase: tetrahydrofuran/ water/ methanol/ acetonitrile mixture, 5: 60: 10: 25 (v/v)

5.2.1.2 Flow rate: 1.5 mL /minute

5.2.1.3 Detection wavelength: 280 nm

5.2.2 Calibration

Inject 20 µL of each of the standard preservative solutions.

5.2.2.1 From the chromatograms obtained determine the ratios of the peak heights (or areas) of the standard preservative solutions to the peak height (or area) of the internal standard.

5.2.2.2 Plot a curve for each preservative relating these ratios to the concentrations of the standard solutions.

5.2.2.3 Perform linear calibration for each of the preservatives

5.2.3 System suitability

5.2.3.1 Inject 6 times 20 µL of the internal standard and/or standard solutions (tolerance: ≤ 2%)

5.2.3.2 Determine the asymmetric factor A_S = b/a, (see further for details).

5.2.4 Determination

5.2.4.1 Inject 20 µL of the sample solution into the chromatograph.

5.2.4.2 Record the chromatogram and measure the peak heights (or area).

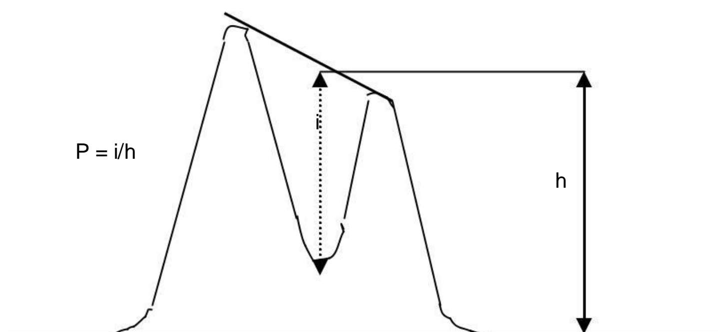
5.2.4.3 Calculate the ratios of the peak heights (or areas) of the investigated preservatives to the peak height (or areas) of the internal standard.

5.2.4.4 Ascertain that for the standard solutions used in the calibration procedure a linear response is obtained.

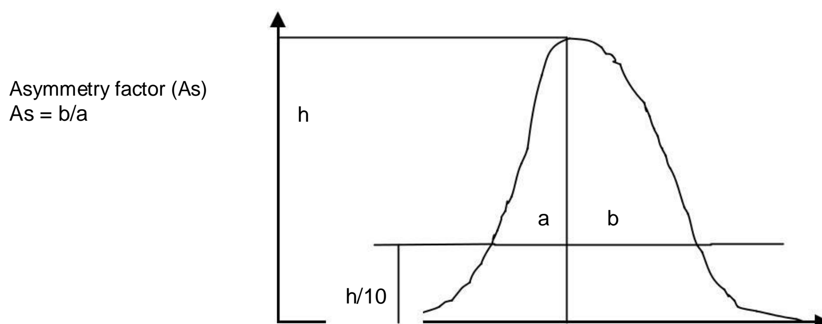
5.2.4.5 If the peak area of the sample is too low or too high, increase or decrease the amount of the sample so that the peak area falls within the calibration range.

5.2.4.6 Ascertain whether the chromatograms obtained for a standard solution and the sample solution meet the following requirements:

- the peak separation of the worst separated pair shall be at least 0,90. (For definition of peak separation, see Figure)



- if the required separation is not achieved, either a more efficient column should be used, or the mobile phase composition should be adjusted until the requirement is met.
- the asymmetry factor A_S of all peaks obtained shall range between 0.9 to 1.5. (For definition of the peak asymmetry factor, see Figure). To record the chromatogram for the determination of the asymmetry factor a chart speed of at least 2 cm/minute is recommended.



- A steady baseline shall be obtained.
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6. CALCULATION

Use the calibration curve and the ratios of the peak heights (or areas) of the investigated preservatives to the peak height (or area) of the internal standard to calculate the concentration of the preservatives in the sample solution.

Calculate the 2-phenoxyethanol, methyl 4-hydroxybenzoate, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate and w_i , as percentage by weight (% w/w), using the formula:

$$\% \ w_i \ (w/w) = b_i * 51 / (10000 \times a)$$

in which:
b_i = the concentration (µg/mL) of preservative i in the test solution as read from the calibration curve a = the weight (g) of sample

7. REMARKS

7.1 Stationary phase

- The retention behaviour of the solutes in HPLC determinations is strongly dependent on the type, the brand and the history of the stationary phase. Whether a column can be used for the separation of the preservatives under examination, can be concluded from the results obtained for standard solutions (see 5.2.4). In addition to the proposed column packing material Hypersil ODS, Zorbax ODS and Shimpack VpODS. were also found to be suitable.
- Alternatively, the recommended mobile phase composition can be optimised in order to obtain the required separation.

7.2 Method validation information

7.2.1 Specificity

PARAMETER	Retention Time (minutes)	Resolution	Requirement
a. 2-Phenoxy-ethanol	4.40	-	Resolution ≥ 1. 5
b. Methyl-paraben	5.04	2.34	
c. Ethyl-paraben	7.87	7.86	
d. Propyl-paraben	13.92	11.80	
e. Butyl-paraben	26.37	15.09	
f. Benzophenone	28.86	2.35	

7.2.2 Repeatability

PARAMETER	RSD (%)	Horrat	Requirement
a. 2-Phenoxy-ethanol	1.383	0.27	Horrat ≤ 2.0
b. Methyl-paraben	1.417	0.22	
c. Ethyl-paraben	1.470	0.23	
d. Propyl-paraben	1.588	0.25	
e. Butyl-paraben	1.499	0.23	

7.2.3 Accuracy

PARAMETER	Recovery (%)	Requirement
a. 2-Phenoxy-ethanol	104.95	Recovery = (90.0 - 110.0)%
b. Methyl-paraben	105.59	
c. Ethyl-paraben	105.60	
d. Propyl-paraben	105.56	
e. Butyl-paraben	105.48	

7.2.4 Range and Linearity

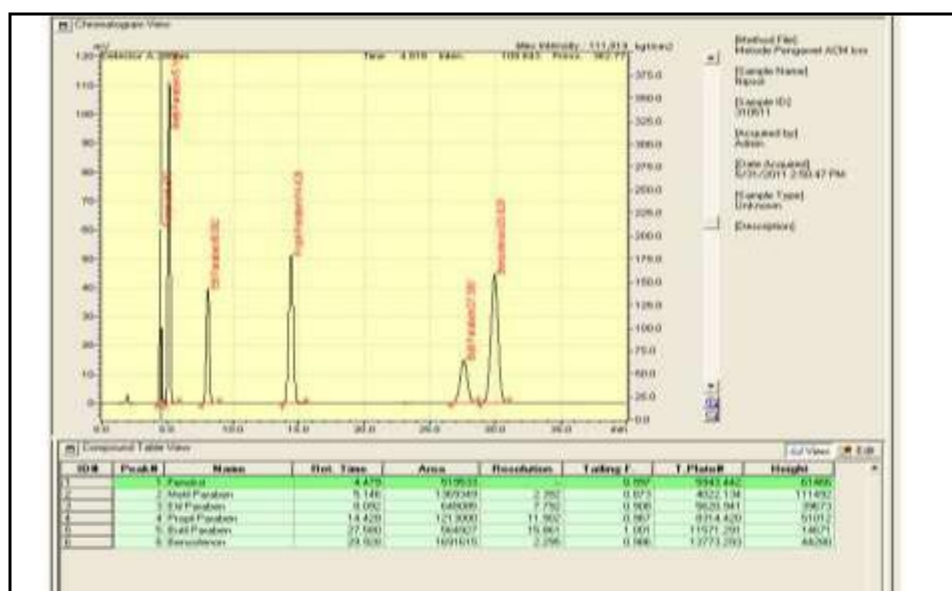
PARAMETER	Range (ppm)	Correlation coefficient (r)	V_{xo}	Requirements of r and V_{xo}
a. 2-Phenoxy-ethanol	40 - 400	0.99959	1.2	$r \geq 0.995$ $V_{xo} \leq 5.0$
b. Methyl-paraben	10 - 100	0.99973	1.0	
c. Ethyl-paraben	10 - 100	0.99980	0.8	
d. Propyl-paraben	10 - 100	0.99982	0.8	
e. Butyl-paraben	10 - 100	0.99982	0.8	

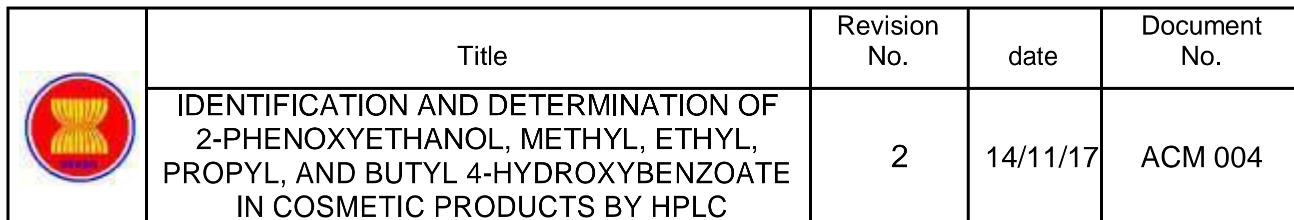
7.2.5 Limit of Quantitation (LOQ)

PARAMETER	Result (µg/g)
a. 2-Phenoxy-ethanol	3.69
b. Methyl-paraben	2.17
c. Ethyl-paraben	1.95
d. Propyl-paraben	0.94
e. Butyl-paraben	7.07

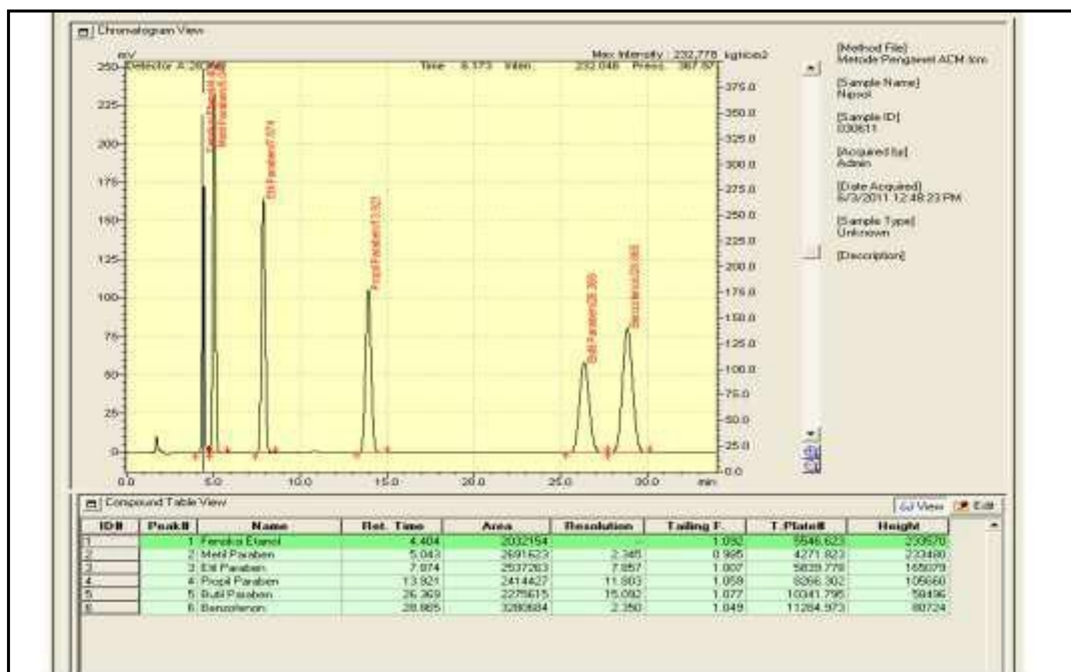
7.3 Profile of Chromatograms

7.3.1 Chromatogram of Standard Solution





7.3.2 Chromatogram of Spiked Sample Solution



ID#	Peak#	Name	Ret. Time	Area	Resolution	Tailing F.	T.Plate#	Height
1	1	Fenoksi Etanol	4.404	2032154	--	1.092	5546.623	233570
2	2	Metil Paraben	5.043	2691623	2.345	0.985	4271.823	223480
3	3	Etil Paraben	7.874	2537263	7.857	1.007	5839.778	165079
4	4	Propil Paraben	13.921	2414427	11.803	1.059	8266.302	105660
5	5	Butil Paraben	26.369	2275615	15.092	1.077	10341.795	59496
6	6	Benzofenon	28.885	3280684	2.350	1.049	11284.973	80724

Harmonised method:

- Issued by the chemical analysis group at the harmonization workshop in Kuala-Lumpur, on September 13th to 17th, 2004
- Approved by the harmonization workshop delegates workshop in Kuala-Lumpur, on September 13th to 17th, 2004,
- Modified after the Jakarta training, Nov 22nd to 26th, 2004
- Modified and approved after the Brunei workshop, Aug 30th & 31st, 2005
- Modified and approved after the final review in Singapore, Nov 30th to Dec 2nd, 2005
- Modified and approved after the 1st ACTLC Meeting in Solo (Indonesia), Nov 20nd, 2012