	Title	Revision n°	date	Document N°
	DETERMINATION OF HEAVY METALS (ARSENIC CADMIUM, LEAD AND MERCURY) IN COSMETIC PRODUCTS	1	12/07/06	ACM THA 05

1. SCOPE AND FIELD OF APPLICATION

The method describes the determination of heavy metals (arsenic, cadmium, lead and mercury) in cosmetic products.

2. PRINCIPLE

Organic matter in sample is digested by wet digestion or dry digestion or high pressure microwave digestion and determine the amount of heavy metals, i.e. arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) by using graphite furnace atomic absorption spectrophotometer (GF-AAS) and flow injection analysis system -atomic absorption spectrophotometer (FIAS-AAS).

3. REAGENTS

All reagents must be of analytical grade

3.1 Nitric acid
3.2 Hydrochloric acid
3.3 Hydrogen peroxide 30% v/v
3.4 reductant
  3.4.1 For Hg either
    3.4.1.1 1.1 % w/v stannous chloride in 3%v/v hydrochloric acid or
    3.4.1.2 0.2 % w/v sodium borohydride in 0.05% sodium hydroxide
3.5 50% w/v Magnesium nitrate.
3.6 Deionised water,resistivity ≥ 18.2 Mohm.
3.7 Standard calibration solutions
  As, Cd, Pb and Hg standard stock solutions conc. 1000 μg/mL
  3.7.1 As :
    3.7.1.1 For GF-AAS
      Prepare As standard calibration solutions concentration of 5, 10, 20, 30 and 50μg/L in 0.5 % v/v nitric acid , respectively.
    3.7.1.2 For FIAS-AAS (Hydride generation technique)
      3.7.1.2.1 Prepare As standard calibration solutions concentration of 1 μg/mL
      3.7.1.2.2 Pipette 200, 400, 600, 800 μl from 3.7.1.2.1 into separate 100 mL volumetric flask and continue under 5.2.
  3.7.2 Cd : Prepare Cd standard calibration solutions concentration of 0.5, 1, 2, 3 and 5 μg/L in 0.5 % v/v nitric acid , respectively.
  3.7.3 Pb : Prepare Pb standard calibration solutions concentration of 5, 10, 20, 30 and 50 μg/L in 0.5 % v/v nitric acid , respectively.
  3.7.4 Hg : Prepare Hg standard calibration solutions concentration of 0. 5, 1, 2, 3 and 5 μg/L in 3 % v/v hydrochloric acid, respectively.
3.8 Modifier for GF-AAS
  3.8.1 For As : 1,000 μg/mL Pd-modifier
  3.8.2 For Pb and Cd : Mix 1:1 of 0.2% w/v $Mg(NO_3)_2.6H_2O$ in 0.5% v/v nitric acid and 0.2 % w/v $NH_4H_2PO_4$ in 0.5% v/v nitric acid
3.9 Reagent for pretreatment of As
  Mix 1:1 of 10 % w/v potassium iodide and 10 % w/v ascorbic acid

4. APPARATUS

Normal laboratory equipment, and:

4.1 Silica dish
4.2 Muffle furnace
4.3 Water bath
4.4 Heating mantle
4.5 Block heater
4.6 Digestion tube 50 mL
4.7 Refrigerator

4.8 Whatman paper No. 1 and No.40

4.9 Microwave digestion,
Condition :

sample type	max. power (W)	max. temp. (^{o}C)	max. pressure (bar)	time (min.)
cream	800	200	75	50
powder	1000	200	75	40
lipstick	900	200	75	50

4.10 quartz or Tetrafluoromethane (TFM) vessel 50 mL

4.11 Graphite Furnace Atomic Absorption Spectrophotometer (As,Cd, Pb)
Condition :

Element	wavelength (nm)	pyrolysis (^{o}C)	atomized temp. (^{o}C)	injection volume (µL)
As	193.7	1250	2100	20
Cd	228.8	550	1550	20
Pb	283.3	550	1550	20

4.12 Flow Injection Analysis System - Atomic Absorption Spectrophotometer (Hydride generation Technique)
Condition :

Element	wavelength (nm)	reducing agent	carrier	atomization temp. (^{o}C)	injection volume (µL)
As	193.7	0.2 % w/v $NaBH_4$	10% v/v HCl	900	500

4.13 Flow Injection Analysis System - Atomic Absorption Spectrophotometer (Cold Vapour Technique)
Condition :

Element	wavelength (nm)	reducing agent	carrier	atomization temp. (^{o}C)	injection volume (µL)
Hg	253.7	1.1% w/v $SnCl_2$ or 0.2 % w/v $NaBH_4$	3% v/v HCl	300	500

4.14 Electrodeless Discharge Lamp or Hollow Cathode Lamp : As, Cd, Pb, Hg

5. PROCEDURE

5.1 Sample Preparation :
Prepare the Reagent Blank as in sample preparation but without adding the sample. Sample preparation can be carried out by either one of the following methods.

5.1.1 Microwave digestion (for As, Cd, Pb, Hg)

5.1.1.1 Accurately weigh, to the nearest mg in duplicate, 0.15 – 0.20 gm of sample into a high pressure resistance 50 mL quartz or TFM vessel. Avoid contact with the side of the vessel. Add 3 mL conc. nitric acid and 30 % hydrogen peroxide 1 mL by using graduated pipette. If sample contain talcum or pigment add conc. hydrochloric acid 1 mL.

5.1.1.2 Close the vessel lid. Leave for about 15 minutes to ensure complete reaction. Digest in microwave digestion system at the specified program.

5.1.1.3 After cooling to room temperature, add deionised water 20 mL to the digested solution, rinse the inner wall and lid thoroughly. Filter through Whatman paper no.1 into 50 mL volumetric flask and dilute to volume with deionised water.

5.1.2 Dry ashing (for As, Cd, Pb)

5.1.2.1 Accurately weigh 2.5 g sample into a silica dish and add 3 mL of 50% w/v magnesium nitrate.

5.1.2.2 Dry on the water bath and ash the residue first in the heating mantle until no more fume and then in the muffle furnace at 500^{o}C for 3 hours.

5.1.2.3 Cool, add 25 mL 6M hydrochloric acid, filter into a 50 mL volumetric flask and dilute to volume with water. For As, continue under 5.2.

5.1.3 Wet digestion (for Hg)

Warning: this technique involves a low recovery of Hg as compared to the microwave digestion technique.

5.1.3.1 Accurately weigh 0.5 g sample into a digestion tube with screw cap and add 7 mL of conc. nitric acid.

5.1.3.2 Heat the sample solution in a block heater at 60°C maximum for at least 3 hours.

5.1.3.3 Cool and dilute to volume (50 mL) with water. Stand for 24 hours in the refrigerator for cream and lipstick samples. Filter the solution through Whatman paper No. 40.

5.1.3.4 The digested solutions are used for analyses by FIAS-AAS (cold vapour mercury technique).

5.2 Pretreatment for As

5.2.1 Pipette 10 mL each of deionised water (as standard blank), the reagent blank, the standard solutions and the sample solution into separate 100 mL volumetric flasks.

5.2.2 Add 10 mL of concentrated hydrochloric acid and 10 mL of reagent for pretreatment of As(3.9) to each of the solutions and allow them to stand for 45 minutes at ambient temperature. Dilute to volume with water. The final concentrations of the standard solutions are 2.0, 4.0, 6.0 and 8.0 μg/L respectively.

5.2.3 These solutions are used for analyses by FIAS-AAS (Hydride Generation Technique).

5.3 Calibration curve

Inject standard calibration solutions into the GF-AAS or FIAS-AAS (Cold vapor Technique) or FIAS-AAS (Hydride Generation System)at the specified condition (4.11 to 4.13). Plot the response (absorbance or peak height or area) versus concentration of each standard solution.

5.4 Inject sample solutions into GF-AAS or FIAS-AAS (Cold vapour Technique) or FIAS-AAS (Hydride Generation System). Record the response and concentration (μg/L) of As, Cd, Pb, Hg in sample solution, then calculate μg/g of As, Cd, Pb, Hg in sample.

6. CALCULATION

$$As,Cd,Pb,Hg = \frac{\text{conc. of As,Cd,Pb,Hg in sample soln (μg/L) x mL of sample}}{\text{sample weight (g) x 1,000}} \text{ μg/g}$$

7. REMARKS

7.1 Method validation information

7.1.1 Precision

7.1.1.1 Within day

Metal	Content in cream (µg/g)	% Relative standard deviation
As	10	10
Cd	1	10
Pb	40	5
Hg	1	15

7.1.1.2 Different days

Metal	Content in cream (µg/g)	% Relative standard deviation
As	10	15
Cd	1	10.0
Pb	40	4
Hg	1	25

7.1.1.3 Different analysts

Metal	Content in cream (µg/g)	p-Value (n1 = n2 = 5, α = 0.05)
As	10	0.47
Cd	1	0.09
Pb	40	0.49
Hg	1	0.22

7.1.2 Accuracy

Percent recovery of As, Cd, Pb and Hg from spiked cream are 84-86%, 66-71%, 85-99% and 95-108% respectively.

7.1.3 Linearity and Range

Linearity of response over the range of concentration are as follows

Metal	Range (µg/L)	Correlation coefficient, r
As	5.0-50	0.99921
Cd	0.5-5.0	0.99997
Pb	5.0-50	0.99925
Hg	0.5-5.0	0.99815

7.1.4 Determination limits:

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Metal	Determination limit (µg/g)
As	2.5
Cd	1
Pb	10
Hg	0.5

7.2 Results report

7.2.1 According to the ASEAN Cosmetic Documents. 2003. Annex II – Part 1 List of substances which must not form part of the composition cosmetics products, As, Cd, Pb and Hg are prohibited..

If the result is below the determination limit listed above, it shall be reported as:
" below the determination limit".

If the result is higher than the determination limit listed above, the amount will be reported.

7.2.2 When metals content is high level, the sample extract (5.1) should be diluted to concentration within the calibration range.

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,**
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