

	Title	Revision No	date	Document No
	DETERMINATION OF HEAVY METALS (ARSENIC, CADMIUM, LEAD AND MERCURY) IN COSMETIC PRODUCTS	2	20/11/12	ACM 005

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 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), cold vapour-atomic absorption spectrophotometer (CV-AAS) and hydride generation-atomic absorption spectrophotometer (HG-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, prepare fresh solution of 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 % w/v sodium hydroxide

3.5 Deionised water, resistivity ≥ 18.2 megaohm-cm

3.6 Standard calibration solutions

As, Cd, Pb and Hg standard stock solutions concentration of 1,000 $\mu\text{g/mL}$

3.6.1 As :

3.6.1.1 For GF-AAS

Prepare As standard calibration solutions concentration of 10, 20, 30, 40 and 50 $\mu\text{g/L}$ in 0.5% v/v nitric acid.

3.6.1.2 For HG-AAS

3.6.1.2.1 Prepare As intermediate standard solution concentration of 1 $\mu\text{g/mL}$.

3.6.1.2.2 Pipette 200, 400, 600, 800 and 1000 μL from 3.6.1.2.1 into five separate 100 mL volumetric flasks and continue under 5.3.

3.6.2 Cd :

Prepare Cd standard calibration solutions concentration of 1, 2, 3, 4 and 5 $\mu\text{g/L}$ in 0.5 % v/v nitric acid.

3.6.3 Pb :

Prepare Pb standard calibration solutions concentration of 10, 20, 30, 40 and 50 $\mu\text{g/L}$ in 0.5 % v/v nitric acid.

3.6.4 Hg :

Prepare Hg standard calibration solutions concentration of 1, 2, 3, 4 and 5 $\mu\text{g/L}$ in 3 % v/v hydrochloric acid.

Note : It is suggested that for different system e.g. different instrument, the manufacturer's recommendation for concentration of standard calibration solutions should be followed.

3.7 Modifier for GF-AAS

3.7.1 For As :

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Palladium modifier solution (Pd-modifier) concentration of 10 mg/μL. Prepare Pd-modifier concentration 1,000 μg/mL in 0.5 % v/v nitric acid.

3.7.2 For Pb and Cd :

Mix 1:1 of 0.2 % w/v Mg(NO₃)₂·6H₂O in 0.5 % v/v nitric acid and 0.2 % w/v NH₄H₂PO₄ in 0.5 % v/v nitric acid.

Note : It is suggested that for different system e.g. different instrument, the manufacturer's recommendation for concentration of reductant and modifier should be followed.

3.8 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 Whatman paper No. 1

4.2 Microwave digestion :

4.2.1 Condition :

sample type	max. power (W)	max. temp. (°C)	max. pressure (bar)	time (mins)
cream	800	200	75	55
powder	1000	200	75	55
lipstick	900	200	75	55

4.2.2 Example of the microwave digestion condition

For Anton Paar microwave digester, set the condition as follows :

Sample type : cream

Phase	Power (W)	Time (mins) Ramp	Final Power (W)	Max. Temp (°C)	Hold time (mins)	Fan speed
1	250	5	400	200	0	1
2	400	0	400	200	20	1
3	400	10	800	200	0	1
4	800	0	800	200	5	1
5	0	0	0	-	15	3

Sample type : powder

Phase	Power (W)	Time (mins) Ramp	Final Power (W)	Max. Temp (°C)	Hold time (mins)	Fan speed
1	250	5	450	200	0	1
2	450	0	450	200	20	1
3	450	10	1,000	200	0	1
4	1,000	0	1,000	200	5	1
5	0	0	0	-	15	3

Sample type : lipstick

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Phase	Power (W)	Time (mins) Ramp	Final Power (W)	Max. Temp (°C)	Hold time (mins)	Fan speed
1	250	5	400	200	0	1
2	400	0	400	200	20	1
3	400	10	900	200	0	1
4	900	0	900	200	5	1
5	0	0	0	-	15	3

Note: It is suggested that for other microwave digester equipment, the manufacturer's recommendations should be followed.

4.3 Quartz or Tetrafluoromethane (TFM) vessel 50 mL

4.4 Graphite Furnace-Atomic Absorption Spectrophotometer (As, Cd and Pb)

4.4.1 Condition :

Element	wavelength (nm)	pyrolysis (°C)	atomized temp. (°C)	Modifier (µL)	injection volume (µL)
As	193.7	1250	2100	5	20
Cd	228.8	650	1650	5	20
Pb	283.3	550	1550	5	20

4.4.2 Example of the GF-AAS condition

For Perkin Elmer GF-AAS, set the condition as follows :

Element : As

Step	Temperature (°C)	Time (mins) Ramp	Hold Time (mins)	Internal Flow mL/min
1	110	11	30	250
2	130	15	30	250
3	1250	10	20	250
4	2100	0	5	0
5	2450	1	4	250
6	-	-	-	250

Element : Cd

Step	Temperature (°C)	Time (mins) Ramp	Hold Time (mins)	Internal Flow mL/min
1	110	1	30	250
2	130	15	30	250
3	650	10	20	250
4	1650	0	5	0
5	2350	1	3	250
6	-	-	-	250

Element : Pb

Step	Temperature	Time (mins)	Hold Time	Internal Flow
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	(°C)	Ramp	(mins)	mL/min
1	110	1	30	250
2	130	15	30	250
3	550	10	20	250
4	1550	0	5	0
5	2350	1	3	250
6	-	-	-	250

Note: It is suggested that for GF-AAS instrument, the manufacturer's recommendation should be followed.

4.5 Hydride Generation-Atomic Absorption Spectrophotometer

4.5.1 Example of the HG-AAS condition

For Perkin Elmer HG-AAS with Flow Injection Analysis System (FIAS), set the condition as follows :

Element	wavelength (nm)	reducing agent	carrier	atomization temp. (°C)	injection volume (µL)
As	193.7	0.2 % w/v NaBH ₄	10 % v/v HCl	900	500

Note: It is suggested that for other HG-AAS instrument, the manufacturer's recommendation should be followed.

4.6 Cold vapour-Atomic Absorption Spectrophotometer

4.6.1 Example of the CV-AAS condition

For Perkin Elmer CV-AAS with Flow Injection Analysis System (FIAS), set the condition as follows :

Element	wavelength (nm)	reducing agent	carrier	atomization temp. (°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

4.6.2 For GBC CV-AAS, set the condition as follows :

Element	wavelength (nm)	reducing agent	carrier
Hg	253.7	1.1 %w/w SnCl ₂ or 0.2 % w/v NaBH ₄	3 %v/v HCl

Note : It is suggested that for other CV-AAS instrument, the manufacturer's recommendation should be followed.

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

5. PROCEDURE

5.1 Sample Preparation :

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Prepare the Method Blank as in sample preparation but without adding the sample. Sample preparation can be carried out as follows :

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

5.1.1.1 Accurately weigh, to the nearest 0.1 mg in duplicate, 0.15 - 0.20 g of sample into a high pressure resistance 50 mL quartz or TFM vessel. Avoid contact with the side of the vessel.

5.1.1.2 Add 3 mL conc. nitric acid and 30 % v/v hydrogen peroxide 1 mL by using graduate pipette. If sample contain talcum or pigment, add conc. hydrochloric acid 1 mL.

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

5.1.1.4 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.

Note : If the sample is not completely digested, further re-digest the uncomplete digested sample by putting the original vessel into microwave under the condition as specified in 4.2.

5.2 Spiked sample preparation for determination of percent recovery

Percent recovery of elements in sample should be carried out at least one sample in a set of analysis.

5.2.1 Accurately weigh, to the nearest 0.1 mg in duplicate, 0.15 – 0.20 g of sample into a 50 mL quartz or TFM vessel.

5.2.2 Pipette standard solutions into the vessel as follows :

Element	Conc. (µg/mL)	Volume (µL)	Final conc.(ug/L)
As	5	300	30
Cd	0.5	300	3.0
Pb	5	300	30
Hg	0.5	300	3.0

Note : It is suggested that for different system e.g. different instrument, the manufacturer's recommendation for concentration of standard calibration solutions should be followed.

5.2.3 Perform as in 5.1.1.1 to 5.1.1.4.

5.3 Pretreatment for As (for analyses by HG-AAS)

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

5.3.2 Add 10 mL of conc. hydrochloric acid and 10 mL of reagent for pretreatment of As (3.8) to each of the solutions in 5.3.1 and five levels of stand solutions from 3.6.1.2.2. Allow them to stand for 45 minutes at ambient temperature. Dilute to volume with water. The final concentrations of the standard solutions are 2, 4, 6, 8 and 10 µg/L, respectively.

5.3.3 These solutions are used for analyses by HG-AAS.

5.4 Running sequence

Sequentially inject or aspirate reagent blank, standard and sample solutions into the GF-AAS or CV-AAS or HG-AAS at the specified condition (4.4 to 4.6) as follows :

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- 0.5 % v/v nitric acid or 3 % v/v hydrochloric acid
- Standard calibration solutions (5 levels)
- Standard check (Standard calibration solution ; 1 level)
(i.e. As 20 or 6 µg/L, Cd 2 µg/L, Pb 20 µg/L and Hg 2 µg/L)
- 0.5 % v/v nitric acid for GF-AAS or 3 % v/v hydrochloric acid for CV-AAS or 10% v/v hydrochloric acid for CV-AAS
- Method blank, replicates 1 and 2
- Sample solution, replicates 1 and 2
- Spiked sample, replicates 1 and 2
- Standard check

Note : the difference of response of standard solution for standard check should not differ from calibration curve by not more than 10 %

5.5 Response (absorbance; peak height or peak area) of standard calibration solutions are plotted versus the concentration of each standard solution by the instrument. The concentration of As, Cd, Pb and Hg in sample solutions are calculated and recorded. Then calculate µg/g of As, Cd, Pb and Hg in sample.

6. CALCULATION

$$\text{As, Cd, Pb, Hg} = \frac{\text{conc. of As, Cd, Pb, Hg in sample sol}^n (\mu\text{g/L}) \times \text{sample solution volume (mL)}}{\text{sample weight (g)} \times 1,000} \mu\text{g/g}$$

$$\% \text{ recovery} = \frac{\text{conc. of As, Cd, Pb, Hg in sample } (\mu\text{g/g}) - \text{conc. of As, Cd, Pb, Hg in unspiked sample } (\mu\text{g/g}) \times 100}{\text{conc. of As, Cd, Pb, Hg added in sample } (\mu\text{g/g})}$$

7. REMARKS

7.1 Method validation information

7.1.1 Precision

7.1.1.1 Within day

Element	Content in cream (µg/g)	% Relative standard deviation
As	1.7	2
Cd	1	3.8
Pb	40	2.9
Hg	0.1	4.4

7.1.1.2 Different days

Element	Content in cream (µg/g)	p-value (n=5, α = 0.05)
As	1.7	0.94
Cd	1	0.86
Pb	40	0.96
Hg	0.1	0.62

7.1.2 Recovery

Percent recovery of As, Cd, Pb and Hg from spiked cream are 97-115 %, 86-105 %, 88-110 % and 88-111 %, respectively.

7.1.3 Linearity and Range

Linearity of response over the range of concentration are as follows :

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Element	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 Limit of quantitation (LOQ) and limit of detection (LOD)

Element	Limit of quantitation, LOQ (µg/g)	Limit of Detection, LOD (µg/g)
As	2.5	0.5
Cd	0.5	0.1
Pb	5.0	1.0
Hg	0.5	0.1

7.2 The requirement of As, Cd, Pb and Hg in cosmetic products according to the ASEAN Cosmetic Decretive (ACD) are as follows :

- 1) ACD : September, 2007 Annex II - Part 1 List of substances which must not form part of the composition cosmetic products, As, Cd, Pb and Hg are prohibited substances.
- 2) On 13 December 2007, the ASEAN Cosmetic Committee (ACC) accepted the maximum contaminated level of As, Pb, and Hg of 5, 20 and 1 µg/g respectively.
- 3) Unavoidable level for cadmium is not available in ACD.

7.3 Results report.

It is recommended 2 decimal places of test results should be reported.

7.3.1 If the result is higher than the LOD, but below the LOQ listed above, it shall be reported as "Concentration of As, Cd, Pb or Hg is below 2.5, 0.5, 5.0 or 0.5 µg/g" as stated in the table 7.1.4.

7.3.2 If the result is higher than the LOQ listed above, the concentration of As, Cd, Pb or Hg in sample will be reported.

7.3.3 If the result is below the LOD i.e. 0.5, 0.1, 1.0 and 0.1 µg/g for As, Cd, Pb and Hg, respectively, it shall be reported as "As, Cd, Pb or Hg is not detected"

7.4 When concentration is high level, the sample extract (5.1 and 5.2) should be diluted to concentration within the calibration range.

7.5 Reductant for Hg

7.5.1 Using sodium borohydride as a reductant may cause some abnormal phenomena i.e. false positive peak of mercury could be observed in particular for eye shadow sample.

7.5.2 Using stannous chloride as a reductant may cause some abnormal phenomena i.e. dilution of high concentration of mercury in sample solution could not recover the correspond concentration (e.g. face cream or mask sample).

Appendix A Informative Wet digestion for determination of mercury

Sample solution for determination of Hg may be prepared by wet digestion. However, this technique involves a low recovery of Hg as compared to the microwave digestion technique.

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A.1 REAGENTS

All reagents must be of analytical grade

A.1.1 Nitric acid

A.1.2 Deionised water, resistivity ≥ 18.2 megaohm-cm

A.2 APPARATUS

Normal laboratory equipment, and

A.2.1 Water bath

A.2.2 Block heater

A.2.3 Digestion tube 50 mL

A.2.4 Refrigerator

A.2.5 Whatman paper No.40

A.3 PROCEDURE

A.3.1 Accurately weigh 0.5 g sample into a digestion tube with screw cap and add 7 mL of conc. nitric acid.

A.3.2 Heat the sample solution in a block heater at 60°C maximum for at least 3 hours.

A.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.

A.3.4 The digested solutions are used for analyses by CV-AAS.

Appendix B Informative Sample preparation by dry ashing

Sample solution for determination of As, Cd and Pb may be prepared by dry ashing.

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B.1 REAGENTS

All reagents must be of analytical grade

B.1.1 50 % w/v Magnesium nitrate

B.1.2 Hydrochloric acid , 6 M

B.1.3 Deionised water, resistivity ≥ 18.2 megaohm-cm

B.2 APPARATUS

Normal laboratory equipment, and

B.2.1 Silica dish

B.2.2 Muffle furnace

B.2.3 Water bath

B.2.4 Heating mantle

A.2.5 Whatman paper No.1

B.3 PROCEDURE

B.3.1 Accurately weigh 2.5 g sample into a silica dish and add 3 mL of 50 % w/v magnesium nitrate.

B.3.2 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 ° C for 3 hours.

B.3.3 Cool, add 25 mL 6 M hydrochloric acid, filter into a 50 mL volumetric flask and dilute to volume with water. For As, continue under 5.2 to 5.3

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 Bangkok training, Nov 29th to Dec 3rd, 2004

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- Modified and approved after the Brunei workshop, Aug 30th to 31st, 2005
- Modified and approved after the final review in Singapore, Nov 30th to Dec 2nd, 2005
- Modified and approved after the Malaysia workshop, Jul 10th to 12th, 2006
- Modified and approved after the ACTLC meeting in Laos, May 20th to 24th, 2013