Annexure 2(d)

HEAVY METALS

Heavy metals in herbal drugs and herbal drug preparation:

Apparatus:

Determine by Atomic Absorption Spectroscopy (2.4.2)

Method:

In case alternative apparatus is used, an adjustment of the instrument parameters may be necessary.

Clean all the glassware and laboratory equipment with a 10.0 g per litre solution of nitric acid before use.

Test solution: In a digestion flask, place the prescribed quantity of the substance to be examined (about 0.50 gm of powdered drug). Add 4.0 ml of hydrochloric acid (heavy metal free) and 6 ml of nitric acid (heavy metal free). Make the flask airtight.

Place the digestion flask in the microwave oven. Carry out the digestion in 3 steps according to the following programme, used for 7 flasks each containing the test solution: 80 per cent for 15 min, 100 per cent for 5 min, 80 per cent for 20 min.

After the completion of cycle, allow flask to cool in air or water. After cooling, open each digestion flask and introduce the clear, colourless solution obtained into 50.0 ml volumetric flask. Rinse each digestion flask with 2 quantities, each of 15.0 ml of dilute nitric acid (heavy metal free). Collect the rinsing in the volumetric flask and dilute to 50.0 ml with water. Modifiers (e.g. in the case of AAS with electrothermal atomisation, 1.0ml of a 10.0 g per litre solution of magnesium nitrate and 1.0 ml of 100 g per litre solution of ammonium dihydrogen phosphate) and stabilising agents may be used, if necessary.

Blank solution: Mix 4.0 ml of Hydrochloric acid (heavy metal-free) and 6.0 ml of nitric acid (heavy metal-free) in a digestion flask. Carry out the digestion in the same manner as for the test solution.

Determination of arsenic, cadmium, copper, nickel and lead using AAS (2.4.2)

Measure the content of arsenic, cadmium, copper, nickel and lead by direct calibration (Method A 2.4.2) or by the standard additions method (Method B 2.4.2), using reference solutions of each heavy metal and the instrumental parameters given in the table 1 (A).

The absorbance value of the blank solution is subtracted from the value obtained with the test solution. Table 1 (A) – Instrumental parameters for AAS with electrothermal atomisation

		As	Cd	Cu	Ni	Pb
Wavelength	**nm**	**193.5**	**228.8**	**324.8**	**232**	**283.5**
Slit width	**nm**	**0.5**	**0.5**	**0.5**	**0.2**	**0.5**
Lamp Current	**mA**	**10**	**6**	**7**	**10**	**5**
Ignition temperature	**˚C**	**1400**	**800**	**800**	**800**	**800**
Atomisation temperature	**˚C**	**2600**	**1800**	**2300**	**2500**	**2200**
Gas flow rate	**l/min**	**3**	**3**	**3**	**3**	**3**

Arsenic and Mercury:

Determination of Arsenic and Mercury using AAS (2.4.2) with cold – vapour or Hydride Atomisation:

Measure the content of Arsenic and Mercury by direct calibration (Method A 2.4.2) or by the standard additions method (Method B 2.4.2) using reference solution of arsenic or mercury and an automated continuous- flow hydride vapour generation system.

The absorbance value of the blank solution is subtracted from the value obtained with the test solution.

Arsenic

Sample solution: Take a 19.0 ml of test solution or of the blank solution as prescribed above, add 1.0 ml of 200 g per ml solution of potassium iodide. Allow the test solution to stand at room temperature for about 50 min or at 70° for about 4 min.

Acid regent. Hydrochloric acid (Heavy metal-free).

Reducing reagent. 6.0 g per litre solution of sodium tetra hydroborate in a 5.0 g per litre solution of sodium hydroxide as describe in table 1(B).

Mercury

Sample solution. Test solution or blank solution, as prescribed above.

Acid reagent. Take 515.0 g per litre solution of hydrochloric acid (heavy metal-free).

Reducing reagent. Take 10.0 g per litre solution of stannous chloride in dilute hydrochloric acid (heavy metal-free).

Table 1(B)- Instrumental parameter foe AAS with cold-vapour of hydride atomisation

		As	Hg
Wavelength	nm	193.7	253.7
Slit Width	nm	0.2	0.5
Lamp current	mA	10	4
Acid regent flow rate	ml/min	1.0	1.0
Reducing reagent flow rate	ml/min	1.0	1.0
Sample solution flow rate	ml/min	7.0	7.0
Absorption cell		Quartz (heated)	Quartz (unheated)
Nitrogen flow rate	l/min	0.1	0.1

Determination of Arsenic, Cadmium, Copper, Mercury, Nickel and Lead using ICP-MS. (2.4.42)

Measure the content of Arsenic, Cadmium, Copper, Mercury, Nickel and Lead by direct calibration (Method A 2.4.2) using reference solutions of each heavy metal and the analytical isotopes and additional masses recommended in table 1 (C).

The signal intensity of the blank solution is subtracted from the value obtained with the test solution.

System Suitability:

A system Suitability test must be carried out on the day of the analysis to ensure that the sample preparation and measurement system are accurate.

Acceptance criteria for preparation of sample solution: A clear solution is obtained.

Acceptance criteria for measurement system: The measured concentration of a standard solution of the metal at a concentration within the range of the used calibration curve does not differ from the actual concentration by more than 20 per cent.

Table 1 (C)- Recommended analytical isotopes and additional masses for ICP-MS.

Isotope	Element of Interest
75	Arsenic
106,108,111,114	Cadmium
63,65	Copper
202	Mercury
60,62	Nickel
206,207,208	Lead

Validation Requirement:

The analytical procedure used must be validated in accordance with the relevant general method AAS (2.4.2), and ICP-MS (2.4.42). Additionally, the following criteria must be fulfilled.

Specificity:

Specificity is the ability to ensure that the analytical procedures for sample preparation and measurement allow a reliable determination of the metals in the presence of components (e.g. carrier gas, impurities, and matrix) that may be expected to be present.

Acceptance Criteria: The procedure must be able to assess each heavy metal to be determined with this procedure in the presence of components that may be expected to be present, including other heavy metals, matrix components and other sources of interference; specificity is demonstrated by complying with the accuracy requirement for the metals to be determined.

Range:

The calibration range of each metal is within the linear range of the method; test solutions containing residues at a level outside the calibration range may be diluted to concentrations within the calibration range.

Acceptance Criteria: Range is demonstrated by complying with the recovery requirement.

Accuracy:

Verify the accuracy using a certified reference material or by performing a test of recovery.

Recovery. Recovery may be determined on a sample of the substance to be examined, spiked with a known quantity of a reference standard of the metal (3 concentration levels in the range of 50-150 per cent of the intended specification limit, even if the original concentration of the reference standard is at the specified value), in triplicate.

Acceptance Criteria: Spike recovery is within 70 per cent and 150 per cent for the mean of 3 replicates at each concentration.

Intermediate Precision:

The effect of random events (intra-laboratory variation) on the analytical precision of the method described. Acceptable experiments for established intermediate precision include performing the repeatability analysis on different days, or with different instrumentation, or with different analyst. Only 1 of the 3 experiments is required to demonstrate intermediate precision.

Acceptance Criteria: The relative standard deviation is not greater than the value indicated in Table -1(D).

Repeatability:

Test sample: Either 6 independent samples of the substance to be examined spiked with a suitable reference standard at the specified concentration level, or 3 concentration levels prepared in triplicate.

Acceptance Criteria: The relative standard deviation is in both cases not greater than the value indicated in Table -1 (D).

Table 1(D)

Concentration range of the metal	Repeatability (RSD) (per Cent)	Intermediate Precision (RSD) (Per Cent)
0.01-1	20	32
>1	10	16

Limit of Detection

Determine the lowest concentration giving a signal clearly distinct from that obtained with a blank solution.

Acceptance Criteria: The limit of detection is not more than 0.1 times the concentration of the specification limit.

Limit of Quantification:

Determine the lowest concentration meeting the acceptance criterion. Use the results from the accuracy study.

Acceptance Criteria: The limit of quantification is below the specification limit.