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Evaluation of Heavy Metals Present in Marketed Herbal Cosmetic Formulations

D. H. Dongre[1], M. P. Puranik[2], P. R. Tathe[3]
[1]*Assistant Professor, Department of Quality Assurance, Samarth College of Pharmacy, Deulgaon Raja, India*
[2]*Assoc. Prof., Dept. of Quality Assurance, Institute of pharmaceutical Education and Research , Wardha, India*
[3]*Assistant Professor, Department of Pharmacology, Samarth College of Pharmacy, Deulgaon Raja, India*

***Abstract*: In present study, Seven Patanjali Herbal Cosmetic formulations were studied by using colorimetry and AAS method for heavy metal, namely, Arsenic, Lead, Mercury, Copper and cadmium. Herbal cosmetic samples were subjected to Acid digestion. Heavy metals all were estimated sample acid digestion methodology, using nitrogen as carrier gas in seven herbal cosmetic Formulations. Out of seven selected brands for arsenic content exceeds. Lead, Mercury, Cadmium, Copper was found within limit. Cadmium was found in four brands namely, Rose Facewash, Drusthi Eye drop, Crack Heal cream and Beauty cream below the detectable limit.**

***Keywords*: Analysis, Atomic absorption spectrophotometer, Colorimetry, Heavy metal ions, Herbal cosmetic formulation.**

1. Introduction

Cosmetic is defined as "Any article intended to be rubbed, poured, sprinkled or sprayed on, or introduced into, or otherwise applied to, the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and includes any article intended for use as a component of cosmetic.

Herbal Cosmetics are formulated, using various permissible cosmetic ingredients to form the base in which one or more herbal ingredients are used to provide defined cosmetic benefits. Herbal cosmetics are the valuable products consisting of botanical or their bioactive ingredients or extracts which enrich the skin with trace elements and other useful minerals to prevent infection and hence responsible for their cosmetic effects.

Herbal cosmetic are used worldwide since ancient time, but in the last decade, there has been a renewed craze of herbal cosmetics and personal care products, especially in the skin care segment with the growing belief that chemical-based cosmetics are harmful and herbal cosmetics are safe being natural. It has been reported that the absorption of toxic metals through skin is very insignificant, still can cause deleterious effects.

So, there is an increase in scientific concern over the issue of heavy metals, unfortunately no sufficient data is available regarding the status of herbal cosmetics for the presence of heavy. Thus the safety of herbal cosmetics becomes doubtful and need further attention of the scientific community and the regulatory agencies.[1]

A. *Quality Assurance* [2]

Elements of quality assurance are:

- ✓ Adherence to, GMP and GLP guidelines,
- ✓ Setting specifications,
- ✓ Quality control measures.

National drug regulatory authorities have established guidelines on all elements of quality assurance, evaluate dossiers and data submitted by the producers, and check post-marketing compliance of products with the specifications set out by the producers as well as compliance with Good Manufacturing Practices (GMP). A cooperative approach between different manufacturers, e.g. by establishing drug master-files for specifications and quality control, should be encouraged.

B. *Quality control*[3]

Herbal products are usually mixtures of many constituents. The active principles are in most cases unknown. Selective analytical methods or reference compounds may not be available commercially. Plant materials are chemically and naturally variable, chemo-varieties and chemo cultivars exist.

C. *Regulations for Cosmetics in India*[4,5]

Cosmetics products in India are regulated under the Drugs and cosmetics Act 1940 and Rules 1945 and Labeling Declarations by Bureau of Indian Standards (BIS). BIS sets the standards for cosmetics for the products listed under Schedule 'S' of the Drugs and cosmetics Rules 1945.

Bureau of Indian Standards (BIS) has provided the specification for cosmetic Indian Standards says that, all the raw materials and finished product must be tested for heavy metals been so tested and must comply with the requirements.

Table 1
Limits of Heavy Metals in Herbal Cosmetics by different regulatory agencies in ppm.

S. No.	Regulatory Agency	As	Pb	Hg	Cu	Cd
1	BIS	2	20	70	100	100
2	D & C act 1948	2	20	100	100	100

D. *Heavy Metals in Cosmetics*[6,7,8,9]

The toxicological properties of lead, arsenic, cadmium, mercury and copper are described very well and impurity limits are identified. These impurity limits encompass a variety of products that differ significantly from cosmetics, and it would not necessarily be appropriate for cosmetics to have a similar heavy metal impurity limit. It is acknowledged that heavy metal impurities in cosmetic products are unavoidable, but should be removed wherever technically feasible. Heavy metals are naturally occurring, are present in the environment and can make their way in trace quantities into raw materials. These substances end up in the products we consume and use every day.

E. *Analytical methods for testing of heavy metals*[10, 11]

There are two basic types of analytical methods for assaying heavy metals. The classical ones are colorimetric and atomic absorption spectrometric method.

1) *Colorimetric methods*

Colorimetric method is measurements of colour of a solution and its correlation to the determination of its concentration. The measurement is made by comparing or reading the colour produced for substance with same colour produced by known substance standard under well-defined condition.

2) *Atomic absorption spectroscopy*

AAS is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of option radiation (light) by free atoms in the gaseous state. In analytical chemistry the technique is used for determining the concentration of a particular element (analyte) in a sample to analyze. AAS can be used to determine over 70 different elements in solution or directly in solids samples used in pharmacology, biophysics and toxicology research. Atomic absorption spectroscopy relies on Beer-Lambert law to determine the concentration of a particular analyte in a sample. The absorption spectrum and molar absorbance of the desire sample element are known, a known amount of energy is passed through the atomized sample and by then measuring the quantity of light it is possible to determine the concentration of the element being measured.

2. Plan of Work

- Literature survey
- Selection of Herbal cosmetics.
- Procurement of herbal cosmetic samples and other chemicals.
- Sample Digestion.
- Colorimetric estimation.
    - ✓ Preparation of standard calibration curve.
        - Arsenic
        - Mercury
        - Lead
        - Cadmium
        - Copper
    - ✓ Metal quantification in coloured sample.
    - ✓ Statistical analysis.
    - ✓ Compilation of data.
- Atomic spectroscopic estimation of heavy metal.
    - ✓ Preparation of standard calibration curve using AAS for:
        - Arsenic
        - Mercury
        - Lead
        - Copper
        - Cadmium
    - ✓ Metal quantification in digested sample.
    - ✓ Statistical analysis.
    - ✓ Compilation of data.

3. Experimental and results

A. *Instruments*

- Electronic weighing balance Model No.AW-220 & BX-620 S, Shimadzu Corporation, Koyto, Japan.
- Atomic Absorption Spectrophotometer Model No. AA 7000 LABENDIA
- Double Beam UV spectrophotometer Model No. UV2401 PC Secore Scientific Engineering Work.
- All required chemicals were purchased from Loba Chemie Pvt. Ltd, Mumbai.
- Deionised water was purchased from Local Market, Wardha.
- Whatman No. 41 Filter paper was used for experimental work.

Table 2
Herbal cosmetic samples selected for study

Sr. No	Name of samples	Sample Code	Product information
01	Kesh kanti Shampoo	A	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No: KK09 Mfg Date: 12/15 Exp Date: 12/17
02	Rose Facewash	B	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No:018 Mfg Date: 10/15 Exp Date: 04/17
03	Drusthi Eye drop	C	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No: B15/136 Mfg Date: 10/15 Exp Date: 10/16
04	Dant kanti junior Dental Cream	D	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No:CD38 Mfg Date: 12/15 Exp Date: 05/17
05	Haldi chandan kanti Soap	E	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No: BQ436 Mfg Date: 11/15 Exp Date: 11/17
06	Crack Heal cream	F	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No:AE10 Mfg Date: 10/15 Exp Date: 10/17
07	Beauty cream	G	Patanjali Ayurved Ltd. Mfg Lic No: Uttra/Ayu-181/2009 Batch No: BQ436 Mfg Date: 11/15 Exp Date: 11/17

B. *Digestion of Sample*

Samples were prepared by acid digestion method.

1. 2 gm of accurately weighed Herbals Cosmetics sample was placed in a kjeldahl flask.
2. 5mL of concentrated Nitric acid and Perchloric acid was added carefully in the ratio (3:1).
3. The Mixture was heated continuously in water bath for two hours.
4. 3 mL of Hydrogen Peroxide was added and heated strongly for one hour.
5. The mixture was allowed to cool, and then 10 mL of deionised water was added.
6. Filtered through Whatman No.41 Filter paper in 100 mL volumetric flask and diluted to mark with deionised water.

C. Estimation of Arsenic

1) Colorimetric Method for detection of Arsenic[27]

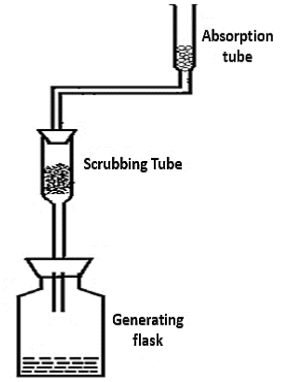

Fig. 1. Modified Gutzeit Apparatus

a) Preparation of standard curve

Generating tube

1. Arsenic working solution was transferred in generator.
2. 7 mL of 50 % Sulphuric acid was added to it and cooled.
3. 5 mL of Potassium iodide solution was added.
4. Followed by 5 drops of stannous chloride solution.
5. Finally, 3-5 g of Zinc was added to generator.

Scrubbing tube

6. A piece of cotton moistened with lead acetate solution was kept.

Absorption tube

7. Glass beads were kept and 3 mL of Sodium hydroxide was added.
8. Generator, scrubbing tube and Absorption tube was connected properly.
9. Kept at temperature of 20°– 25° C for 60- 90 min.
10. The apparatus was disconnected and contents of scrubbing tube were washed 2×2 mL of deionised water.
11. The washings was filtered in 25 mL volumetric flask.
12. 5 mL of 1N sulphuric acid, 1 mL of the ammonium molybdate solution and 1 mL of hydrazine sulphate solution was added. Finally volume was made up to the mark with deionised water.
13. The solution was kept for 1 hour and the blue colour was scanned in the range of 400 to 800 nm against reagent blank. UV-VIS absorption spectrum was shown in Figure 2.

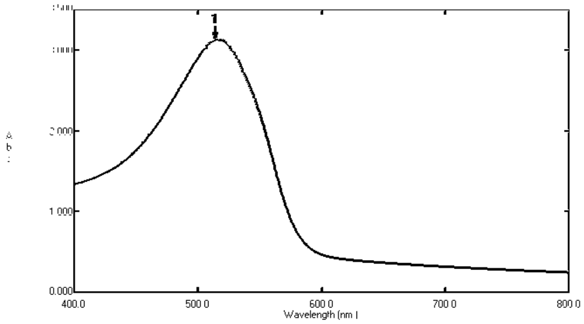

Fig. 2. UV-VIS Absorption Spectrum of Arsenic

The same procedure was repeated for arsenic working solution in the concentration range of 2 to 12 ppm, the absorbance was noted at 514nm. The result of various concentrations and their corresponding absorption are shown in Table 4 and the standard calibration curve was shown in Fig. 3.

Table 4
Readings of Standard curve for Arsenic by colorimetry

Sr. No	Concentration (ppm)	Absorbance
1	0	0
2	2	0.033±0.0042
3	4	0.071±0.0077
4	6	0.104±0.0062
5	8	0.142±0.0064
6	10	0.178±0.0027
7	12	0.209±0.0044

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

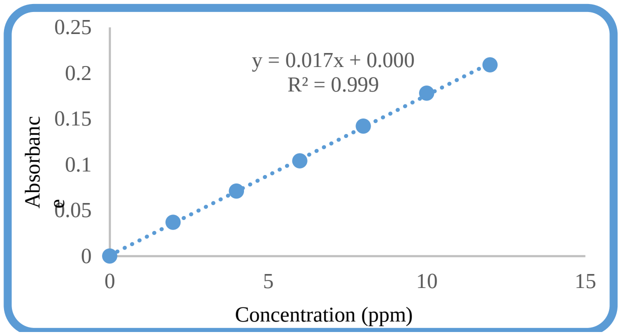

Fig. 3. Standard curve of Arsenic by colorimetry

b) Preparation of sample by Colorimetry

1 mL digested sample was taken in generator flask. Remaining procedure similar to (a) was carried out for each cosmetic sample. Absorbance was noted at 514 nm against reagent blank.

Table 5
Content of Arsenic by colorimetry

Sample Code	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.034±0.096	2.14
B	Rose Facewash	0.049±0.0026	3.25
C	Drusthi Eye drop	0.075±0.0056	4.10
D	Dant kanti junior Dental Cream	0.095±0.0085	4.91
E	Haldi chandan kanti Soap	0.101±0.0089	5.42
F	Crack Heal cream	0.069±0.0045	3.81
G	Beauty cream	0.041±0.0079	2.61

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

2) Atomic Absorption Spectrometric Method for detection of Arsenic

a) Preparation of standard curve of arsenic by AAS

- *Stock solution:* 1000 mg/Lit arsenic solution in 0.1 M HCl. Pipette out 10mL and diluted upto 100 mL using deionised water.
- *Standard solution:* From stock solution pipette out 2.5, 5, 7.5,10 and 12.5 mL and volume was made upto 50 mL using deionised water.

Operational Condition

- Wavelength:193.7 nm
- Method: Hydride Generation
- Lamp: Hallow cathode lamp.
- Flame colour: Blue
- Type of flame: Air/C_2H_2
- Fuel Gas Flow Rate: 1.50 L/min

The procedure was repeated for arsenic standard solution in the concentration range of 5 to 25 ppm, the absorbance was noted at 193.7nm. The result of various concentrations and their corresponding absorption are shown in Table 6 and the standard calibration curve was shown in Figure 4.

Table 6
Standard curve for Arsenic by AAS

Sr. No	Concentration (ppm)	Absorbance
1	0	0
2	5	0.011±0.0056
3	10	0.024±0.0019
4	15	0.035±0.0045
5	20	0.049±0.0079
6	25	0.054±0.0085

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

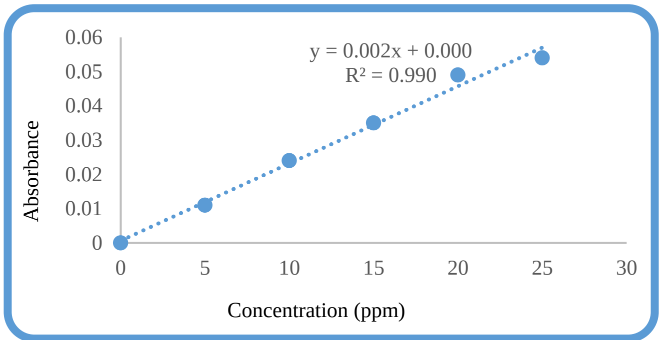

Fig. 4. Standard curve of Arsenic by AAS

b) Preparation of Sample for AAS

1mL of digested sample was pipetted out and transferred to 100 mL volumetric flask and diluted up to the mark with deionized water.

Table 7
Content of Arsenic in Herbal cosmetic by AAS

Sample Code	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.005±0.00012	2.95
B	Rose Facewash	0.008±0.00045	3.45
C	Drusthi Eye drop	0.009±0.0089	4.66
D	Dant kanti junior Dental Cream	0.012±0.00045	5.54
E	Haldi chandan kanti Soap	0.013±0.00012	5.92
F	Crack Heal cream	0.010±0.00010	4.19
G	Beauty cream	0.005±0.00012	2.79

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

D. Determination of Lead

1) Colorimetric Method for detection of Lead[28]

a) Reagents as per IP

- ✓ *Calcium chloride solution:* 10 gm of calcium chloride was dissolved in 100 mL of deionised water.
- ✓ *Potassium cyanide solution (10%):* 10 gm of potassium cyanide reagent was dissolved in 100 mL of deionised water.
- ✓ *Thymol blue solution:* 0.1 gm of thymol blue reagent was dissolved in 2.15 mL of 0.1 M sodium hydroxide and 20 mL of ethanol (95%). Then sufficient deionised water was added to produce 100 mL.
- ✓ *Dithiazone solution:* 10 mg of dithizone reagent was dissolved in 1000 mL of chloroform.
- ✓ *Sodium chloride solution in Hydrochloric acid:* Saturated solution of sodium chloride in Hydrochloride acid.

b) Standard solution of Lead

- ✓ *Stock Solution (1000 ppm):* 0.1598 gm of lead was accurately weighed in 1000 mL volumetric flask, 1 mL concentrated nitric acid was added to it and then volume was made up to 1000 mL with deionised water.
- ✓ *Working solution (100 ppm):* From the above stock

solution 10 mL was pipetted and volume was made up to 100 mL with distilled water.

c) Preparation of standard curve:

1. The working solution was pippeted in the glass beaker.
2. 1 drop of Thymol blue indicator was added followed by 5 mL of 10% Potassium cyanide solution.
3. Ammonium hydroxide solution was added drop wise until the pH of 8.5 was attained.
4. It was transferred in separating funnel along with 5 mL dithiazone solution and extracted vigorously.
5. The Dithiazone layer (organic layer) was separated into beaker and extraction was further continued with a fresh 5 mL of dithizone solution.
6. The process of extraction was continued till the green colour of the added dithizone remains unchanged.
7. The dithizone (organic layer) collected was diluted up to 50 mL with chloroform and scanned in the range of 400 to 800 nm against reagent blank. The UV - VIS absorption spectrum was shown in Fig. 5.

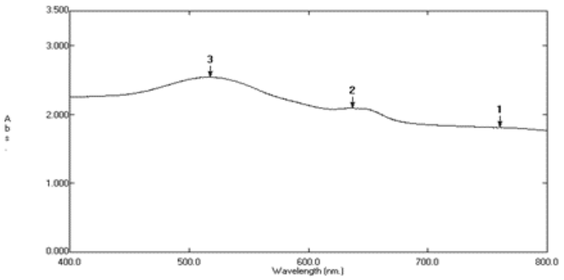

Fig. 5. UV-VIS Absorption Spectrum of Lead

Different series of working solutions having concentrations in the range of 5 to 30 ppm were taken and the absorbance were noted at 513 nm, as shown in Table 8 and the corresponding standard curve was shown in Fig. 6.

Table 8

Reading for standard calibration of lead by colorimetry.

Sr. No	Concentration (ppm)	Absorbance
1	0	0
2	5	0.024±0.0015
3	10	0.059±0.0019
4	15	0.082±0.0016
5	20	0.102±0..0026
6	25	0.131±0.0056
7	30	0.15±0.0019

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

d) Preparation of sample for colorimetry

1.0 mL digested sample was accurately pippeted in the Glass beaker and procedure similar to 4.4.1.3. was carried out for each cosmetic sample. Absorbance was noted at 513 nm against reagent blank.

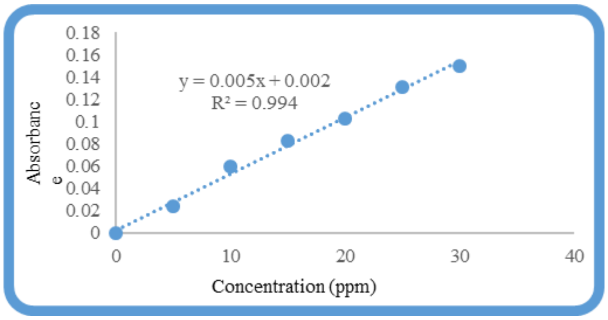

Fig. 6. Standard Calibration curve of lead by Colorimetry

Table 9

Content of lead in cosmetic samples by colorimetry

Sample code	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.056±0.0025	10.5
B	Rose Facewash	0.041±0.0036	9.2
C	Drusthi Eye drop	0.060±0.0056	11.6
D	Dant kanti junior Dental Cream	0.027±0.0011	6.6
E	Haldi chandan kanti Soap	0.012±0.0086	2.5
F	Crack Heal cream	0.062±0.0089	12.0
G	Beauty cream	0.068±0.0079	12.61

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

2) Atomic Absorption Spectrometric method for estimation of Lead

a) Preparation of standard curve of Lead by AAS

- *Stock solution:* 1000 µg/L Cadmium solution in 0.1 M HCl. 10 mL pipetted and diluted up to 100 mL using deionised water.
- *Working solution:* From stock solution pipette out 2.5, 5, 7.5, 10 and 12.5 L and volume was made up to 50 mL using deionised water.

Operational Condition

- *Wavelength:* 217 nm
- *Method:* Flame Absorption
- *Lamp:* Electron Discharge Lamp.
- *Flame colour:* Blue
- *Type of flame:* Air/C_2H_2
- *Fuel Gas Flow Rate:* 1.50 L/min

The procedure was repeated for lead standard solution in the concentration range of 5 to 25 ppm, the absorbance was noted at 217 nm. The result of various concentrations and their corresponding absorption are shown in Table 10 and the standard calibration curve was shown in Fig. 7.

Table 10
Reading for standard calibration of lead by AAS

Sr. No	Concentration (ppm)	Absorbance
1	0	0
2	5	0.00404±0.00011
3	10	0.00622±0.00036
4	15	0.00998±0.00055
5	20	0.01261±0.00077
6	25	0.01561±0.00049

Values are expressed as arithmetic mean: (*n* = 5).
±SD: Standard Deviation

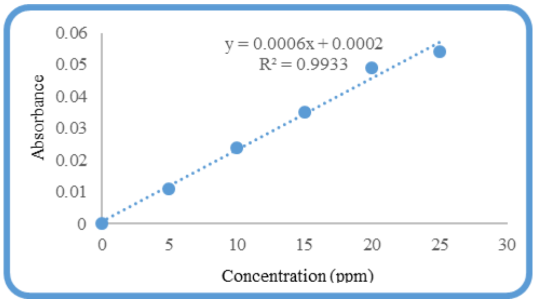

Fig. 7. Standard Calibration curve of lead by AAS

b) Preparation of Sample for AAS

1mL of digested sample was pipetted out and transferred to 100 mL volumetric flask and diluted up to the mark with deionized water.

Table 11
Content of Lead in Herbal cosmetic samples by AAS

Sample Code	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.00641±0.0089	11.0
B	Rose Facewash	0.00622±0.0041	10.02
C	Drusthi Eye drop	0.00781±0.0045	12.55
D	Dant kanti junior Dental Cream	0.00512±0.0085	6.90
E	Haldi chandan kanti Soap	0.00312±0.0051	2.6
F	Crack Heal cream	0.00792±0.0056	12.6
G	Beauty cream	0.00821±0.0012	12.79

Values expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

E. Determination of Mercury

1) Colorimetric Method for determination of Mercury[29]

a) Reagent as per IP

- *Dithiazone solution:* 10 mg of dithizone reagent was dissolved in 1000 mL of Chloroform.

b) Standard solution of Mercury

- *Stock solution (1000 ppm):* Accurately weighed 1.3541 gm of Mercuric chloride was transferred in 1000 mL volumetric flask. 500 mL of 0.5 N hydrochloric acid was added to it. Then diluted to 1000 mL with water.
- *Working solution (100 ppm):* 10 mL of mercury stock solution was taken in 100 mL volumetric flask. 50 mL of 0.5 N hydrochloric acid was added to it and then diluted to 100 mL with Deionized water.

c) Preparation of standard curve

- The working solution was pipetted in the separating funnel along with 10 mL of Dithiazone reagent and shaken vigorously.
- The Dithiazone extract was separated and the extraction process was continued until the green colour of the added dithiazone remained unchanged. Dithizone extract was collected and diluted upon 50 mL with chloroform.

The dithizone extract was scanned in the range of 400 to 800 nm against chloroform as blank and the UV-VIS absorption spectrum was shown in Fig. 8.

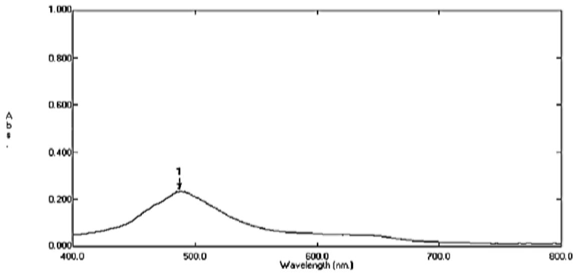

Fig. 8. UV-VIS Absorption spectrum of Mercury

Different concentration of working solution was taken and the absorbance was noted 487 nm using chloroform as blank. The absorbance is noted in Table 12 and the corresponding standard curve was shown in Fig. 9.

Table 12
Readings for standard calibration curve of Mercury by colorimeter

Sr. No	Concentration (ppm)	Absorbance
1	0	0
2	10	0.078±0.0052
3	20	0.131±0.0089
4	30	0.185±0.0056
5	40	0.241±0.0014
6	50	0.282±0.0029

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

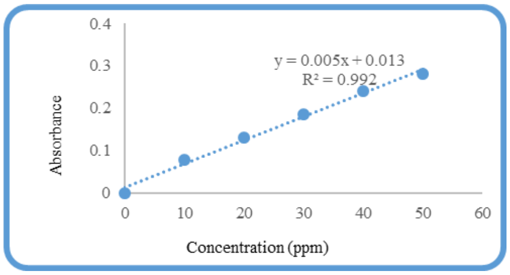

Fig. 9. Standard calibration curve of Mercury by colorimeter

d) Preparation of sample for colorimetry

1. 1 mL digested sample was taken in generator flask.
2. Remaining procedure similar to 4.5.1.3 was carried out for each cosmetic sample.
3. Absorbance was noted at 487 nm against reagent blank.

Table 13
Content of Mercury in Herbal cosmetic samples by colorimeter

Sample Code.	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.321±0.0040	59.59
B	Rose Facewash	0.189±0.0078	31.6
C	Drusthi Eye drop	0.279±0.0012	49.11
D	Dant kanti junior Dental Cream	0.239±0.0056	39.91
E	Haldi chandan kanti Soap	0.135±0.0045	21.11
F	Crack Heal cream	0.192±0.0046	31.99
G	Beauty cream	0.084±0.0079	12.61

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation**.**

2) Atomic Absorption Spectrometric method for estimation of Mercury

a) Preparation of standard curve of Mercury by AAS

- *Stock solution:* 1000 mg/Lit Mercury solution in 0.1 M HCl. Pipette out 10mL and diluted upto 100 mL using deionised water.
- *Standard solution:* From stock solution pipette out 2.5,5,7.5,10 and 12.5 mL and volume was made upto 50 mL using deionised water.

Operational Condition

- *Wavelength:* 253.6 nm
- *Method:* Hydride Generation
- *Lamp:* Hollow Cathode Lamp.
- *Flame colour:* Blue
- *Type of flame:* Air/C_2H_2
- *Fuel Gas Flow Rate:* 1.50 L/min

The procedure was repeated for mercury standard solution in the concentration range of 5 to 25 ppm, the absorbance was noted at 253.6 nm. The result of various concentrations and their corresponding absorption are shown in Table 14 and the standard calibration curve was shown in Fig. 10.

Table 14
Readings for standard calibration curve of Mercury by AAS

S. No.	Concentration (ppm)	Absorbance
1	0	0
2	5	0.343±0.00025
3	10	0.653±0.00056
4	15	0.899±0.00023
5	20	1.195±0.00046
6	25	1.541±0.0025

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

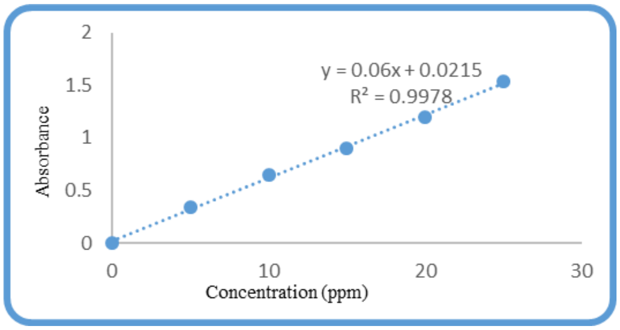

Fig. 10. Standard calibration curve of Mercury by AAS

b) Preparation of Sample for AAS

1 mL of digested sample was pipetted out and transferred to 100 mL volumetric flask and diluted up to the mark with deionised water.

Table 15
Content of Mercury in Herbal cosmetic samples by AAS

Sample Code.	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	2.381±0.0156	61.11
B	Rose Facewash	1.992±0.0125	41.01
C	Drusthi Eye drop	2.164±0.0789	49.10
D	Dant kanti junior Dental Cream	1.999±0.0126	41.06
E	Haldi chandan kanti Soap	1.206±0.0423	22.42
F	Crack Heal cream	1.789±0.0723	32.0
F	Beauty cream	1.231±0.0126	22.79

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

F. Determination of Copper

1) Colorimetric Estimation of Copper[30]

a) Reagents as per IP

- *Dilute Sulphuric acid:* 57mL conc. sulphuric acid and diluted to 1000 mL with deionised water.
- *Phenol Red indicator:* 0.1gm Phenol red, 0.28 mL of 0.1 N. NaOH was dissolved in 2.0 mL of ethanol and diluted to 10 mL with deionized water.
- *Buffer solution:* Dissolved 8.3gm of anhydrous sodium phosphate and 38g of citric acid in 250 mL of deionised water.
- *Dithizone Solution: 10* mg of dithizone reagent was dissolved in 1000 mL of Chloroform.

b) Standard solution of Copper

- *Stock Solution of Copper (100 ppm):* 0.1 gm Dissolved Copper was dissolved in 5mL of Nitric acid and diluted to 1000 mL with dilute Sulphuric acid.
- *Working Solution (10 ppm):* 10 mL from stock solution was diluted to 100 mL with 0.1 N sulphuric acid.

c) Preparation of Standard Calibration Curve

1. Take series of copper solutions in the range of 2 to 10 ppm. 5 mL of dilute sulphuric acid was added and diluted to 20 mL with deionised water.

2. Two drops of phenol red indicator were added and concentrated ammonium hydroxide, drop wise till yellow colour was produced.
3. 2 mL of buffer solution was added in above solution and mixed well then10 mL of dithizone solution was added, shaken well and allowed to separate.
4. The dithizone extract was collected throughout the process and diluted upto 50 mL with chloroform. Scanned in range of 400 to 800 nm against reagent blank. The UV-VIS absorption spectrum of copper was shown in Fig. 11.

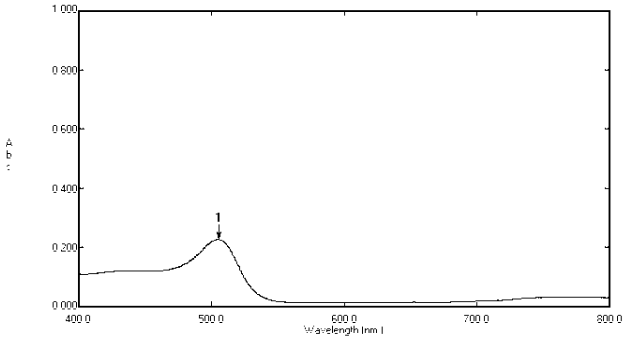

Fig. 11. UV-VIS Absorption Spectrum of Copper

Different concentrations of copper solution was taken and the absorbance was noted at 505 nm against reagent blank and recorded in Table 16 and standard calibration curve was plotted as shown in Fig. 12.

Table 16
Reading of standard calibration curve by colorimetry

S.No.	Concentration (ppm)	Absorbance
1	0	0
2	2	0.040±0.0001
3	4	0.084±0.0008
4	6	0.121±0.0008
5	8	0.168±0.0008
6	10	0.203±0.0002

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

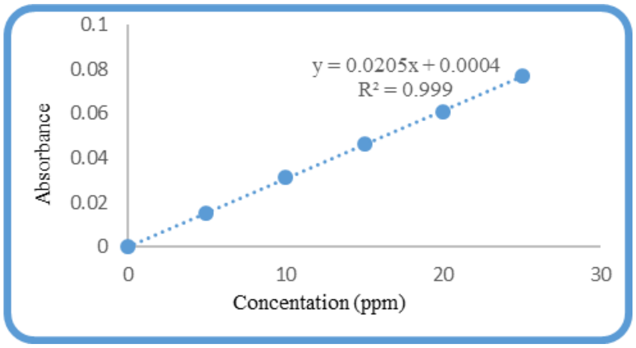

Fig. 12. Standard Calibration curve of Copper by Colorimetry

d) Preparation of sample for Colorimetry

1. 1 mL digested sample was taken in generator flask.
2. Remaining procedure similar to 4.6.1.3was carried out for each cosmetic sample.
3. Absorbance was noted at 505 nm against reagent blank.

Table 17
Content of copper in samples by colorimetry

Sample Code.	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.214±0.0012	11.56
B	Rose Facewash	0.419±0.0056	21.53
C	Drusthi Eye drop	0.612±0.0013	31.79
D	Dant kanti junior Dental Cream	0.274±0.0045	17.41
E	Haldi chandan kanti Soap	0.231±0.0089	15.69
F	Crack Heal cream	0.221±0.0065	12.91
G	Beauty cream	0.394±0.0015	19.0

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation.

2) Atomic Absorption Spectrometric Method for estimation of Copper

a) Standard calibration curve of Copper

- *Stock solution:* 1000 mg/Lit Copper solution in 0.1 M HCl. Pipette out 10mL and diluted upto 100 mL using deionised water.
- *Standard solution:* From stock solution pipette out 2.5,5,7.5,10 and 12.5 mL and volume was made upto 50 mL using deionised water.

Operational Condition

- *Wavelength:* 324.7 nm
- *Method:* Flame Absorption
- *Lamp:* Electron Discharge Lamp.
- *Flame colour:* Blue
- *Type of flame:* Air/C_2H_2
- *Fuel Gas Flow Rate:* 1.50 L/min

The procedure was repeated for copper standard solution in the concentration range of 5 to 25 ppm, the absorbance was noted at 324.7 nm. The result of various concentrations and their corresponding absorption are shown in Table 18 and the standard calibration curve was shown in Fig. 13.

Table 18
Reading of Copper by AAS

S.No.	Concentration (ppm)	Absorbance
1	0	0
2	5	0.0151±0.00012
3	10	0.0315±0.00089
4	15	0.0465±0.00085
5	20	0.0606±0.00012
6	25	0.0769±0.00056

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

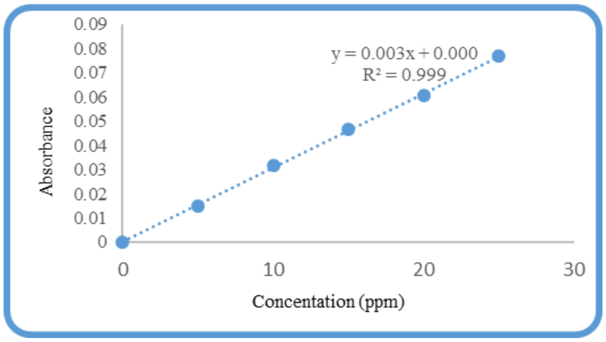

Fig. 13. Readings for standard calibration of Copper by AAS

b) Preparation of Sample for AAS

1 mL of digested sample was pipetted and transferred to 100 mL volumetric flask and diluted up to the mark with deionised water.

Table 19
Content of Copper in Herbal cosmetic by AAS

Sample Code.	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.0345±0.0025	11.70
B	Rose Facewash	0.0672±0.0026	21.77
C	Drusthi Eye drop	0.0812±0.0056	31.82
D	Dant kanti junior Dental Cream	0.0474±0.0025	16.52
E	Haldi chandan kanti Soap	0.0498±0.0085	16.83
F	Crack Heal cream	0.0451±0.0014	13.93
G	Beauty cream	0.584±0.0089	18.21

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

G. *Determination of Cadmium*

1) Colorimetric Method for Estimation of Cadmium[31]

a) Reagents as per IP

- *Potassium tartrate solution:* 250 gm of potassium tartrate was dissolved in deionised water and diluted to 1000 mL.
- *Hydroxylamine hydrochloride solution:* 1.0 gm of Hydroxylamine hydrochloride was dissolved in 50 mL of ethanol (95%) and 1.0 mL of bromophenol blue solution was added to it, 0.1 M Sodium hydroxide was added until the solution became green. and diluted to 100 mL with deionised water.
- *Dithiazone solution:* 10 mg dithiazone dissolved in 1000 mL with Chloroform.
- *Tartaric acid solution:* 20 gm Tartaric acid was dissolved in deionised water and make up to 1000 mL.
- *Thymol blue indicator solution:* 0.1 gm of thymol blue reagent was dissolved in 2.15 mL of 0.1 M sodium hydroxide and 20 mL of ethanol (95%), sufficient deionised water was added to produce 100 mL.
- *Potassium cyanide solution in 0.1 N Sodium hydroxide:* 10 gm of potassium cyanide was dissolved in 0.1 N Sodium hydroxide and diluted to 1000 mL.
- *Sodium hydroxide 6N:* 24.0 gm of Sodium hydroxide dissolved in 100 mL of deionised water.

b) Standard solutions of cadmium

- *Standard solution:* 10 mg of cadmium sulphate was weighed and dissolved in 20 mL water and 5 mLHCl diluted to 100 mL with deionised water.
- *Working solution:* 1 mL of stock cadmium solution pippeted in 100 mL volumetric flask. To it 1 mLHCl was added and diluted to 100 mL with deionised water.

c) Preparation of standard calibration curve

1. Working solution was pipetted in the separating funnel.
2. 1 mL of potassium tartrate solution, 5 mL of potassium cyanide (0.1N sodium hydroxide) solution was added.
3. 1.0 mL of Hydroxylamine hydrochloride solution and 1-2 drops of thymol blue indicator was added, colour of solution was changed to blue.
4. 25 mL of tartaric acid solution was added.
5. The solution become colourless,
6. The colourless solution was transferred to separating funnel along with 15 mL of Dithizone solution.
7. The content were shaken vigorously and the dithiazone layer was separated and extraction was continued with a fresh 10 mL of dithiazone.
8. The dithiazone extract was collected through the process and diluted up to 50 mL with chloroform.
9. The pink colour of the dithiazone extract was read in the colorimeter against the chloroform as a blank and scanned in range of 400 to 800 nm as shown in Fig. 14.

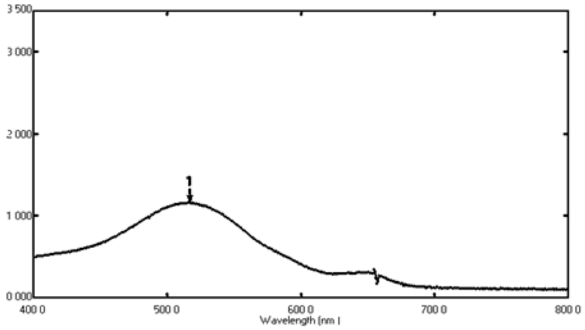

Fig. 14. UV-VIS Absorption maxima of Cadmium

The same procedure was repeated by taking different concentrations of the standard Cadmium solution. The readings are shown in Table 20. The standard curve was plotted and shown in Fig. 15.

Table 20
Readings of Standard curve for Cadmium by colorimetry

Sr. No	Concentration (ppm)	Absorbance (nm)
1	0	0
2	10	0.078±0.0025
3	20	0.131±0.0069
4	30	0.185± 0.0056
5	40	0.241±0.0025
6	50	0.282±0.0025

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

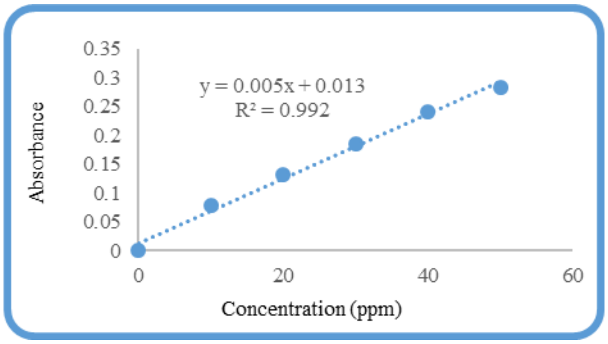

Fig. 15. Standard curve of Cadmium by colorimetry

c) Preparation of sample for colorimetry

1.0 mL digested sample was accurately pippeted in the Glass beaker and procedure similar to 4.7.1.3. was carried out for each cosmetic sample. Absorbance was noted at 517 nm against reagent blank.

Table 21
Content of cadmium in cosmetic samples by colorimetry

Sample Code	Sample Name	Absorbance	Concentration (ppm)
A	Kesh kanti Shampoo	0.0143±0.0052	1.12
B	Rose Facewash	-	BDL
C	Drusthi Eye drop	0.0015±0.0056	0.19
D	Dant kanti junior Dental Cream	0.0021±0.0053	0.26
E	Haldi chandan kanti Soap	0.0079±0.0069	0.82
F	Crack Heal cream	0.0052±0.0025	0.64
G	Beauty cream	0.0082±0.0056	0.89

Values are expressed as arithmetic mean: (*n* = 5).
BDL: Below detectable limit.
±: Standard Deviation

2) Atomic Absorption Spectrometric method for Estimation of Cadmium

a) Preparation of standard curve by AAS

- *Stock solution:* 1000 mg/Lit Cadmium solution in 0.1 M HCl. Pipette out 10mL and diluted upto 100 mL using deionised water.
- *Standard solution:* From stock solution pipette out 2.5, 5, 7.5,10 and 12.5 mL and volume were made upto 50 mL using deionised water.

Operational condition

- *Wavelength:* 228.8 nm
- *Method:* Flame Absorption
- *Lamp:* Electron Discharge Lamp.
- *Flame colour:* Blue
- *Type of flame:* Air/C_2H_2
- *Fuel Gas Flow Rate:* 1.50 L/min

The procedure was repeated for cadmium standard solution in the concentration range of 5 to 25 ppm, the absorbance was noted at 228.8 nm. The result of various concentrations and their corresponding absorption are shown in Table 22 and the standard calibration curve was shown in Fig. 16.

Table 22
Readings of Standard curve for cadmium by AAS

Sr. No	Concentration (ppm)	Absorbance (nm)
1	0	0
2	5	0.059±0.023
3	10	0.126±0.052
4	15	0.186±0.01
5	20	0.231±0.018
6	25	0.301±0.018

Values are expressed as arithmetic mean: (*n* = 5).
±: Standard Deviation

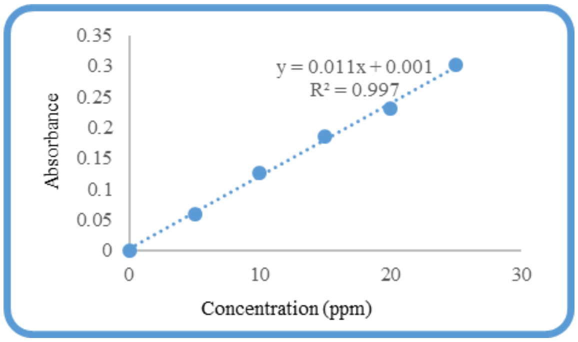

Fig. 16. Standard curve of Cadmium by AAS

3) Preparation of Sample for AAS

1 mL of digested sample was pipetted and transferred to 100 mL volumetric flask and diluted up to the mark with deionized water.

Table 23
Content of Cadmium in Herbal cosmetic sample by AAS

Sample Code.	Sample Name	Absorbance (nm)	Concentration (ppm)
A	Kesh kanti Shampoo	0.015	1.26±0.0056
B	Rose Facewash	0.0072	0.63±0.0056
C	Drusthi Eye drop	-	BDL
D	Dant kanti junior Dental Cream	0.0032	0.28±0.0089
E	Haldi chandan kanti Soap	0.0048	0.38±0.0026
F	Crack Heal cream	-	BDL
G	Beauty cream	-	BDL

Values are expressed as arithmetic mean: (*n* = 5).
BDL: Below detectable limit.±: Standard Deviation

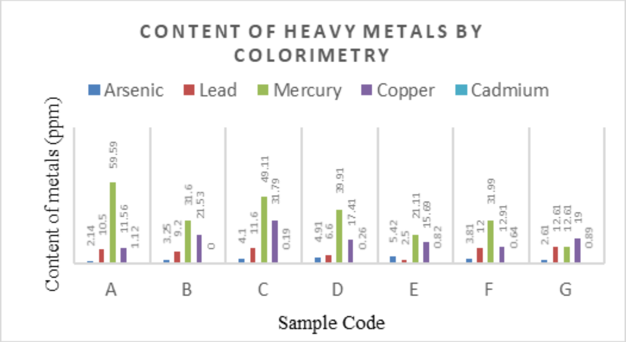

Fig. 17. Content of Heavy metals by colorimetric methods

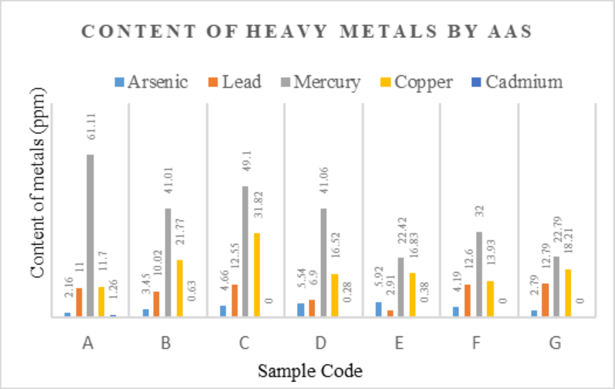

Fig. 18. Content of Heavy metals by AAS

4. Discussion and conclusion

A. Discussion

Seven Patanjali Herbal Cosmetic formulations were studied by using colorimetry and AAS method for heavy metal, namely, Arsenic, Lead, Mercury, Copper and cadmium. Herbal cosmetic samples were subjected to Acid digestion. Each Samples was run five times and their mean was used.

1) Arsenic

In the limit test of arsenic standard stain was compared with sample stain, distinct spots for arsenic was observed. Arsenic was found in the range of 2.14 to 5.42 ppm, by colorimetric method. By AAS in Herbal cosmetic Arsenic was found in range of 2.79 to 5.54 ppm. The lowest amount of arsenic was found in shampoo (A) and highest found in Haldi chandan soap (E) by AAS and colorimetry both. All the seven samples showed exceeding limits when compared with limits prescribed BIS and D & C act i.e. of 2 ppm.

2) Lead

By colorimetric method, Lead was found in the range of 2.5 to 12.61 ppm. And by AAS method. Lead was found in the range of 2.6 to 12.79 ppm. The lowest amount of lead was found in Facewash (B) and highest found in Beauty cream (G). In the seven samples lead was found within limits when compared with limits prescribed by BIS and D & C act i.e. of 20 ppm.

3) Mercury

By colorimetric method, mercury was found in the range 9.1 to 59.61 ppm. And by AAS method, mercury was found in the range of 12.42 to 61.18 ppm. The lowest amount of mercury was found in Beauty cream (G) and highest found in shampoo (A) by both methods. In the seven samples mercury was found within limits when compared with limits prescribed by BIS and D & C act i.e. of 100 ppm.

4) Copper

By colorimetric method, copper was found in the range 11.56 to 31 ppm. And by AAS method, copper was found in the range 11.70 to 31.32 ppm. The lowest amount of Copper was found in shampoo (A) and highest found in Eye drop (C) by colorimetry and AAS method. In the seven samples copper was found within limits when compared with limits prescribed by BIS and D & C act i.e. of 100 ppm.

5) Cadmium

By colorimetric method, cadmium was found in the range 0.19 to 1.12 ppm. And by AAS method, cadmium was found in the range 0.38 to 1.26 ppm. The lowest amount of Cadmium was found in Dant Kanti junior dental cream (D) and highest found in shampoo (A). Out of seven sample studied by colorimetry cadmium was found BDL in Rose Facewash, whereas by AAS cadmium was found BDL in Drusthi eye drop, Crack heal cream, Beauty cream and Rose Facewash. But all in within limit when compared with limits prescribed by BIS and D & C act i.e. of 100 ppm

The toxic heavy metals, arsenic, lead, copper and mercury were detected in all cosmetic products. But, only arsenic exceeds the limit, whereas others found in safe limit. The presence of heavy metal in cosmetic can cause serious problem to consumer as they can cause premature ageing of the skin, skin allergies and skin cancer.

B. Conclusion

The study concluded that, the herbal cosmetic product contained toxic heavy metal arsenic above the permissible limit where as other are within limit prescribed by BIS and D & C Act. The presence of heavy metal in cosmetic can cause serious problem to consumer, thus there is an urgent need for constant quality assessment in order to ensure safety.

5. Summary

Herbal Cosmetics are very popular in India because of their fever side effects, effectiveness, and availability and low cost. In this study we evaluated the Herbal Cosmetics preparations the presence of toxic heavy elements. Out of seven selected brands for arsenic content exceeds. Lead, Mercury, Cadmium, Copper was found within limit. Cadmium was found in four brands namely, Rose Facewash, Drusthi Eye drop, Crack Heal cream and Beauty cream below the detectable limit.

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