Determination of heavy metals in toothpastes containing tin as an active ingredient

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This study discusses the technical rationale behind the development and validation of an alternative AAS and colorimetric methods for the determination of heavy metals in toothpastes containing tin as an active ingredient. Using BIS 6356:2001 method, it is found to be difficult to analyze toothpastes containing <100 ppm Pb, based on the PbS color formed by addition of sodium sulfide. In the present investigation an alternative, highly sensitive and specific AAS method has been developed and validated. The minimum detection limits are found to be 0.222 µg/mL, 0.010 µg/mL, 1.873 ng/mL, 5.465 ng/mL, 2.949 µg/mL for Pb, Cd, Hg, As and Sn respectively.

Keywords: Atomic absorption spectrophotometric method, Colorimetry, Heavy metals, Toothpastes

Heavy metals such as lead, mercury, arsenic and cadmium are known to have negative impact on human health. For example, lead (Pb) is toxic even when present at a very low level and causes abnormal behavior in humans. Characteristics of lead toxicity include anemia, colic, neuropathy, sterility and coma. Mercury compounds are known for fatal damage of human nervous system. Cadmium and its compounds are carcinogenic and arsenic poisoning and gangrene disease are well-known. Organo tin compounds are as toxic as cyanides. Therefore, the regulatory agencies have set certain standards for the maximum limit of heavy metals in cosmetics, detergents and toothpastes. Toothpastes are defined as dentifrices in the form of a smooth, semisolid and homogeneous mass containing acceptable ingredients such as abrasives/polishing agents, surface active agents, humectants, binding agents, and other appropriate substances for oral health maintenance. These products shall comply with BIS: 6356:2001 specifications according to which the maximum limit of heavy metals in toothpastes is 20 ppm (ref.3). Thus, determination of heavy metals in toothpastes necessitates the establishment of rapid, reliable and accurate methods free from matrix interferences. Although a few methods for the determination of fluoride and monofluorophosphate in toothpaste have been reported in literature, there are a limited number of reports available on the determination of heavy metals in toothpastes.

Chauhan et al. have reported the determination of lead and cadmium in different cosmetics, viz, soap, face cream, shampoo and shaving cream by atomic absorption spectrometry. Popova et al. have discussed the application of atomic absorption spectrometry (AAS) for control of heavy metals in toothpastes. United States Pharmacopeia (USP) adopts a colorimetric method for the determination of heavy metals in drugs and pharmaceuticals. However, these methods are neither specific nor sensitive enough and also do not provide adequate recovery of the elements being tested. The colorimetric methods are based on 100 years old wet chemistry principles involving the precipitation of metal sulfides and do not work well for the modern pharmaceutical industry. The current method of testing heavy metals in dentifrice in India is as per BIS 6356:2001. Using this method, heavy metals in toothpaste are analyzed by a simple colorimetric method, which relies on the visual observation of dark color of metal sulfides formed on addition of sodium sulfide. However, with newer dentifrice formulations containing tin as one of the active ingredients, the results obtained by colorimetric method do not give the real content of heavy metals in toothpastes. These methods often give results as out of specification (≥ 20 ppm), which may not be true in most of the formulations containing tin in the formula. Although, tin may be considered as one of the heavy metals but it is one of the desired ingredients of such newer formulations. It necessitates development of specific methods which can assess the levels of...
toxic metals Pb, Cd, Hg and As in formulations with high content of tin. In such cases, methods based on AAS and ICP-MS are preferred for control of heavy metals in toothpastes. Since toothpastes are considered to be highly complex disperse systems with different organic constituents, such as glycerin, aerosyl and formaldehyde, their sample preparation is quite difficult. The present paper reports the results of an investigation proposed to understand the technical rationale behind the validity of colorimetric methods and development of an alternative AAS method for the determination of heavy metals in toothpastes.

**Experimental Procedure**

**Chemicals and reagents**

Nitric acid, hydrochloric acid, sulphuric acid, hydrofluoric acid, acetic acid, ammonium hydroxide, sodium hydroxide, lead nitrate, glycerol, thioacetamide, hydrogen peroxide, sodium borohydride, chloroform, ammonium pyrroline dithio carbamate, cadmium carbonate, arsenic pentaoxide, mercury (II) iodide (S.D.Fine Chemicals, Mumbai, India) and ICP-OES standards for Pb, Cd, As, Hg & Sn (MERCK- KGaA, Darmstadt, Germany) were used. Platinum and silica crucibles were used for sample digestion. Five samples of toothpastes, such as Sample A containing high level of tin, sorbitol, water, SLSS, stannous salt, CMC, sodium gluconate, flavor; Sample B without tin, sorbitol, SLSS, silica, water, MSP, CMC, flavour; Sample C containing low level of tin, glycerin, flavor, hydroxymethylcellulose, stannous salt; Sample D without tin, sorbitol, silica, SLSS, copolymer, flavor, titanium dioxide, pigment; and Sample E containing high level of tin were used. These samples were obtained from one of the toothpaste manufacturing industries, Mumbai, India.

**Preparation of reagents**

Reagents, viz. thioacetamide, glycerol mixture and acetate buffer were prepared according to the procedures given by BIS: 6356:2001. A stock solution of 100 ppm lead nitrate was prepared by dissolving 0.1599 g of lead nitrate in 1000 mL of water containing 1 mL of nitric acid. 10 mL of the stock solution was diluted to 100 mL to prepare 10 ppm standard solution of lead nitrate. Similarly other standard solutions of cadmium, mercury and arsenic were prepared.

**Sample preparation**

Due to the complex nature of toothpaste five different methods of sample preparation were investigated. Five sample preparation methods are discussed below:

**Procedure 1**

Sample preparation was carried out according to BIS method IS: 6356:2001. Around 2 g of toothpaste sample was weighed in a platinum crucible and incinerated at 550°C for 2 h. The crucible was allowed to cool at room temperature; 2 mL of aqua-regia was added and solution was heated on steam bath till dryness. The residue obtained was dissolved in 5 mL hot water, evaporated to dryness, treated with hydrofluoric acid, and evaporated again to dryness. It was diluted with water up to 100 mL, filtered, and then washed. A blank was also prepared similarly without using toothpaste.

**Procedure 2**

Sample preparation was carried out according to USP 231. Around 2 g of toothpaste sample was weighed in a silica crucible and then 2 mL of conc. HCl and 6 mL of conc. HNO₃ were added, heated at 140°C, and cooled to room temp. To the above solution, 2 mL of HF was added and heated at 140°C till the HF is ceased. To the residue obtained, 2 mL of conc. H₂SO₄ was added and heated at 140°C. Later, 10 mL of HNO₃ was added and heated until the solution becomes clear. The solution obtained was diluted to 25 mL with deionized water and used for the determination of heavy metals by AAS.

**Procedure 3**

Sample digestion was carried out in a crucible made up of silica instead of platinum. Around 2 g of toothpaste sample was weighed in a silica crucible and incinerated for about 2 h at 550°C. The solution was then cooled followed by the addition of 1.5 mL hydrochloric acid and 0.5 mL nitric acid and finally the evaporation to dryness on the steam bath. The residue obtained was dissolved in 5 mL hot water, evaporated to dryness, treated with hydrofluoric acid, and evaporated again to dryness. It was diluted with water, filtered, washed and made up to 25 mL.

**Procedure 4**

Around 2 g of toothpaste sample was weighed in 150 mL conical flask containing 5 mL of water and 5 mL of HNO₃ and stirred until toothpaste dissolved at low heat. The solution was then cooled and added...
with 1.5 mL of 30% H₂O₂ solution and stirred until 
H₂O₂ decomposes. The pH was adjusted at 1.1-1.2 
with 1% NH₄OH solution and solution was filtered.

**Extraction of lead**

To the above solution, 1 mL of 2% ammonium 
pyrroline dithiocarbamate (APDC) solutions was 
added, shaken and kept as such for 3 min followed 
by the addition of 10 mL CHCl₃ and shaking for 2 min. 
From the above separated solutions, CHCl₃ was 
transferred to another separating funnel, and with 
another 10 mL of CHCl₃ the extraction process was 
repeated. All extracted solutions were combined and 
10 mL of Hg²⁺ was added and shaken for 2 min. The 
upper layer separated was used for the determination 
of Pb content using AAS.

**Procedure 5**

Sample digestion was carried out in a microwave 
oven. Around 250 mg of the toothpaste was weighed 
into the digestion vessel, and 5 mL of HNO₃ and 
2 mL of HF were added. The mixture was then shaken 
carefully with a clean glass rod for 10 min before the 
vessel is closed and heated in a microwave oven as 
per the operating program given below. Blank 
solutions were prepared using following respective 
conditions without taking the sample:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td>170</td>
<td>190</td>
<td>100</td>
</tr>
<tr>
<td>Ta, min</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Time, min</td>
<td>5</td>
<td>30</td>
<td>15</td>
</tr>
</tbody>
</table>

**Colour development**

An aliquot of 25.0 mL of the above test solutions 
was taken separately in a 50.0 mL Nessler cylinder. 
Now to 2.0 mL of test solution, 2.0 mL acetate buffer 
(pH 3.5) was added and mixed well. 1.2 mL of 
thioacetamide reagent was added, diluted to 50.0 mL 
and allowed to stand for 2 min. In the 2nd Nessler 
cylinder, 1 mL of standard lead solution (10 ppm) 
and 2.0 mL of test solution were taken and diluted with 
water to 25.0 mL then 2.0 mL of acetate buffer 
(pH 3.5) was added and mixed well. After that 1.2 mL 
of thioacetamide reagent was added, diluted with 
water to 50 mL and allowed to stand for 2 min. The 
color produced in the 2 Nessler cylinders 
was compared.

**Apparatus**

Analyst 300 model atomic absorption spectrometer 
(Perkin Elmer, Massachusetts, USA) attached with

MHS 15 model mercury hydride generator and PerkinElmer® Model ELAN® DRC™ II ICP mass spectrometer (PerkinElmer, Inc., Shelton, CT, USA) were used. Nessler® cylinders procured from local market were used for the comparison of colours.

**Results and Discussion**

**Evaluation using BIS: 6356:2001 method**

Initially, the colorimetric method adopted by BIS: 
6356:2001 was followed to determine the content of 
heavy metals in toothpastes. The samples were 
prepared as per Procedure 1. During sample 
preparation, it is observed that all the solutions have 
become orange-red in colour. Probably, it could be 
due to the reaction of aquaregia with Pt and formation 
of orange-red coloured chloroplatinic acid (H₂PtCl₄), 
as given below:

\[
\text{Pt} [s] + 2 \text{HNO}_3 [aq] + 4 \text{HCl} [aq] \rightsquigarrow [\text{NO}]_2 \text{PtCl}_4 [s] + 3 \text{H}_2\text{O} [l] + \frac{1}{2} \text{O}_2 [g] \tag{1}
\]

\[
[\text{NO}]_2 \text{PtCl}_4 [s] + 2 \text{HCl} [aq] \rightsquigarrow \text{H}_2\text{PtCl}_4 [aq] + 2 \text{NOCl} [g] \tag{2}
\]

After adding thioacetamide reagent, the samples 
developed dark colours as shown in Fig. 1. Reaction 
mechanism is given below:

\[
\text{M}^{2+} + \text{CH}_3\text{C}[\text{S}] \text{NH}_2 + \text{H}_2\text{O} \rightsquigarrow \text{MS} + \text{CH}_3\text{C}[\text{O}] \text{NH}_2 + 2 \text{H}^+ \quad (M = \text{Ni, Pb, Cd, Hg, etc.})
\]

Accordingly, an inference could be drawn that all 
the samples (A-E) contain heavy metals out of 
specification, i.e. >20 ppm. However, this is found to 
be not true when the same samples were analyzed by 
the method adopted by USP for testing heavy metals 
in drugs and pharmaceuticals. When samples were 
prepared as per Procedure 2, clear solutions are

![Fig. 10 Colour developed in samples prepared as per Procedure 1](image-url)
obtained and the solutions do not develop orange-red colour, as in case of BIS method. This is also confirmed by digesting the toothpaste samples using BIS procedure not in a Pt but silica crucible. It shows that there is no reaction of Pt with the acids that are used in Procedure 2. This is also confirmed by digesting the same samples in a crucible made up of silica instead of platinum as described in Procedure 3. Clear solutions are obtained without any colour. On addition of thioacetamide, dark colours are developed. These are compared with the standard and it is inferred that Samples A & E may contain heavy metals out of specification. However, this is also not true because Samples A & E have high content of tin and it is possible that the thioacetamide might react with tin to form a brown-black SnS precipitate along with PbS, as shown below:

\[ \text{Sn}^{2+} + \text{CH}_3\text{C}[\text{S}]\text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{SnS} + \text{CH}_3\text{C}[\text{O}]\text{NH}_2 + 2 \text{H}^+. \]

A calibration with different concentrations of Pb and Sn standard solutions indicates that sensitivity of the colorimetric method varies for different elements (Fig. 2). Further, the colours of metal sulphides that arise in test solutions are found to be different, i.e., white, yellow, orange, brown or black, complicate the comparison to dark brown lead sulphide. Further, it is found difficult to distinguish the color of PbS in a toothpaste containing < 100 ppm Pb. Thus, the colorimetric methods adopted by BIS and USP are found to be not selective and sensitive enough for determination of heavy metals in toothpastes. Moreover, these are not quantitative but are qualitative in nature. To fulfill the requirement of accuracy, sensitivity, specificity and limits of detection based on toxicology of individual metals, USP describes the need for new testing approaches and suggests the use of either plasma or atomic absorption spectroscopic (AAS) techniques. AAS is quite preferable for this purpose due to the high sensitivity of determinations combined at the same time with high selectivity. However, its application requires a judicious choice of sample preparation due to the complex nature of toothpastes.

Development of AAS method

The contents of Pb, Cd, Sn, Hg and As in toothpastes were determined at different wavelengths (Table 1). Due to the volatile nature of Hg and As, a hydride generator was used for their determination by AAS. The optimum conditions used for the determination of heavy metals in toothpastes are given in Table 1. Four different procedures (Procedures 2, 3, 4 and 5) were used for the preparation of samples. The results are given in Table 2. It could be observed that the impact of sample preparation procedure (Procedures 2 & 3) on determination of Pb is minimum as compared to other elements, viz., Cd, Sn, Hg and As. The variation in Pb content obtained by Procedures 2 & 3 was found to be very insignificant. These results support that the sample preparation procedure has no effect on the determination of Pb by AAS. However, in case of Hg and As, the effect of sample preparation procedure is found to be significant. Sample preparation Procedure 4 is specific and involves extraction of Pb by forming a complex with ammonium pyrroline dithio carbamate (APDC). AAS analysis of the organic layer for Pb has been investigated and the possibilities of the chemical and spectral interferences are noticed. The limited stability of lead complex in the organic layer, the stability of the solvent in water and incompleteness of the extraction may contribute to errors in determination of lead. Furthermore, standard solutions must be extracted in the same manner for calibration, so the method is time consuming and laborious. Sample preparation Procedure 5 involving microwave digestion yields low recovery of heavy metals from
toothpaste. As the microwave digester (Procedure 5) needs very low quantity of sample (< 0.2g), the concentration of heavy metals in the prepared solutions does not reach the detection limits of AAS. It could be due to the formation of turbidity and incomplete digestion of samples. A greater part of the elements might have been captured in the sediments and hence this method is not found to be suitable for sample preparation for AAS. Even though Procedures 2 and 3 are found to be suitable for determination of Pb, Procedure 2 is followed for the determination of all the elements such as Pb, Cd, Hg, As and Sn in toothpastes because of the loss of Hg and As using Procedure 3. Therefore, in all cases Procedure 2 is followed for the determination of Pb, Cd, Sn, Hg and As in toothpastes. The results obtained as an average of 3 determinations are given in Table 3. As the sample concentration is found to be below the detection limits of AAS for Pb, it is cross checked by ICP-MS and the results are given in Table 3. It may be noted that the detection limit for Pb by ICP-MS is 1.2 ng/g.

Validation

Accuracy

Accuracy of the method was determined by studying the recovery of all elements from the sample solutions spiked with each element at three different concentration levels in triplicate. After sample digestion and preparation of solutions as per procedure 2, different concentrations of Pb, Cd, Hg, and As are spiked to the solutions. The recoveries and RSDs of all the elements are found to be in between 71.9-114.5% and 0.9-25.3% respectively Table 3.

Precision

The precision of the method is studied with respect to both repeatability and intermediate precision. Toothpaste sample solution was analysed to evaluate

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Gas</th>
<th>Atomized temp. °C</th>
<th>Sample A µg/g</th>
<th>Sample B µg/g</th>
<th>Sample C µg/g</th>
<th>Sample D µg/g</th>
<th>Sample E µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb 283.3</td>
<td>Air-acetylene 2300</td>
<td>1.57 (1.86)*</td>
<td>0.92 (0.87)*</td>
<td>1.58 (1.34)*</td>
<td>0.84 (1.02)*</td>
<td>1.26 (1.08)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cd 228.8</td>
<td>Air-acetylene 2300</td>
<td>&lt;0.031</td>
<td>&lt;0.031</td>
<td>&lt;0.031</td>
<td>&lt;0.031</td>
<td>&lt;0.031</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hg 253.6</td>
<td>Argon Cold-vapor technique</td>
<td>0.008</td>
<td>0.050</td>
<td>0.060</td>
<td>0.032</td>
<td>0.014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As 193.7</td>
<td>Air-acetylene 2300</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sn 224.6</td>
<td>Nitrous oxide-acetylene 2900</td>
<td>5658</td>
<td>&lt;8.84</td>
<td>2013</td>
<td>&lt;8.84</td>
<td>5795</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*By ICP-MS.

Table 2 Impact of sample preparation methods on Pb analysis by AAS

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Sample A, µg/g</th>
<th>Sample B, µg/g</th>
<th>Sample C, µg/g</th>
<th>Sample D, µg/g</th>
<th>Sample E, µg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure 2</td>
<td>1.68</td>
<td>0.85</td>
<td>1.25</td>
<td>0.89</td>
<td>1.63</td>
</tr>
<tr>
<td>Procedure 3</td>
<td>1.44</td>
<td>0.78</td>
<td>1.12</td>
<td>0.99</td>
<td>1.45</td>
</tr>
<tr>
<td>Procedure 4</td>
<td>1.57</td>
<td>0.92</td>
<td>1.58</td>
<td>0.84</td>
<td>1.26</td>
</tr>
</tbody>
</table>

Table 3 Accuracy with % recovery and RSD of samples [No. of observations (n) = 3]

<table>
<thead>
<tr>
<th>Metal</th>
<th>Sample A</th>
<th>Sample B</th>
<th>Sample C</th>
<th>Sample D</th>
<th>Sample E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb 1 ppm spiking</td>
<td>% Recovery</td>
<td>% RSD</td>
<td>% Recovery</td>
<td>% RSD</td>
<td>% Recovery</td>
</tr>
<tr>
<td>Cd 0.5 ppm spiking</td>
<td>112.197</td>
<td>10.290</td>
<td>113.182</td>
<td>10.840</td>
<td>112.939</td>
</tr>
<tr>
<td>Hg 10 ppb spiking</td>
<td>75.145</td>
<td>19.322</td>
<td>87.680</td>
<td>6.656</td>
<td>77.579</td>
</tr>
<tr>
<td>As 30 ppb spiking</td>
<td>89.749</td>
<td>9.410</td>
<td>77.658</td>
<td>11.631</td>
<td>71.910</td>
</tr>
<tr>
<td>Sn 50 ppm spiking</td>
<td>95.342</td>
<td>5.879</td>
<td>100.242</td>
<td>1.354</td>
<td>94.776</td>
</tr>
</tbody>
</table>
the precision of the system. Repeatability was demonstrated by analyzing separate sample solutions spiked with all the elements at 10 ng/mL-50 µg/mL levels. The intermediate precision of the method was determined on separate sample solutions prepared by spiking the substances at specification level on three consecutive days Table 4.

Linearity, range, LOD and LOQ

Limit of detection (LOD) was measured as the lowest amount of the analyte that could be detected to produce a significant response. It is approved by calculations based on the standard deviation of the response (û) and the slope (S) of the calibration curve at the levels approaching the limits according to equation LOD = 3.3 (û/S) and Limit of quantification (LOQ) = 10 (û/S). The LOQ and LOD for Pb are set as 0.12 and 0.03% respectively. The linearity, range, LOD and LOQ are given in Table 5.

Conclusion

It is inferred that the sample preparation Procedure 2 gives reasonable results for the determination of heavy metals in toothpastes. It eliminates the problems and errors associated with extraction of heavy metals. The method is relatively free from matrix interferences and shortness of the analysis time. Since the sample digestion is carried out at a relatively low temperature, compared to procedure 1, good recoveries for volatile elements such as mercury and arsenic are obtained. Sample preparation procedure 4 involves dissolution of the ashed samples in aqueous medium, formation of ammonium pyrrolidine dithiocarbamate (APDC) complex and extraction into chloroform. AAS analysis of the organic layer for lead has been investigated and the possibilities of the chemical and spectral interferences have been noticed. The limited stability of lead complex in the organic layer, the stability of the solvent in water and incompleteness of the extraction may contribute to errors in determination of lead. Furthermore, standard solutions are also extracted in the same manner for calibration, and the method is found to be time consuming and laborious. Sample preparation procedure 5 involving microwave digestion yields low recovery of heavy metals from toothpaste. It could be due to the formation of turbidity and incomplete digestion of samples. A greater part of the elements might have been captured in the sediments and therefore this method is found not to be suitable for sample preparation for AAS.

References


<table>
<thead>
<tr>
<th>Table 4</th>
<th>Intra-day and Inter-day precession data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Content in toothpaste, µg/g</td>
</tr>
<tr>
<td>Pb</td>
<td>1.68</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;0.031</td>
</tr>
<tr>
<td>Hg</td>
<td>0.008</td>
</tr>
<tr>
<td>As</td>
<td>&lt;0.016</td>
</tr>
<tr>
<td>Sn</td>
<td>5658</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Linearity, range, LOD and LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>Range</td>
</tr>
<tr>
<td>Pb</td>
<td>0.66-20 ng/mL</td>
</tr>
<tr>
<td>Cd</td>
<td>0.031-2 ng/mL</td>
</tr>
<tr>
<td>Hg</td>
<td>5.62-25 ng/mL</td>
</tr>
<tr>
<td>As</td>
<td>16.4-50 ng/mL</td>
</tr>
<tr>
<td>Sn</td>
<td>8.85-300 ng/mL</td>
</tr>
</tbody>
</table>