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ICP-MS STUDY OF TRACE ELEMENTAL BUILD-UP IN SOLID PHARMACEUTICALS: POTENTIAL ENVIRONMENTAL AND BIOMEDICAL IMPACT

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ABSTRACT

This paper primarily examines the capability of using ablative laser technology to assess the uniformity of distribution of trace elements in highly processed solid pharmaceutical samples. The study investigated elemental profiles in the solid phase, both spatially and depth-wise using a 55 µm-diameter laser beam coupled to an ICP-MS instrument. Solids were subjected to 213-nm irradiation along a 4-point grid, each point separated by a distance of 1.0 mm. The level of the beam energy was 70%, and the laser was programmed to ablate a depth of 5 µm at each point, with iterative scanning, recording measurements after each ablation to a total depth of 150 µm. The experimentally determined laser results showed fluctuations in characteristic metal intensity and, in some cases, 'hotspots' (or build-up) were observed. The exact mechanism for this random accumulation of trace metals is not clear, but could be partially attributed to uneven mixing. For purposes of general comparison, total concentrations of elements in the individual solid samples, digested in aqueous media, were also determined. These absolute data reflected elevated levels of some toxic metals, which could be linked to potential environmental and biomedical effects.

Keywords: ICP-MS, biomedical, environmental, pharmaceutical, trace metals.

INTRODUCTION

This study explores an ultrasensitive laser technique for assessing the distribution of selected trace and toxic elements in solid pharmaceutical samples, and pinpointing inner areas and regions on the surfaces of such samples to study depth and spatial dispersion of these elements. Laser ablation (LA) depth-profiling has the capability of 'drilling' through a solid matrix and obtaining relevant information on the distribution of undesirable contaminants. The laser itself is linked to an inductively coupled plasma-mass spectrometry instrument (LA-ICP-MS). The technique is semi-quantitative and capable of detection over a wide range of elemental levels. Very few contemporary instrumental methods have the capability to study metal intensity with depth without the scientist first manually cutting the sample beforehand (which increases the risk of contamination). X-ray methods are useful, but lack the ability to control depth penetration. Nuclear particle irradiation, SEM, SIMS and XPS are equally useful, but such techniques tend to be limited to only a few microns below the surface. The competence, therefore, of the laser approach to delve to discreet depths below the surface of a sample is attractive for homogeneity studies in bulk materials.

To date, there have been relatively few studies published on the use of LA-ICP-MS for the depth profiling of elements in pharmaceutical samples. In one example (Lam and Salin, 2004), the technique was used on Neusilin tablets. They found the detection limits for Al and Mg were 40 µg g^{-1} and 6 µg g^{-1} (ppm), respectively. Typically, relative standard deviations of 47-61% were obtained, improving to 29% when signal ratios were used. They concluded, however, that LA-ICP techniques may find some use for the analysis of tablets. In another study (Santamaria-Fernandez *et al.*, 2008), a variation on the theme of LA-ICP-MS was used by incorporating a multicollector (MC) for the analysis of counterfeit tablets. The active ingredient of the tablets was a sulphur-containing substance. This fact was exploited by using the characteristic sulphur isotopic signature of the compound. More specifically, $\delta^{34}S$ measurements were made with silicon internal standardization. The authors concluded that the laser ablation MC-ICP-MS technique has possible use as a quick screening method for the detection and classification of counterfeit pharmaceuticals. In the present study, the authors subjected suitable tablets/lozenges (painkillers, antipyretics) of common, readily-available pharmaceutical formulations to chemical analysis by LA-ICP-MS. This is of interest in certain environmental and biomedical studies, because it not only informs of metal levels in the tablets and lozenges, but also how the concentrations vary with depth. A study of this nature could be of use in the pharmaceutical industry for: quality control; producing more homogeneous mixtures; identifying counterfeit batches of tablets; and constructing a database on competitors' products. Our

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research is original from the perspective that new and essential knowledge is gained on random accumulation of trace metals in a range of solid pharmaceutical products. In addition, absolute ICP-MS information is presented for many elements, which could serve as useful baseline data for the pharmaceutical industry.

MATERIALS AND METHODS

LA-ICP-MS/Sample Handling

Laser ablation technology coupled to ICP-MS has been described before (Jarvis *et al*., 1992; Harris, 1999; Robinson *et al*., 2005). In this study, 48 laser data points were recorded from six solid pharmaceutical products obtained from local retail outlets. Each product possessed separate active ingredients as follows: Product 1, diclofenac diethylamine; Product 2, paracetamol; Product 3, ibuprofen; Product 4, dichlorophenyl carbinol and amylmetacresol; Product 5, acetylsalicylic acid; Product 6, calcium carbonate and magnesium carbonate. No serious sample treatment was necessary prior to irradiation of the solids. Samples were investigated with a Perkin Elmer SCIEX DRC-e ICP-MS (Ontario, Canada) fitted with a New Wave UP-213 laser ablation system. The instrument settings have been described elsewhere (Pillay *et al*., 2011), except in this study it was programmed to continuously ablate successive depths of **5** µm at each point and 'drilled' through the sample to a total depth of 150 µm. Depth-profiling spectra were recorded for each measurement. Depth profiles were estimated from real-time data.

All the samples of interest were subsequently digested in mild acidic media (<3% HNO_3 solution) and total concentrations were determined against certified reference standards. As in the case of the solids, all measurements were performed under the same experimental conditions.

Instrumental Performance

Characteristic intensities emanating from the targeted elements were measured, and considerations were given to possible interferences and matrix effects. Before each run, the instrument was calibrated and corrected for background. Validation and standardization of the technique were conducted on solid and aqueous samples.

Validation of the laser technique using certified solid standard. The validation procedure was identical to that described in an earlier paper (Pillay *et al*., 2011) and involved the use of a glass bead (NIST, Certificate 613, Gaithersburg, MD). Relative standard deviations of less than 5% were attained for a wide range of elements (Table 1) indicating that the performance of the instrument was satisfactory.

Validation of the technique using certified aqueous standard. An aqueous standard (Fluka standard, 70007; 10.00 ppb per element, St. Louis, MO) was available to test the performance of the instrument on homogeneous aqueous solutions. A point to note is that the Perkin Elmer ICP-MS is linear over several orders of magnitude for aqueous samples, and can cover a wide range of concentrations in a single measurement. A measure of the accuracy in terms of the relative error was computed and, in general, values less than 10% were attained demonstrating that the accuracy of the system for aqueous samples was satisfactory (Table 2).

RESULTS AND DISCUSSION

Depth-Profiling Solid Samples – Homogeneity of Mixing

Depth-profiling is an ultra-sensitive tool that has the capability of 'drilling' through samples and obtaining relevant information on the distribution of trace metals. Signal intensities were compared with surface metals and those occurring internally, within the sample, and appropriate spectra were generated to observe fluctuations in characteristic metal intensity with penetration depth. Characterizing solid samples in terms of metal distribution is particularly important to obtain knowledge of the completeness of mixing and the level of metallic residues. Poor mixing could result in unequal trace concentrations in the solid sample, which could affect the purpose for which the pharmaceutical is intended - for example, one half of a tablet may be more potent than the other half, thus containing a higher dosage. This could be important, because some parents give their children half a tablet of common, over the counter medicines to treat minor ailments. Uneven elemental distribution on the surface could be a reflection of uneven blending. As previously stated, such non-uniformity in the integrity of the sample could create unwanted problems, especially if they are used for highly sensitive medical purposes. Figure 1, for example, displays depth-profiles of Na from two different tablets (referred to here as samples) of the same product. Inspection of these spectra shows clearly that there are significant gaps between peaks, evidently demonstrating imperfections in the mixing of these pharmaceuticals. Figure 2 is another good example and represents depth-profiles of Mg, where there is unmistakable evidence of intermittent signals denoting inhomogeneity of the matrix. The capability, therefore, of ablative laser technology to penetrate to controlled depths below the surface of a sample and detect imperfect mixing or blending is attractive for a variety of studies in bulk materials. It is imperative to emphasize that this investigation was conducted in the absence of identical reference standards, for the obvious reason that it was not possible to obtain suitable standards to match the pharmaceutical matrix. The results are therefore relative and compared in terms of intensities (counts sec^{-1}).

'Hotspots'

Apart from evaluating homogeneity, characterization of pharmaceutical matrices is useful to locate 'hotspots' or areas of unusually high metal intensity. In this respect, the metal residue itself could play an intricate role in its distribution. As the solid sample is processed, some metals with greater mobility and affinity for the matrix could find themselves bound within the matrix. Others with fewer predilections to remain ingrained could migrate to the surface and accumulate at certain points. This is significant from the perspective of matrix purity, and the possibility of contamination could be marked if extraneous ingrained metals are present at pronounced levels at certain spots within the matrix. The location of

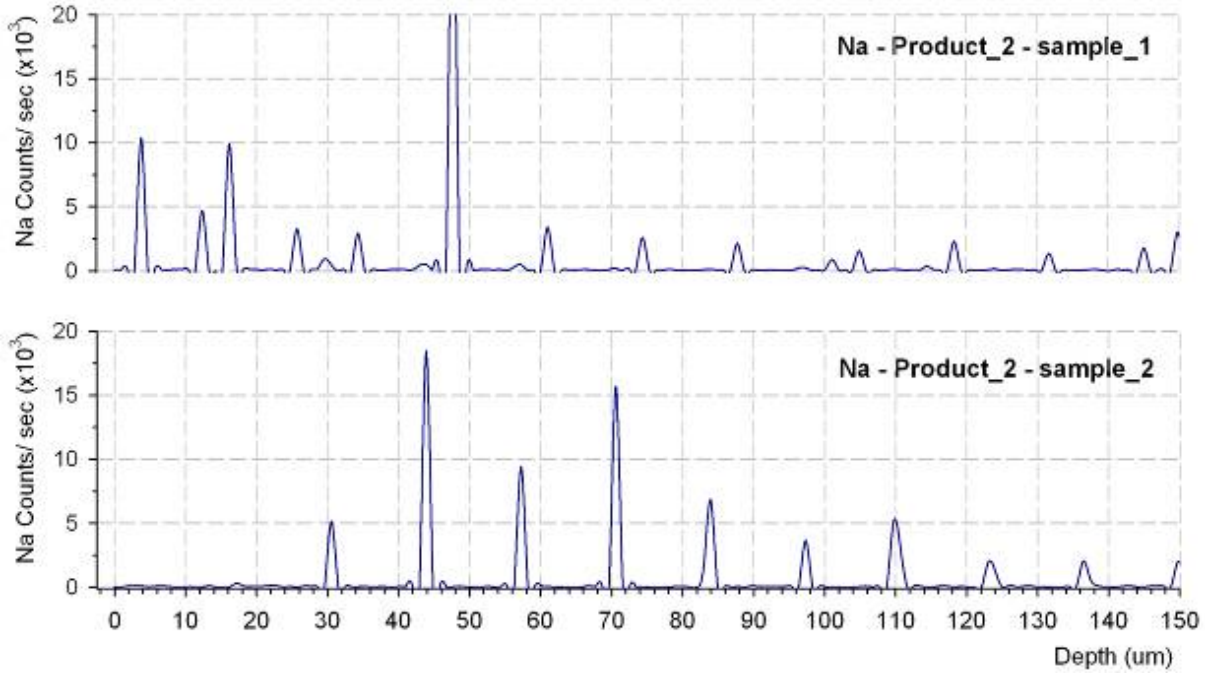

Fig. 1. Depth-profiles of Na displaying imperfect mixing with depth.

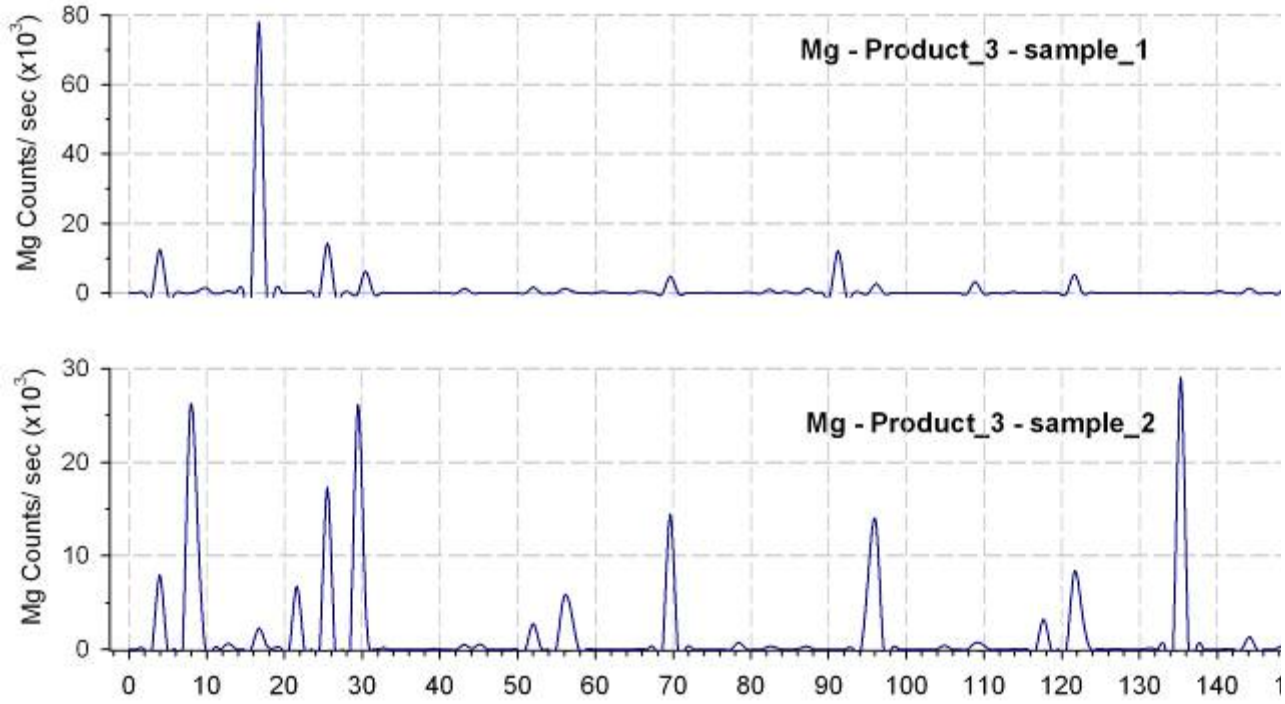

Fig. 2. Depth-profiles of Mg showing intermittent signals with depth.

such metal components, therefore, depends not only on the competency of mixing, but also on the potential of some metals with the capacity to migrate. Figure 3 represents a typical depth-profiling spectrum of a sample from Product 3 showing a 'hot-spot' of Zr with progressive depth below the surface. The tall peak in the spectrum, therefore, represents a 'hotspot' or region of abnormally high metal intensity, and probably pinpoints an area in the matrix where the metal accumulated. It is not clear at this stage why the trace metal would accumulate at this point; but such 'hotspots' provide distinct evidence that the mixing process could be improved.

Simple inspection of figure 3 shows that the other peaks are much lower in intensity, and eventually taper off to considerably diminished levels producing an overall differentiation of extreme intensities by factors of roughly 100, at certain points. These data highlight two important factors: (i) that the trace metal is unevenly distributed throughout the matrix; and (ii) that to model this depth-distribution, other factors (such as migration of the contaminant in the matrix under certain physical and chemical conditions) have to be taken into account. The implication for clinical purposes is quite marked, and suggests that if part of the pharmaceutical tablet or lozenge containing the 'hotspot' is consumed a splurge of trace metals could be released into the body creating undesirable (and probably) deleterious effects. As aforementioned, this effect could be more pronounced in children.

Total Concentrations

As shown above, depth-profiling in itself is appropriate for detecting imperfect mixing, but does not lend itself to the attainment of reliable levels of trace metals at internal spots within the solid matrix for the simple reason that suitable homogeneous standards of matching matrix are unavailable. Digesting samples to obtain total concentrations of trace metals and elements is highly accurate with ICP-MS. It provides a useful picture of the holistic elemental concentrations in pharmaceutical

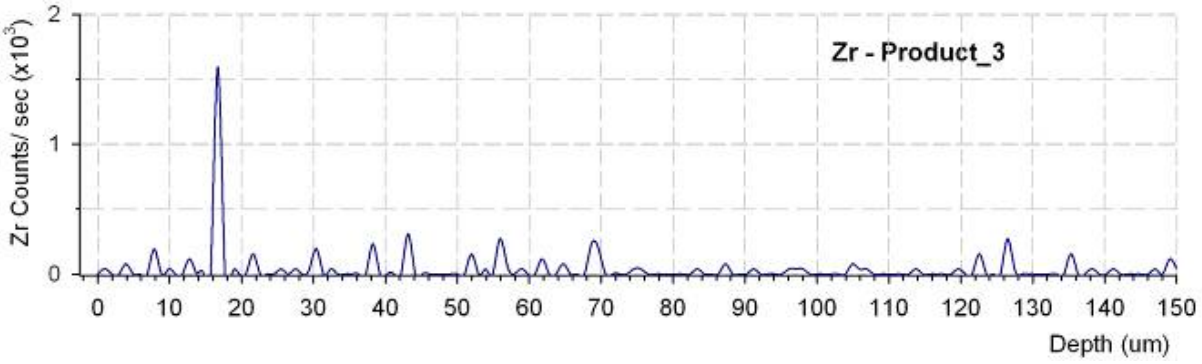

Fig. 3. Depth-profile of Zr showing 'hotspot'about 16 µm from the surface.

Table 1. Measurement of reproducibility in a glass standard (counts sec^{-1}).

Measurement	^{39}K	^{47}Ti	^{88}Sr	^{138}Ba	^{140}Ce	^{238}U
1	49072	6162	3688	1618	1023	1805
2	49172	6142	3672	1624	1141	1814
3	49775	6082	3587	1620	1036	1792
Mean ± RSD	49340 ± 0.8%	6129 ± 0.7%	3649 ± 1.2%	1621 ± 0.2%	1067 ± 4.9%	1804 ± 0.5%

Table 2. Accuracy for aqueous standard: +/- denote measured values above and below the certified values.

Element	Certified value (ppb)	Measurement 1 Fluka 70007 (ppb)	Relative Error (%)	Measurement 2 Fluka 70007 (ppb)	Relative Error (%)
Be	10.00	10.20	+2.0	10.75	+7.5
Mg	10.00	9.32	-6.8	9.92	-0.80
Co	10.00	9.74	-2.6	9.98	-0.2
Ni	10.00	9.93	-0.70	10.10	+1.0
In	10.00	9.94	-0.60	9.94	-0.60
Pb	10.00	10.70	+7.0	11.19	+11.2
Bi	10.00	10.06	+0.60	10.36	+3.6

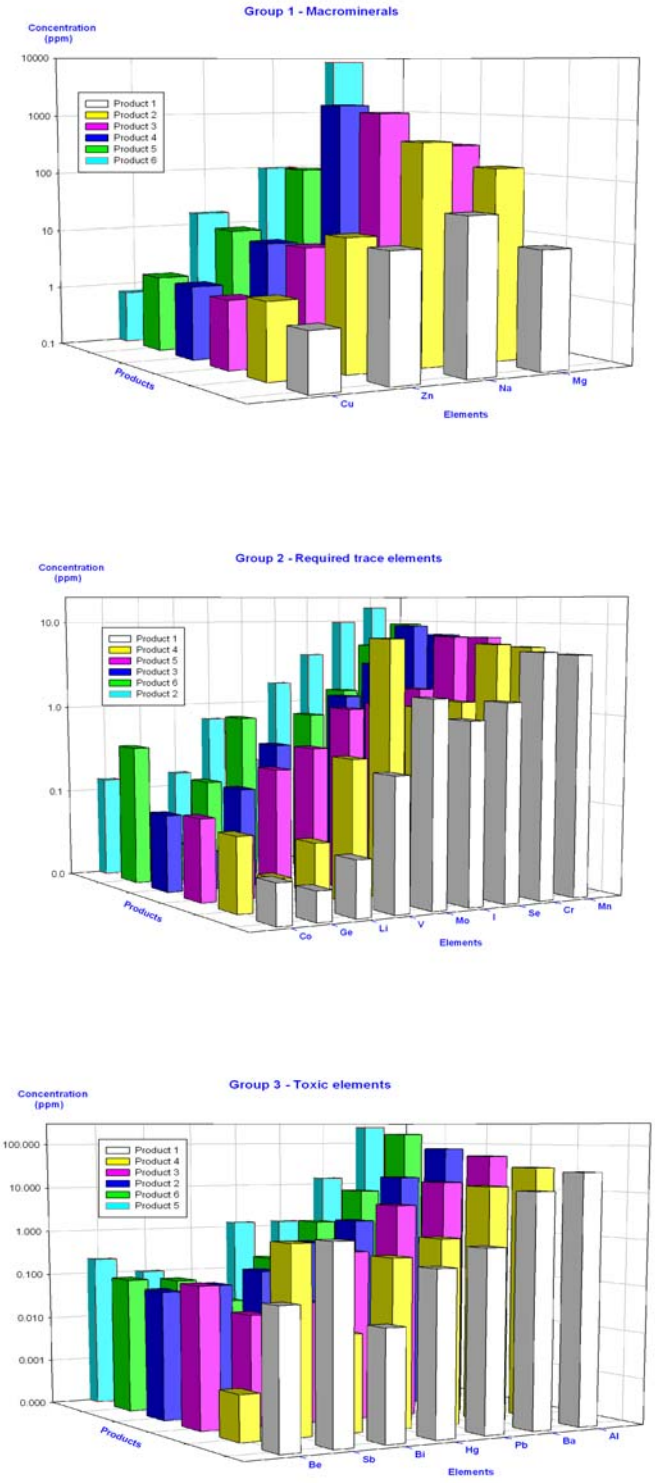

Fig. 4. ICP-MS elemental profiles of pharmaceuticals in aqueous media.

samples and gives an idea of whether these elements are present at toxic levels or not. Figure 4 displays (as bar graphs) the concentrations of some of the elements considered in the six products. For convenience these elements are classified into three separate groups. The capability of ICP-MS for detecting 'exotic' elements such

as Bi and La is superior to other contemporary instrumental techniques. It is evident from the profiles shown in figure 4 that the range of elemental concentrations is considerable in certain cases, clearly indicating that the level of purity of some pharmaceuticals is evidently higher than others. For example, the ranges of the following 'exotic' elements (ppm) exceed a factor of 40 in some cases demonstrating that undesirable contaminants unmistakably infiltrate the processing techniques of some medications: Bi: 0.01-0.16; Tl: 0.02-0.12; W: 0.09-0.23; Ce: 0.01-0.18; La: 0.01-0.36; Sb: 0.03-1.44; Sn: 0.16-0.63; Ag: 0.06-0.56; Mo: 0.20-7.06; Zr: 0.15-0.51; Co: 0.03-0.37 ppm. The biological effects and maximum permissible levels of some of these trace elements are not well established and are still under clinical research. On the other hand, some other elements such as iodine are present at abnormal levels and exceed the natural concentration in the human body (0.4 ppm). The mean concentration of total iodine in drinking-water in the USA is approximately 4 ppb. Iodine is known as a thyroid stimulator and unusually high levels could lead to unwanted effects. Another element, Sr, occurs at about 5 ppm in the body, but we found levels in some samples that seem relatively high at up to 28 ppm (unpublished results). This could be beneficial to bone growth, though abnormal levels could be undesirable. The mechanism and source of infiltration of these contaminants into pharmaceutical formulations is not clear and could be the subject of future study.

Toxicology/Environmental Implications/Suggested Safety Measures

Many of the levels of the detected elements in figure 4 exceed the permissible levels in drinking water (De, 1994). For purposes of comparison, the acceptable levels of certain toxic metals in drinking water are as follows (De, 1994): Al: 10 ppb; Cr: 50 ppb; Ni: 2 ppb; Se: 10 ppb; Cd: 5 ppb; Hg: 1 ppb; Pb: 10 ppb. It must be borne in mind though, that unlike drinking water, pharmaceutical medications are consumed in small doses. However, excess bioaccumulation of trace toxic metals in the human body through ingestion of contaminated medicines and foodstuffs could ultimately lead to serious disorders (particularly in children). For example, when abnormal amounts of Cd^{2+} are ingested, it replaces Zn^{2+} at prominent enzymatic sites leading to metabolic disorders (Kurt, 1990; Suwazano *et al*., 2000). Aluminium toxicity is also a good example which has been associated with various disorders such as Alzheimer's disease, kidney and liver problems, memory loss, and osteoporosis. Another possibility is Bi poisoning, which in small doses can cause severe renal failure, gastrointestinal problems and encephalopathy. Selenium is an essential nutrient, but in high doses could be poisonous. Acute oral exposure to Se compounds could result in lung disorders and cardiovascular and gastrointestinal problems including nausea, vomiting and serious effects on the liver and nervous system. Chronic (long-term) exposure to high levels of Se in food, water or medicines results in skin discoloration, baldness, excessive tooth decay and lethargy. The clinical effects and toxicity of Be and Sb are cloudy and are currently the subject of rigorous medical research. Exact clinical evidence of the detrimental impact of these toxic metals on humans is still forthcoming. Be is one of the most toxic elements and it can produce an allergic immune response, an acute Be disease or a chronic Be disease in susceptible people (McCleskey *et al*., 2009). In the worst cases of exposure to Sb, several health problems can occur including respiratory irritation, pneumoconiosis, spots and pancreatitis (Sundar and Chakravarty, 2010). On the other hand, Pb poisoning is well documented and has been the intense focus of environmental health research for decades. And Hg, originally thought to be non-toxic in its elemental form is now considered harmful to the central nervous system in small doses.

From the environmental viewpoint, disposal of unused medications could contaminate the ecosystem. Pharmaceuticals could enter the soil or water pathways through excretion or disposal. This could lead to undue pollution, especially if the formulations contain elevated levels of toxic metals. It is known (Kolpin *et al*., 2002) that dumped pharmaceuticals could infiltrate potable streams, underground aquatic resources and landfills. Also, pharmaceutical residues in body wastes could enter the sewers and contaminate waste water treatment plants. Hence, the general impact on the environment is a cause for concern, and our work could make a useful contribution to ongoing sustainable development (Shearman, 1990). Essentially, the overall potential impact is firstly, the effect on public health, livestock and crops; and secondly on wildlife such as rare birds and animals (Robinson, 1993).

Suggested safety measures include limiting the additives used in such pharmaceuticals. Another possibility is to dilute the sample with suitable edible additives (negligible in toxins) to reduce the level of contamination. To further restrict the hazard, the method of mixing could be re-visited and improved. These pre-emptive remedial measures could help control any potential threat to the environment and ensure sustainable living.

CONCLUSIONS

Laser technology has the capability of delineating spatial and depth distribution of embedded metals and elements in pharmaceutical matrices. It appears that such distributions would depend on several variables such as the nature of the metal, its affinity for the matrix, the matrix itself and the additives used. It is not clear at this stage whether such distributions could be treated mathematically and modeled. Depth-distributions

displayed inconsistencies in signal intensity thus denoting uneven blending and mixing of the solid sample. Analysis of aqueous solutions of the digested samples indicated that many of the levels are elevated and an extension of this study would be to trace the source and mechanism of entry of contaminants into pharmaceutical formulations. The potential environmental impact of pharmaceuticals creating pollution must be given due consideration. It could be useful to apply the laser ablation ICP-MS technique to tablets or powders of drugs of abuse. Individual bags of drugs or batches of tablets may have characteristic metal profiles, therefore, providing investigators with evidence on whether samples in a captured haul are from the same or different batches.

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