

DETECTION OF HEAVY METALS IN AZATHIOPRINE API DRUGS

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Abstract

This paper described analysis of concentrations of heavy metals in Azathioprine drug. Azathioprine is active pharmaceutical ingredient which having immunosuppressive action given either orally or intravenously and can be used to prevent rejection in organ transplantation. CEM Microwave digestion system (MARS6 iWave) was applied for the digestion of the samples and heavy metals concentration was determined using Teledyne Leeman labs Inductive Coupled Plasma (ICP) instrument (Prodigy7). Metals were found to be present in varied concentrations in the azathioprine samples. The concentration ranges were found in limits. The findings of the study suggest that azathioprine contained safe levels of heavy metals and within the permissible limits (PL) of the World Health Organization (WHO).

Key-words: Azathioprine, Anti-cancer, Heavy-metals, ICP, Immunosuppressive Medication

Introduction

Azathioprine, 6-[(1-Methyl-4-Nitro-1HimidAzathioprinel-5yl) sulfanyl]-7H-purine is having marked effect on T-lymphocytes and immunosuppressive action which is given orally or by I.V route. It is also used in systemic anti-inflammatory states, such as rheumatoid arthritis, lupus erythematosus, colitis ulcerosa, auto immunological hepatitis and Crohn's disease. Azathioprine acts as a pro-drug for Mercaptopurine, inhibiting an enzyme that is required for the synthesis of DNA.¹⁻⁵ Thus it most strongly affects proliferating cells; such as the T cells and B cells of the immune system. More than 50 years of widespread clinical use of azathioprine the understanding of its mechanism of action is still incomplete.¹ Several methods have been described for determination of Azathioprine in pharmaceutical preparations including HPTLC, HPLC and NMR had been used for the determination of Azathioprine. Moreover the literature survey revealed that, so far no simple method has been reported for estimation heavy metals from Azathioprine. Therefore, it was thought worthwhile to develop simple, precise, accurate heavy metal analysis method for the estimation heavy methods in Azathioprine. Validation was carried out in compliance with International Conference on Harmonization guidelines. The term "heavy metal" is defined based on their specific gravity, atomic weight, atomic number and chemical properties. Lead, cadmium and nickel are chemical elements with a specific gravity at least 5times higher than that of water hence they are considered as heavy metals. Heavy metals are usually present in nature either as

elementary compounds or mineral deposits. During extraction and processing of these metals they come in contact with atmosphere, soils, water, plants and animals, causing environmental pollution and producing toxicity.²⁻⁶

Heavy metals in medicinal products may be present as impurities. A list of heavy metals which are likely to be present in a finished pharmaceutical substance can come from catalysts that are deliberately added to the process, from raw materials or reagents that are employed in the manufacturing process and leaching from equipments or vessels that are used in the manufacturing process.⁷ Hence, heavy metals may enter the human body via manufacturing, pharmaceutical, industrial, or residential settings. Determination of heavy metals from pharmaceutical dosage form needs a very sensitive and selective analytical technique that can quantify trace level of metals present as contaminants in the complex matrices.

Experimental Work

A total of 5 samples of five different types of manufacturers were purchased from different sources in the local markets of Mumbai. The samples used in this study were purchased from authenticated medical shops/supermarkets approved by FDA, Mumbai. Table 1 shows a list of the name and the number of samples analyzed. All glassware and digestion vessels were soaked in 20% nitric acid and rinsed with ultrapure water. Multi-element standard solutions of lead (Pb), cadmium (Cd), iron (Fe), zinc (Zn), and copper (Cu) were prepared by dilution of 1000mg/L stock solutions (Fluka TraceCert Ultra, Sigma-Aldrich) with 5% nitric acid (HNO₃) solution. The calibration curve for each element was linear and a correlation coefficient of 0.995 was obtained.

Table 1.

Sr. No.	Drugs name	Brand Name	Manufacturer name
1	Azathioprine	Azoran 50mg Tablet	RPG Life Sciences Ltd
2	Azathioprine	Aretha 50mg Tablet	Biocon
3	Azathioprine	Imuza 50mg Tablet	Panacea Biotec Ltd
4	Azathioprine	Azr 50mg Tablet	Ipca Laboratories Ltd
5	Azathioprine	Azapure 50mg Tablet	Intas Pharmaceuticals Ltd

Procedure

Sample Digestion- For microwave digestion, 1 Tablet of the sample was accurately weighed into a digestion vessel (MARSXpress), followed by addition of 1mL of 37% hydrochloric acid (HCl) (trace metal concentrated, Suprapur, Merk), 7.0mL of 69% nitric acid (HNO₃) (trace metal concentrated, Suprapur, Merk), and 0.5mL of 30% hydrogen peroxide (H₂O₂) (Sigma-Aldrich). The mixture was digested using MASR 6 Microwave digestion system (CEM Corporation, Matthews, USA).

Metal Analysis

At the end of the digestion program, the samples were quantitatively transferred to 25mL volumetric flask and diluted with distilled water. The concentration of metals in the sample was determined using Inductive coupled plasma PRODIGY 7 instrument manufactured by Teledyne Leeman Lab. All quality control and assurance measures were taken including calibration check measures, determination of Method Quantification Limits (MQL), and replicate analysis of samples.

Observations

LOD (limit of detection), LOQ (limit of quantification), linearity, selectivity, precision and assay these parameters covered in current studies.

Table 2

Sr. No.	Metal Name	LOD ppm	LOQ ppm	Linearity Correlation coefficient (r)	Precision Intraassay (%R.S.D.)	Precision Interday (%R.S.D.)
1	Lead	0.3ppm	0.9 ppm	0.9976	97.16	91.35
2	Arsenic	0.1ppm	0.3 ppm	0.9964	98.32	95.43
3	Cadmium	0.3ppm	0.9 ppm	0.9954	95.74	93.13
4	Mercury	0.3ppm	0.9 ppm	0.9973	94.13	93.71
5	Platinum	0.1ppm	0.3 ppm	0.9983	93.41	91.32

LOD, LOQ, Linearity, Intraassay and intraday precision, repeatability of Inductive coupled plasma method for the determination of Lead, Arsenic, Cadmium, mercury & platinum.

Table 3

Sr. No.	Metal Name	Sample Name				
		Azoran 50mg Tablet	Aretha 50mg Tablet	Imuza 50mg Tablet	Azr 50mg Tablet	Azapure 50mg Tablet
1	Lead	0.713 ppm	0.735 ppm	0.772 ppm	0.784 ppm	0.708 ppm
2	Arsenic	0.865 ppm	0.813 ppm	0.863 ppm	0.874 ppm	0.884 ppm
3	Cadmium	0.756 ppm	0.775 ppm	0.759 ppm	0.723 ppm	0.811 ppm
4	Mercury	0.613 ppm	0.682 ppm	0.676 ppm	0.652 ppm	0.687 ppm
5	Platinum	0.974 ppm	0.908 ppm	0.983 ppm	0.934 ppm	0.921 ppm

Metal contents are in ppm.

Conclusion

The present method is applicable over a wider concentration range; where 0.9 -15 ppm solution of std. for lead, cadmium and mercury and 0.3 – 5.0 ppm solution of std. for arsenic and platinum can be determined, from Azathioprine. In pharmaceutical analysis it is important to test the selectivity toward the excipients and the fillers added to the pharmaceutical preparations. Fortunately, such materials mostly do not interfere. This is clear from the results obtained for the pharmaceutical preparations that these excipients do not interfere. As with any medication, it is possible to overdose on azathioprine. Sensitivity, accuracy, convenience and the reproducibility of the results are superior to those obtained from other methods like AAS, the method should be useful for routine analytical and quality control assay of the investigated drug in dosage forms.

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