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ZINC ESTIMATION IN HERBAL FORMULATIONS, BY COMPLEXOMETRIC METHOD: AN ALTERNATIVE TO ATOMIC ABSORPTION SPECTROMETRY

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ABSTRACT
Zinc is a metallic chemical element. Generally, Atomic Absorption Spectrometry (AAS) is used to measure the concentration of metallic elements. Although this technique is reliable and sensitive, it is expensive and thus not easily affordable by most pharmaceutical colleges and institutes. An alternative, reliable, easy and affordable method to estimate the amount of zinc in different types of herbal formulations is the proposed complexometric method. In this proposed EDTA method, Eriochrome Black – T is used as indicator (EBT). Zinc was estimated in two batches each of two different types of herbal formulations and their corresponding Active Pharmaceutical Ingredients (API). The first type was an oral dosage form (Aphrodisiac capsule) and it's API (Yashad bhasma), and the second type was a topical dosage form (Anti-acne Gel) and it's API (a zinc oxide dispersion Pushpanjan). The Aphrodisiac capsule is used for vigour and vitality and Anti-acne gel is used against black heads, pimples and red patches on the skin. The samples were first subjected to incineration to remove other impurities and subsequently estimated for the content of zinc by EDTA complexometric method. The content of zinc in the first batch of Aphrodisiac capsule was 18.00 mg/capsule and that in the second batch was 17.55 mg/capsule. The content of zinc in the corresponding API (Yashad bhasma) was 74.36 % w/w. Thus, the practical values were 102.85 % and 100.28 % of the theoretically expected values, in both batches respectively. Similarly, the content of zinc in first batch of anti-acne gel was 1.55 mg/g and that in the second batch was 1.58 mg/g. The content in corresponding API (Pushpanjan) was 60.50 % w/w. Thus, the practical values were 103.33 % and 105.33 % of the theoretically expected values in both batches respectively.
Keywords: Zinc, Complexometric titration method, EDTA, Eriochrome Black–T (EBT), herbal formulations

INTRODUCTION

Herbal medicines are used rapidly across the world. Herbs are staging a come-back and herbal renaissance is happening all over the globe. Herbal medicines symbolize safety in contrast to the synthetics. The blind dependence on synthetics is over and people are returning to the naturals with the hope of safety and security[1]. Medicines are classified into various types based on their route of administration. The two important types are oral (the drug is placed in the mouth and swallowed) and topical (the drug is applied to the skin and mucous membrane for local action)[2]. Herbal medicines are used in both oral as well as topical forms. Zinc is an essential trace mineral. Next to iron, zinc is the most common mineral in the body and is found in every cell. It has been used since ancient times to help heal wounds and plays an important role in the immune system, reproduction, growth, taste, vision, smell, blood clotting and proper insulin and thyroid function. Zinc also has anti-oxidant properties, as it helps protect the cells of the body from damage caused by free radicals. Oral zinc supplements help improve acne. Zinc also slows the process of age-related macular degeneration (occurs when a part of retina responsible for central vision degenerates). During the first signs of cold, zinc lozenges or nasal spray reduce the duration and severity of symptoms. Zinc supplements also reduce the symptoms of sickle cell disease and speed up the healing of stomach ulcers. Zinc is useful in the cure of various other diseases like Attention Deficit Hyperactivity Disorder (ADHD), Herpes simplex (cold sores), HIV/AIDS and Wilson's disease[3]. Similarly, zinc in the form of zinc oxide (Pushpanjan) can be used as a healer. It is externally mild, soothing, astringent and desiccant. It is dusted over as powder in eczema, impetigo, excoriation, bed-sores and cracked nipples or applied as ointment to wounds, burns, vesicular eczema, chronic skin diseases, etc[4]. It acts as a protective coating for mild skin irritations and abrasions. It promotes healing of chapped skin. Zinc is a UV-A and B reflector and thus protects the skin against skin tan and burns. In oral formulations zinc is used in the form of Yashad bhasma (zinc based ayurvedic metallic preparation)[5]. In case of topical formulations, zinc is used as zinc oxide dispersion (Pushpanjan), as it enhances the local absorption of the dosage form. The various uses of Yashad bhasma and Pushpanjan (both containing zinc) have been reported in ancient literature.

Estimation of components in herbal medicines is a complex task due to their heterogenous composition[6]. But we cannot ignore the estimation of components as it is an important step in the standardization of the drugs. Quantitative determination of zinc is extremely essential because it has various important uses and excess concentration of the metal may lead to adverse effects in the body. Atomic Absorption Spectrometry (AAS) is an analytical technique that measures the concentration of elements. It is used in different areas of chemistry like clinical analysis, environmental analysis, pharmaceuticals, mining, etc. and is a very sensitive technique[7]. However, AAS in an expensive technique and thus, is not easily affordable. Hence, we have proposed a complexometric titration method using EDTA, which is alternative, easy, affordable and reliable for the estimation of zinc. The EDTA method of titration is a complexometric titration, in which the titrant (ligand) reacts with the analyte (metal ion zinc in this case) to form a complex, more specifically a chelate. The indicator used in this method is Eriochrome Black-T (EBT), which shows an end point from

wine red to blue[8]. EDTA (Ethylene Diamine Tetra Acetic acid) is a member of a class of compounds called aminopolycarboxylic acids, which undergoes successive acid dissociations to form a negatively charged ion. This ion has the ability to wrap itself around positive metal ions in water solution (called chelation or complex formation). Zinc ion forms a stable water soluble 1:1 complex with EDTA. This reaction of EDTA with metal ions is pH dependent. Thus, the pH of reaction is controlled by using buffer solutions. In this case the complexometric titration method using EDTA is being used to determine the content of zinc in two batches each of two different types of herbal dosage forms viz. an oral dosage form (Aphrodisiac capsule) and a topical dosage form (Anti-acne gel), and their corresponding API's (Yashad bhasma and Pushpanjan respectively).

MATERIALS AND METHODS

Materials

API's like Yashad bhasma and Pushpanjan were procured from Uma Ayurvedic and Pioma Chemicals respectively, and the two batches each of the two types of formulations under study (Aphrodisiac casule and Anti-acne gel) were manufactured in house in Formulation Department of Piramal Enterprises Limited. The reagents used in the experiments viz. hydrochloric acid (A.R. Grade), Ethylene Diamine Tetra Acetic acid (EDTA - A.R. Grade), ammonium chloride (A.R. Grade), ammonia (A.R. Grade) and Eriochrome Black – T (EBT) indicator were obtained from M/s Merck. Distilled water was used throughout the experimental work.

Methods

Metal determination in biological media (plant extracts in this case) needs a previous step of matrix simplification or dissolution[9]. Thus the first step in determination of zinc; is incineration. Estimation method for Content of zinc is as follows:

Residue on Ignition (Incineration)

About 0.2 g, 0.2 g, 1 g and 5 g of Yashad bhasma, Pushpanjan, capsule and gel respectively, were accurately weighed in a previously cleaned, dried and weighed silica crucible. The sample was then gently heated on hot plate in hood with gradually increasing the temperature. The crucible with the burnt residue was transferred to the muffle furnace at the higher temperature. Sample was subjected to ignition.

Content of zinc

Reagents required→ 0.05M EDTA: 18.61 g EDTA powder -→ 1000 ml with water

Buffer pH 10: Dissolve 7 g of pure ammonium chloride in 56.8 ml ammonia and dilute to 100 ml with distilled water.

EBT Indicator: 1: 99 (Triturate Eriochrome black –T: Sodium chloride)

Procedure

The inorganic residue (ash) obtained after ignition was subjected to acid digestion with 10 ml dilute hydrochloric acid and 50 ml distilled water to boiling temperature. Content was allowed to attain the room temperature and diluted to 200 ml with distilled water. Individual sample stock was mixed thoroughly and filtered. Filtrate was considered as "STOCK" solution "S". 50 ml of the filtrate from respective stock solution was titrated against 0.05M EDTA in presence of 10 ml of buffer pH 10 using Eriochrome Black T indicator. End point was wine red to blue color. Burette Test Reading was recorded as "A" ml, Similarly blank was performed omitting the sample and the same procedure was repeated. The burette Blank Reading was recorded as "B" ml. Content of zinc in the formulation can be calculated by applying following formula

Content of zinc in Yashad bhasma and Pushpanjan (%w/w) → = (A -B) X (0.003269 / w) X (200/50) X (M /0.05) x 100

Content of zinc in capsule (% w/w) = (A -B) X (0.003269 / w) X (200/50) X (M /0.05) x 100

Content of zinc in capsule (mg/capsule) = % w/w X Average filled weight of capsule (g) X 10

Content of zinc in gel (% w/w) = (A -B) X (0.003269 / w) X (200/50) X (M /0.05) x 100

Content of zinc in gel (mg/g) = % w/w X 1000/100

Where, w →Weight of the sample in g, M → Actual practical molarity of 0.05M EDTA

RESULTS AND DISCUSSION

The above experiment focuses on the assessment of the applicability of the complexometric EDTA method for the determination of zinc in two different types of herbal formulations (capsule and gel) and their respective APIs (Yashad bhasma and Pushpanjan). The Table shown in the illustrations section, shows the description, content of zinc (observed values), theoretical values and the percentage of observed values with respect to theoretical values, of two batches each, of an oral dosage form (Aphrodisiac capsule), a topical dosage form (Anti-acne gel) and their corresponding APIs, Yashad bhasma and Pushpanjan, respectively. The content of zinc in yashad bhasma was found to be 74.36 % w/w, which is 106.22 % of the theoretical value (Not less than 70.00 % w/w). The zinc content in pushpanjan was found to be 60.50 % w/w, which is 100.83 % of the theoretical value (Not less than 60.00 % w/w). In case of oral dosage form (Aphrodisiac capsule), the contents of zinc in Batch 1 and Batch 2 were found to be 18.00 mg/capsule and 17.55 mg/capsule, which is 102.85 % and 100.28 % of the theoretical value (Not less than 17.50 mg/capsule), respectively. Similarly, in case of topical dosage form (Anti-acne gel), the contents of zinc in Batch 1 and Batch 2 were found to be 1.55 mg/g and 1.58 mg/g, which is 103.33 % and 105.33 % of the theoretical value (Not less than 1.50 mg/g), respectively. The percentage of observed value with respect to theoretical value lying between 80-120 % in both the batches of the two types of dosage forms indicates the accuracy of the proposed method. Also, the deviation in the results of both the batches of the dosage forms is minimal. This further proves the precision of the method.

Table: Content of Zinc in Yashad Bhasma, Pushpanjan, Capsule and Gel Formulations

Test Parameters	Yashad bhasma	Pushpanjan	Aphrodisiac capsule		Anti-acne gel	
Description	Yellowish colored fine powder, odorless and tasteless	Off-white colored thick dispersion	Capsule with light brown colored powder filled in it.		Light brown colored gel with characteristic odor	
Content of Zinc (Observed values)	74.36 % w/w	60.50 % w/w	Batch 1	Batch 2	Batch 1	Batch 2
			18.00 mg/ capsule	17.55 mg/ capsule	1.55 mg/g	1.58 mg/g
Theoretical values	Not less than 70.00 % w/w	Not less than 60.00 % w/w	Not less than 17.50 mg/capsule	Not less than 17.50 mg/capsule	Not less than 1.50 mg/g	Not less than 1.50 mg/g
Percentage of observed values with respect to theoretical values	106.22 %	100.83 %	102.85 %	100.28 %	103.33 %	105.33 %

W = weight, g = gram , mg = milligram

CONCLUSION

Thus, the motive of the experiment to prove the suitability of the proposed method for the determination of zinc in herbal dosage forms by EDTA titration using EBT indicator has been accomplished. In the Indian Pharmacopoeia a complexometric method has been proposed for estimation of zinc oxide. This method involves titration with EDTA, using xylenol orange as an indicator. The end point in this method is violet-pink to yellow which is not as distinct as the end point in the proposed method (wine red to blue). Also the color change observed by using xylenol orange indicator occurs only if the pH of the solution is exactly 5.5, which is fairly difficult to maintain. Hence, the estimation of zinc oxide using xylenol orange is comparatively tedious. The above experiment provides precise and accurate results. Moreover the method is easy, affordable and reliable. Therefore it can be successfully used as an alternative method in the quantitative estimation of zinc.

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