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Extractive Spectrophotometric Determination of Iron as an Impurity in Pharmaceutical Raw Materials

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The objective of the present study is to develop an instrumental method, which is more accurate than the physical method that is followed in Indian Pharmacopoeia. A simple and sensitive spectrophotometric method has been developed for the estimation of iron as an impurity in pharmaceutical raw materials. In this method wine red color complex formed between iron and 1-3-diphenyl-4-carboethoxy pyrazole-5-one (DPCP) was measured at 525 nm. Beer's law was found to obey in the concentration range 0.5-10 µg/ml. Sandell's sensitivity and standard deviations were found to be 0.0483 µg/cm² and±0.035 respectively.

Concept about purity has changed with time and is indispensable for development in analytical chemistry. Hence detection and estimation of impurity is one of the most important subjects in a pharmaceutical industry. The pharmacopoeia places the greatest emphasis on the control of physiologically harmful impurities. Contamination by arsenic and lead is widespread, largely as a result of atmospheric pollution. Iron is also one of the associated impurities. Iron in trace amount is one of the important constituents of the body metabolism. The excess intake of iron may cause siderosis and other toxicological complications. So need arises to limit the presence of iron in raw materials, which are used in pharmaceutical formulations. The chemical test that detects and measures the impurity is called limit test. The limit test of iron is provided to determine that the content of iron as an impurity does not exceed the limit for iron specified in the individual monograph of the pharmacopoeia.

In the existing method given in Indian Pharmacopoeia, the determination of iron is done by concomitant visual comparison with a control, prepared from a

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standard iron solution. Generally the sources of error, if any, arising out of the chemical method or the apparatus employed are negligible, in comparison with the error due to lack of sensitivity of the human eye to variations in luminous intensity. In the absence of screening, the sensitivity of the eye is adversely affected by the illuminated surroundings. The relative error solely due to the sensitivity of the eye may generally be reckoned as 5% and even 10% or 20% under unfavourable conditions[1]. Eye can only judge whether two luminous intensities are equal between two limits of intensity[2]. So it becomes difficult to compare between the color intensities of standard and test solutions containing near about the same impurity concentration.

A simple and sensitive spectrophotometric method in visible region has been developed for the limit test of iron as an impurity in pharmaceutical raw materials. This method gives better accuracy and avoids human error that may arise, in conventional method, in the comparison between standard and test solutions. The method is developed using 1,3-diphenyl,-4-carboethoxy pyrazole,-5-one (DPCP) as a complexing reagent. DPCP is a new developed reagent. The reagent DPCP gives wine red color complex in acidic condition with both Fe (II) as well as Fe (III). The complex formed is extracted in chloroform medium, which can be measured spectrophotometrically at 525 nm. Pyrazolones have been gaining increasing popularity as a potential extractant[3-8] as well as powerful drugs[9].

All chemicals used were of analytical grade. Stock solution of iron was prepared by dissolving 0.1736 g of ferric ammonium sulphate (Himedia) and diluting to 100 ml with 0.05 M sulphuric acid. Standard solution of iron (20 ppm) was prepared by diluting one volume of 0.1726% w/v solution of ferric ammonium sulphate in 0.05 M sulphuric acid to 10 volume with distilled water. This solution contains iron in ferric state. A 20% w/v iron free citric acid (SRL) solution was prepared in distilled water. Citric acid used was ensured iron free by dissolving 0.5 g citric acid in 40 ml water and was made acidic with 0.1N HCl (iron free). To this 2 drops of 0.1% DPCP in alcohol was added and diluted to 50 ml with distilled water. No pink color was produced. Reagent DPCP (0.1% w/v) was prepared by dissolving 100 mg in 100 ml absolute alcohol. UV visible spectrophotometer of Shimadzu 150-02 with 1 cm match quartz cell were used for optical measurements.

In the existing pharmacopoeia method, a 2 ml of 20 ppm iron standard solution is mixed with 2 ml of 20% iron free citric acid IP. One hundred microlitres of thioglycollic acid is added, made alkaline with iron free ammonia solution, diluted to 50 ml with water and allowed to stand for 5 min. The sample is treated similarly. The sample and standard solutions in Nessler cylinders are compared visually.

In the proposed method a 2 ml of 20 ppm iron standard solution was transferred in a separatory funnel. To it, 2 ml of 20% iron free citric acid was added and mixed. Two millilitres of 0.1% reagent DPCP in absolute alcohol was added to the mixture followed by 5 ml of water. It was then extracted with 10 ml of chloroform. The wine red color complex formed was extracted in chloroform layer. The organic layer was collected in a test tube. Anhydrous sodium sulphate was added to absorb water molecules if any. The sample was treated similarly. The absorbance of the sample and standard solutions were measured at 525 nm using chloroform blank and compared. The reagent DPCP does not absorb at this wavelength at the above reaction conditions.

The important analytical parameters and the optimum conditions were evaluated. The acidic condition was sufficient for color development. Variations of reagent concentration showed that 2 ml of 0.1% DPCP was sufficient to extract iron as an impurity at trace levels. The complex was easily extractable within 1 min. Several organic solvents such as chloroform, carbon tetrachloride, xylene, toluene, benzene were tried; of these chloroform was found to give good extraction. The complex was stable for 48 h. The interfering metal ions present in pharmaceutical raw materials did not interfere in above reaction conditions. Beer's law was found to obey in the concentration range 0.5-10 µg/ml. Molar absorptivity and Sandell's sensitivity was found to be 1.156×10^{31} mol^{-1} cm^{-1} and 0.0483 $\mu g/cm^2$ respectively. Relative standard deviation of ± 0.035 was observed for analysis of six replicate analyses.

Advantage of the proposed method is that with DPCP, a redox type of reagent, iron impurity in any state Fe (II) as well as Fe (III) can be determined. In the established method standard and test solutions are compared spectrophotometrically hence the method is more accurate as it helps to avoid errors in visual observations leading

TABLE 1: ANALYSIS OF PHARMACEUTICAL RAW MATERIALS

	Raw Materials	Absorbance (525 nm)	Remark
1.	Glycerin (Hindustan Liver Ltd.)	0.353	*Passes test
2.	Sodium Edetate (Sulab)	0.371	Passes test
3.	Magnesium Chloride (Canton Lab)	0.205	Passes test
4.	Sodium Hydroxide (Caliron)	0.365	Passes test
5.	Maize Starch (Riddhi Siddhi Starch and Chemicals)	0.353	Passes test

(Absorbances of standard iron solution at 525 nm= 0.387)

*Concentration of iron as an impurity, in sample solution, is less than concentration of iron in the standard iron solution.

to inconsistency and subjectivity in the overall performance of the existing pharmacopoeia[1] method[10]. As the complex formed is easily extractable and over all test requires about five minutes to perform, it is a quick and easy method.

The method was applied successfully to the raw materials listed in Table 1. The aliquot of test solution prescribed in Indian Pharmacopoeia was treated as per the developed method. The results of the analysis of pharmaceutical raw materials by proposed method was in good agreement with the pharmacopoeal method. The method is applicable to any raw material which contains iron in Fe (II) or Fe (III) state as an impurity. In this standard solution was treated separately as per the above procedure. If the absorbance of the test solution is less than the standard solution then it passes the limit test.

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