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Development of a Spectrophotometric Method for the Determination of Aspirin in Blood Sample

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The study was conducted for the determination of Aspirin in blood sample by spectrophotometeric method. This is based on the formation of a color complex of the drug in serum with ferric-mercuric reagent. The absorbance of the colored complex was then measured at 540 nm for maximum absorption. This method shows linearity in the range of 0-100μg/ml and useful for the routine analysis of drug in the serum.

Key words: Aspirin, spectrophotometric method, serum

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Introduction

Aspirin, an anti-inflammatory, analgesic and anti-pyretic agent (BNF, 1988) is widely used for the treatment of pain and fever. It is also used as an anti-platelet aggregator (Mazerus *et al*., 1991). The drug is official in USP which specified HPLC method for the determination of salicylic acid in various dosage forms (USP, 1995). Other methods for the determination of salicylic acid in the blood serum include gas chromatography, mass spectra and fluorescence polarization immunoassay methods (Fitz *et al.*, 1991; Rosekanz *et al.*, 1985 and Jolley *et al.*, 1981). But no spectroscopic method has yet been developed for identifying aspirin in blood samples.

In the present work, we have described a spectrophotometric method for the determination of aspirin in blood sample using ferric-mercuric reagent.

Materials and Methods

Aspirin tablets of 300 mg were purchased from Advanced Chemical Industries, Bangladesh and the active ingredient aspirin was supplied by Chemico Laboratories, Rajshahi. All other chemicals were of analytical grade and used without further purification.

Preparation of Reagent: Ferric-mercuric reagent is useful to precipitate protein from human serum (Dasgupta *et al.*, 1992). Ferric-mercuric solution was prepared by dissolving 10g of mercuric chloride in a small amount of distilled water in beaker A and In beaker B, 10 g of ferric nitrate was dissolved in 30 ml of 1N hydrochloric acid(Hcl). Contents in both beakers were then mixed into 250 ml volumetric flask and made upto the mark by distilled water.

Preparation of Standard Aspirin solution: A stock solution containing 0.1 mg/ml of aspirin was prepared in distilled water with moderate heating. The stock solution was further diluted to get 10, 20, 40, 60, 80 and 100 μg/ml standard solutions of the drug.

Preparation of Calibration curve: One milli liter of each of the diluted standard solutions were taken in separate test tubes and heated in a water bath for 3-5 minutes at 45 ± 2°C, and after cooling in a running water. The 5ml of ferric-mercuric reagent was added to each of the standard test tubes and mixed well. 1ml of distilled water was taken in another test tube and the same procedure was followed. This sample was prepared for blank determination. All the test tubes were again heated for 1-2 min. and then cooled.

The absorbance at 540 nm against blank was determined for all of the standard solutions. When absorbance values were plotted against concentrations a straight line was obtained which represented a five-point calibration curve.

Serum Aspirin Analysis: Aspirin tablets of 300mg were ingested by six healthy volunteers, randomly marked from A to F. After one hour, 10 ml of blood sample from each individual was withdrawn into separate test tubes containing 0.5 g sodium oxalate. The sample was then centrifuged at 4500 rpm for 5 min. Each 1 ml of the supernatant serum was transferred into three test tubes and 5ml of ferric-mercuric reagent was added to them and mixed well. The mixture was again centrifuged at 4500 rpm for complete precipitation of plasma protein by forming complex with the reagent. Then the supernatant solutions were collected and absorbances were measured.

Results and Discussion

From the calibration curve the concentrations of salicylic acid in serum sample of six individuals, marked from A to F, were found to be in the range of 24.67 ± 0.86, 32.03 ± 0.99, 23.4 ± 0.75, 30.03 ± 0.47, 24.07 ± 0.86 and 28.60 ± 0.70 μg/ml, respectively The standard deviation of three samples was with in the limits of all volunteers which proved to be significant. The results are shown in Table 1.

Table 1: Concentration of Salicylic acid in blood samples of six volunteers in μg/ml.

Individual	Sample I	Sample II	Sample III	Mean $\pm$ SD
A	24.5	25.6	23.9	24.67 ± 0.86
B	32.7	30.9	32.5	32.03 ± 0.99
C	22.6	23.5	24.1	23.40 ± 0.75
D	30.4	30.2	29.5	30.03 ± 0.47
E	25.0	23.3	23.9	24.07 ± 0.86
F	28.9	27.8	29.1	28.60 ± 0.70

The three results of each individual did not differ significantly. So, the method can be accepted for reproducible results. The system obeys Beer's law in the range of 0-100 μg/ml of aspirin in the final solution. Aspirin is hydrolysed to salicylate in the stomach and as soon as it enters in blood circulation (Brody, 1998). Aspirin in the standard solutions was also hydrolysed to salicylic acid after heating that formed complex with the ferric-mercuric reagent. This complex gave a purple color that was determined for salicylate content in spectrophotometer in the visible range. Similarly, the reagent also formed complex with the salicylate in the blood serum. Ultracentrifugation precipitated in the serum proteins and the supernatant serum contained the salicylate that was determined in the spectrophotometer. We concluded that this method would be simple, sensitive, cost effective and convenient for laboratory analysis of aspirin in blood serum.

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