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Rapid Spectophotometric Determination of Sulphonamide Derivatives with Resorcinol

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A rapid and simple spectrophotometric method for the determination of sulpha drugs is described. The method is based on the formation of a red coloured azo product by the diazotisation of sulphonamides, sulphamethoxazole, sulphadiazene and sulphacetamide followed by a coupling reaction with resorcinol. Absorbance of the resulting red azo product is measured at 500 nm. Beer's law is obeyed in the concentration range of 0.25-7.0 µg/ml at the wavelength of maximum absorption. The method is successfully employed for the determination of sulphonamides in various pharmaceutical preparations.

Sulpha drugs are widely used in the treatment of infections, especially for patients intolerant to antibiotics. The vast commercial success of these medicinal agents has made the chemistry of sulphonamides to become a major area of research and an important branch of commercial importance in pharmaceutical sciences[1]. A survey of literature reveals that there are various methods available for the determination of sulphonamide derivatives. The official method of BP[2,3] and USP[4] describes nitrite titration method for the analysis of sulpha drugs. Among the number of spectrophotometric methods available the most prominent methods are chloramine-T[5], o-chloranil[6], metol-periodate[7], 4-dimethylaminecinnamaldehyde[8,9] and acetylacetone-formaldehyde[10] but all have certain limitations.

The present work describes the diazotisation reaction of sulphanomide derivatives followed by coupling with resorcinol to yield a red azo product with a maximum absorption at 500 nm. The method offers the advantages of rapidity, sensitivity and simplicity without the need for extraction or heating.

A JASCO model UVIDEC-610 UV/VIS spectrophotometer with 1.0 cm matched cells was used for electronic spectral measurements. Sulphonamide derivatives were all purchased from Sigma Chemical Co, St. Louis, MO, and were used without further purification. Sodium nitrite and resorcinol were purchased from BDH and AR sulphuric acid was used. All other reagents were of analytical grade. Commercial dosage forms were purchased from Burroughs Wellcome, Rhone-Poulnec, Nicholas Piramal India Ltd and East India Ltd. Deionized water was used to prepare all solutions. Standard solutions of sulphonamides (1000 µg/ml) were prepared by dissolving 100 mg of each sulphonamide in 2.0 ml of 10 M H_2SO_4 and then diluting to the mark in a 100 ml standard flask. A working standard solution of each sulphonamide containing 25 µg/ml was prepared by further

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dilution and standardised by the USP method[4]. A 1% of solution of $NaNO_2$ in water, 0.1% aqueous solution of resorcinol and 2% aqueous sulphamic acid were used for the experiment.

About 6.25-175 µg (2.5-150 µg for SFA) of sulphonamide derivative solutions were transferred into each of the series of 25 ml standard flasks and 1 ml of 10 M H_2SO_4 was added to each. After cooling in an ice bath, 1.5 ml of 1% $NaNO_2$ solution was added with swirling. The solutions were allowed to stand for 5 min and then 2.5 ml of 2% sulphamic acid solution was added. The solutions were swirled and allowed to stand for 5 min. Then, 4 ml of 0.1% resorcinol solution was added. The solution was made upto the mark with water, mixed thoroughly and after 5 min, the absorbance was measured at 500 nm against the corresponding reagent blank and calibration graphs were constructed.

Twenty tablets were weighed and finely powdered. The powder amount equivalent to 50 mg was dissolved in 2 ml of 10 M H_2SO_4 and filtered. The filterate was made upto 100 ml and appropriate aliquots of the tablet solutions were treated as described above in the recommended procedure. For eye drops, an accurately measured volume was appropriately diluted with 2 ml of 10 M H_2SO_4 and made upto 100 ml and the recommended procedure was followed.

The optical characteristics and precision data for the three sulpha drugs are given in Table 1. It was found that a 10 M solution of H_2SO_4 in the range of 0.5-2.0 ml, 1% solution of sodium nitrite in the range of 0.5-2.0 ml, 2% solution of sulphamic acid in the range of 1.0-3.5 ml and 2.0-6.0 ml of 0.1% solution of resorcinol were necessary to achieve maximum colour intensity. Hence, the required volumes of all the reagents were used as mentioned in the recommended procedure. The excess of nitrite during diazotisation could be removed by the addition of sulphamic acid solution. The use of sulphuric acid as the reaction medium was found to give better results than hydrochloric acid in terms of colour development and stability of the product. For the diazotisation process, sulpha drugs could be readily diazotised in acidic medium and that the diazonium cation would then react with a molecule of resorcinol by electrophilic substitution at the p-position of the coupling agent to produce a red azo product. The stability of the azo product was studied for more than two hours and it was found that the absorbance readings will be 3% off the original value. To increase the stability of the product for more than 2 h was unsuccessful. Some of the common excipients like carboxy methylcellulose, dextrose, glucose, lactose, starch, gum acacia, talc, sodium alginate and vitamin-B_6 which often accompany the pharmaceutical preparations do not interfere in the present method. The percentage recoveries of sulpha drugs in presence of the excipients varied from 99.3

TABLE 1: OPTICAL CHARACTERISTICS AND PRECISION DATA.

Parameters / Characteristics	SFMx	SFD	SFA
Colour	Red	Red	Red
λmax (nm)	500	500	500
Stability (h)	02	02	02
Beer's law range (µg/ml)	0.25-7.0	0.25-7.0	0.1-6.0
Least of detection (µg/ml)	0.0782	0.0530	0.0491
Least of quantification (µg/ml)	0.2592	0.1756	0.1627
Molar absorptivity (l/mol.cm)	3.02×10^4	3×10^4	4.5×10^4
Sandell's sensitivity (µg/cm^2)	0.0084	0.0083	0.0048
Optimum photometric range (µg/ml)	0.5-6.0	0.5-6.0	0.8-5.0
Regression equation (Y=bx+a)			
Slope (b)	0.0058	0.0219	0.0121
Intercept (a)	0.1003	0.1231	0.1062
Correlation coefficient (r)	0.9987	0.9976	0.9971
Relative standard deviation (%)	0.3339	0.7745	0.6365
Range of error	± 0.4635	± 1.075	± 0.8835

to 101.4. The reproducibility of the method was checked by ten replicate determination at 2 µg/ml level of sulpha drug and the relative standard deviation (%) was found to vary between 0.30 to 0.80. The present method has been applied for the analysis of sulpha drugs in pharmaceutical preparations. The results of the analysis of septran, sulphadiazine, albucid and locula compare favourably with those of the official method[3] and the reported methods[8,9].

The proposed method is found to be simple, rapid and sensitive. Thus the method can be adopted as an alternative to the existing spectrophotometric methods. The recommended procedure is well-suited for the assay and evaluation of drugs in pharmaceutical preparations to assure high standard of quality control.

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An Improved Gas Chromatographic Assay Method for HMG-CoA Reductase and Mevalonic Acid

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A simple and accurate gas chromatographic method for the *in vitro* estimation of mevalonic acid (as mevalonolactone) was developed. The method showed good sensitivity, accuracy and linearity over a wide concentration range. Detection at lower concentrations without the use of any radioactivity measurements, and more rapid analysis than other existing methods, are the advantages of this modified method.

Cholesterol is biosynthesised in the body from acetyl-CoA. Though it involves many steps, the most important, rate-limiting step is the formation of mevalonic acid from acetyl-CoA in the presence of enzyme, 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase[1]. Drugs such as lovastatin, simvastatin and gugulipid, which inhibit HMG-CoA reductase, are used to treat hypercholesterolemia. Several methods have been used to detect and estimate mevalonic acid from biological samples. But these methods include complicated procedures like derivative formation and crystallization to constant specific radioactivity[2], hydroxamate formation[3] or paper chromatography followed by assay for radioactivity[4]. Since crystallization of derivatives of mevalonic acid to constant specific radioactivity is time consuming, another method is sought to quantify the mevalonic acid

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