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NOTE

Extractive Spectrophotometric Determination of Balsalazide in Pure Form and Pharmaceutical Formulations by Using Safranin-O and Methylene Blue

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Two simple extractive spectrophotometric methods are described for the determination of balsalazide in pure form and pharmaceutical formulations. These methods are based on the formation of ion association complexes of the balsalazide with basic dyes safranin-O (**method A**) and methylene blue (**method B**) in basic buffer of pH 9.8 followed by their extraction in chloroform. The absorbance of the chloroform layer for each method was measured at its appropriate λ_{max} against the reagent blank. These methods have been statistically evaluated and are found to be precise and accurate.

Key Words: Balsalazide, Extractive spectrophotometry, Safranin-O, Methylene blue.

Balsalazide is chemically (E)-5-[[-4-[[(2-carboxyethyl)amino]carbonyl] phenyl]azo]-2-hydroxy benzoic acid. Balsalazide is an orally administered antiinflammatory[1] (gastrointestinal) drug. It is available in the form of disodium hydrate. It is used in the treatment of mild to moderate active ulcerative colitis. Balsalazide which has one molecule of 5-amino salicylic acid linked to a carrier *via* a diazo bond, is similarly split to release the active drug in the intestine. Balsalazide is a prodrug, converted by bacterial azoreduction to 5-aminosalicylic acid (active), 4-aminobenzoyl-β-alanine (inert) and their metabolites. 5-Aminosalicylic acid may decrease inflammation by blocking the production of arachidonic acid metabolites typically in the colon mucosa[2-4]. Balsalazide insoluble in acid and designed to be delivered to the colon intact.

In present studies, spectrophotometric method for determination of balsalazide has been developed. The proposed methods are based on the formation of ion association complexes of the balsalazide with basic dyes safranin-O (SFN O) and methylene blue (MTB)[5] in basic buffer of pH 9.8 followed by their extraction in chloroform.

All spectral and absorbance measurements were made on a Shimadzu UV/visible double beam spectrophotometer (model 1700) with 1 cm matched quartz cells were used for all the spectral measurements.

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All chemicals used were of analytical reagent grade. Balsalazide was obtained from Sun Pharmaceuticals Ltd. Dadra. Cozabal is the commercial capsule formulation labeled to contain 750 mg per capsule. SFN O 2.857×10^{-4} M and methylene blue 3.12×10^{-4} M were prepared in distilled water. Ammonia-ammonium chloride buffer solution (pH 9.8) was prepared by mixing 7 g of ammonium chloride with 56.8 mL of ammonia solution and diluted to 100 mL with distilled water and pH was adjusted to 9.8.

Standard solution of balsalazide (1 mg/mL) was prepared by dissolving 100 mg of drug in 100 mL of water. It was further diluted with water to get the working standard solution of 100 µg/mL. In case of formulation, one brand of commercially available capsules was analyzed by the proposed methods. Twenty capsules of balsalazide, each containing 750 mg of the drug. Capsule powder equivalent to 100 mg of balsalazide, was taken.

General procedure and calibration

Method A: Different aliquots of standard solution ranging from 0.2-1.0 mL and 1.0 mL of pH 9.8 buffer solution were placed separately in a series of 125 mL separating funnels. A volume of 2.0 mL safranin O was added to each separating funnel. The total volume in each funnel was adjusted to 10 mL with distilled water. Then 10 mL of chloroform was added to each separating funnel and the contents were shaken for 5 min and allowed to separate. The absorbance of the separated organic layer was measured at 520 nm against the reagent blank. The amount of balsalazide present in the sample was computed from calibration curve.

Method B: Different aliquots of standard solution ranging from 0.4-2.0 mL and 0.8 mL of pH 9.8 buffer solution were placed separately in a series of 125 mL separating funnels. A volume of 1.2 mL methylene blue was added to each separating funnel. The total volume in each funnel was adjusted to 10 mL with distilled water. Then 10 mL of chloroform was added to each separating funnel and the contents were shaken for 5 min and allowed to separate. The absorbance of the separated organic layer was measured at 666 nm against the reagent blank. The amount of balsalazide present in the sample was computed from calibration curve.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table-1. The regression analysis using method of least squares was made for the slope (b), intercept (a) and correlation (r) obtained from different concentrations and the results are summarized in Table-1. The per cent relative standard deviation and per cent range of error (0.05 and 0.01 level of confidence limits) calculated from the 8 measurements ¾ of the upper Beer's law limits of balsalazide are shown in Table-1. The results showed that these methods have reasonable precision. To evaluate the validity and reproducibility of the methods, known amounts of the pure drug were added to the previously analyzed pharmaceutical preparations and the mixtures were analyzed by the proposed methods. The per cent recoveries are given in Table-2. Interference studies revealed that the common excipients and other additives are usually present in the capsule dosage forms did not interfered at their regularly added levels.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Parameters	**Method A**	**Method B**
λ_{max} (nm)	520	666
Beer's law limits (µg/mL)	2-10	4-20
Molar absorptivity (L/mol^{-1} cm^{-1})	3.9×10^3	2.9×10^3
Sandell's sensitivity (µg/mL/cm^2/0.001 absorbance unit)	0.0111	0.0221
Regression equation (Y*)		
Slope (b)	0.0863	0.0441
Intercept (a)	0.0045	0.0061
Correlation coefficient (r)	0.9988	0.9985
% RSD	0.6340	1.1250
Range of errors		
Confidence limits with 0.05 level	0.00457	0.0050
Confidence limits with 0.01 level	0.00672	0.0074

*Y = bC + a where C is the concentration of balsalazide in µg/mL and Y is the absorbance unit.

TABLE-2
EVALUATION OF BALSALAZIDE IN PHARMACEUTICAL DOSAGE FORMS

Sample (capsules)	Labeled claim (mg)	Amount obtained (mg)*			Recovery (%)	
		Proposed methods		Reference UV method	Proposed methods	
		A	**B**		**A**	**B**
1	750	749.39 ± 0.04	748.99 ± 0.05	749.67 ± 0.03	99.91 ± 0.01	99.86 ± 0.05

*Mean ± SD of 8 determinations.

The proposed methods were found to be simple, sensitive, selective, accurate, precise and economical. In conclusion the proposed extractive spectrophotometric methods are more accurate and can be used in the determination of balsalazide in bulk drug and its pharmaceutical dosage forms in a routine manner.

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