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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHODS FOR THE ESTIMATION OF BALSALAZIDE IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

Two simple and sensitive visible spectrophotometric methods (A and B) have been developed for the quantitative estimation of balsalazide in bulk drug and dosage form. Method A is based on the oxidation followed by complex formation reaction of balsalazide with 1, 10-phenanthroline in presence of ferric chloride to form blood red colored chromogen with absorption maximum at 509 nm and Beer's law is obeyed in the concentration range of 6-18 µg/mL. Method B is based on the oxidation followed by complex formation reaction of balsalazide with potassium ferricyanide in presence of ferric chloride to form a bluish green colored chromogen with absorption maximum at 790.0 nm and Beer's law is obeyed in the concentration range of 2-12 µg/mL.The developed methods were found to be precise and accurate. The results obtained are statistically validated and found to be reproducible.

Key words: Validation, Balsalazide, Spectrophotometric, 1,10-Phenanthroline, Potassium ferricyanide.

INTRODUCTION

Balsalazide is chemically (E)-5-[[-4-[[(2-carboxyethyl)amino]carbonyl] phenyl]azo]-2-hydroxybenzoic acid. Balsalazide is an orally administered anti-inflammatory[1, 2] (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-aminosalicylic acid linked to a carrier via a diazo bond, splits to release the active drug in the intestine. It can also be used as an enema for local action.

Balsalazide is a prodrug, converted by bacterial azoreduction to 5-aminosalicylic

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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[3-5]. Balsalazide disodium capsules contain granules of balsalazide disodium, which are insoluble in acid and designed to be delivered to the colon intact. Upon reaching the colon, bacterial azo reductases cleave the compound to release 5-aminosalicylic acid, the therapeutically active portion of the molecule, and 4-aminobenzoyl-β-alanine.

Balsalazide is not official in any pharmacopoeia and spectrophotometric analytical reports are not found in literature for its quantitative estimation in bulk drug and pharmaceutical dosage forms. Hence, it was thought worthwhile to develop spectrophotometric method for the same. This paper describes two simple and sensitive visible spectrophotometric methods (A and B) developed for the quantitative estimation of balsalazide.

Method A is based on the oxidation followed by complex formation reaction of balsalazide with 1,10-phenanthroline[6-8] in presence of ferric chloride to form blood red colored chromogen with absorption maximum at 509 nm and Beer's law is obeyed in the concentration range of 6-18 µg/mL. Method B is based on the oxidation followed by complex formation reaction of balsalazide with potassium ferricyanide[9] in presence of ferric chloride to form a bluish green colored chromogen with absorption maximum at 790 nm and Beer's law is obeyed in the concentration range of 2-12 µg/mL. The spectrophotometric parameters are established for the standardisation of the methods including statistical analysis of data. These methods have been extended successfully to the pharmaceutical dosage forms.

EXPERIMENTAL

Instrumentation

A Shimadzu UV/visible double beam spectrophotometer (Model 1700) with 1 cm matched quartz cell was used for all the spectral measurements.

Reagents

All the chemicals used were of A.R. grade from s.d. Fine-Chem Ltd., Mumbai. Authentic sample of balsalazide was gifted by Sun Pharmaceuticals Ltd Dadra.

(i) Double distilled water

(ii) 1,10 – Phenanthroline (0.3 % in water)

(iii) Aqueous ferric chloride (0.5 % w/v)

(iv) Potassium ferricyanide (0.2 % w/v in water)

Standard and sample solutions

About 100 mg of balsalazide (pure or formulation) was accurately weighed and dissolved in water and volume was made up to 100 mL with water (1 mg/mL). The final concentration of balsalazide was made to 100 µg/mL with water. In case of formulation, twenty capsules of balsalazide each containing 750 mg were accurately weighed and powdered and then 100 mg of balsalazide equivalent were taken for the study.

Assay

Method A

Aliquots of balsalazide ranging from 0.6 – 1.8 mL (1 mL = 100 µg) were transferred into a series of 10 mL volumetric flasks. To each flask 0.2 mL of ferric chloride and 1.5 mL of 1,10 – phenanthroline were added. It was heated for 30 minutes on water bath at 95ºC and then cooled to room temperature. The volumes were made up to the mark with water. The absorbance of the blood red colored chromogen was measured at 509 nm against reagent blank. The colored species was stable for more than 4 hours. The amount of balsalazide present in the sample was computed from calibration curve.

Method B

Aliquots of balsalazide ranging from 0.2-1.2 mL (1 mL = 100 µg) were transferred into a series of 10 mL volumetric flasks. To each flask 1.0 mL of ferric chloride and 1.0 mL of potassium ferricyanide were added and set aside for 5 minutes for complete color development. The volumes were made up to the mark with water. The absorbance of the dark green colored chromogen was measured at 790 nm against reagent blank. The colored species was stable for more than 4 hours. The amount of balsalazide present in the sample was computed from calibration curve.

The results of the above methods were compared with the results obtained with UV spectrophotometric method. The solution of balsalazide in water was prepared. Aliquots of balsalazide ranging from 0.2-1.2 mL (1 mL =100 µg) were transferred into a series of 10 mL volumetric flasks. The volumes were made up to the mark with water and the absorbance of the solutions was measured at 358 nm against the solvent blank. The amount of balsalazide present in the sample was computed from the calibration curve.

RESULTS AND DISCUSSION

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity are presented in Table 1.

Table 1. Optical characteristics and precision

Parameters	Method A	Method B
λ_{max} (nm)	509	790
Beer's law limits (µg/mL)	6-18	2-12
Molar absorptivity (L/ $mol^{-1}.cm^{-1}$)	4.425 x 10^3	5.968 x 10^3
Sandell's sensitivity (µg /mL/ cm^2/ 0.001 absorbance unit)	0.0103	0.0109
Regression equation (Y*)		
Slope (b)	0.0491	0.0836
Intercept (a)	0.0161	0.0198
Correlation coefficient (r)	0.9986	0.9981
% RSD	1.16	0.875
Range of errors**		
Confidence limits with 0.05 level	0.00562	0.00399
Confidence limits with 0.01 level	0.00825	0.00591

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

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 summarised in Table 1. The percent relative standard deviation and percent range of error (0.05 and 0.01 level of confidence limits) calculated from the eight 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. The comparison of the results obtained with the proposed and UV methods for the dosage forms (Table 2) confirms the suitability of the methods for pharmaceutical dosage forms. When compared with UV method, the proposed

methods are reaction specific and eliminates interference from impurities.

Table 2. Evaluation of balsalazide in pharmaceutical dosage forms

Sample (capsules)	Labelled amount (mg)	Amount obtained (mg)*			% Recovery**	
		Proposed methods		Reference UV method	Proposed methods	
		A	B		A	B
1	750	748.92 ± 0.03	749.98 ± 0.04	749.67 ± 0.03	99.98 ± 0.02	99.99 ± 0.02

* Mean ± s.d. of eight determinations.

The optimum conditions for colour development for methods A and B were established by varying the parameters one at a time and keeping the other parameters fixed. The effects of product on the absorbance of the colored species were observed and incorporated in the procedures. To evaluate the validity and reproducibility of the methods, known amounts of the pure drug were added to the previously analysed pharmaceutical preparations and the mixtures were analysed by the proposed methods. The percent recoveries are given in Table 2. Interference studies revealed that the common excipients and other additives usually present in the capsule dosage forms do not interfere at their regularly added levels.

The proposed methods were found to be simple, sensitive, selective, accurate, precise and economical. The visible 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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