INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

RP-HPLC METHOD FOR THE ESTIMATION OF BALSALAZIDE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A rapid and reproducible RP-HPLC chromatographic method has been developed for the estimation of Balsalazide in its pure form as well as in pharmaceutical formulation. Chromatography was carried out on a C_{18} column using a mixture of water and acetonitrile as the mobile phase at flow rate of 0.8 ml/min and detection was done at 368 nm. The retention time of the drug was 3.685. The results obtained with the proposed methods are in good agreement with labeled amounts when marketed pharmaceutical preparations are analyzed. The recovery in the present method is in the range of 99.96 - 100.6. Results obtained are found to be reproducible.

Keywords: Balsalazide, reverse –phase liquid chromatography, anti-inflammatory.

INTRODUCTION

Balsalazide is chemically, 5-[(1E)-[4-[[(2-carboxyethyl) amino] carbonyl] phenyl] azo]-2-hydroxybenzoic acid. Balsalazide is chemically (E)-5-[[-4-[[(2-carboxyethyl) amino] carbonyl] phenyl]azo]-2-hydroxy benzoic acid. Balsalazide is an orally administered anti-inflammatory[1-3] (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 liked to a carrier via a diazo bond, is similarly split to release the active drug in the intestine. It is not official in any Pharmacopoeia. A thorough literature survey reveals that no HPLC method are reported however a few analytical methods have been reported for the estimation of balsalazide in bulk and pharmaceutical formulation including UV spectrophotometry[4-6].

EXPERIMENTAL

Instrumentation

An isocratic high performance liquid chromatography (Knauer HPLC) with Wellchrom HPLC - Pump K 501 and with software C2000 version 1.7 and UV/vis detector K 2501(Knauer). Column used was C-18, 250 x 4 mm i.dl; particle size 5 μm and packing material was eurosphere - 100.

Chemical and reagents

Balsalazide was the gift sample obtained from Sun pharmaceuticals Ltd, dadra, India. Acetonitrile HPLC grade are from Merk. Bombay, India.

Chromatographic conditions

The chromatographic column used was a 250 × 4 mm stainless steel with 5 μm particles. The HPLC equipment was operated at ambient temperature. The flow rate of the mobile phase was maintained at 0.8 ml/min in the ratio of

45:55 (Water: Acetonitrile). Detection was carried out by UV detector at 358 nm and the injection volume was 20 µl.

Working Standard of Drug Solution

About 100 mg of pure balsalazide dissolved in distilled water and diluting to 100 ml volumetric flask and diluted up to the mark with distilled water to get the concentration of (1 mg/ml). 10 ml of this solution was diluted up to 100 ml in a volumetric flask with methanol to get a final concentration 100 µg/ml.

Procedure

The solution was prepared on a weight basis and volumetric flasks were used to minimize solvent evaporation. Stock solution of drug was prepared by dissolving 100 mg of balsalazide in 100 ml volumetric flask containing 70 ml of distilled water sonicated for 15 min by using Bandelin sonoplus HD2070 Sonicator and then made upto volume with distilled water. Daily working standard solution of balsalazide was prepared by suitable dilution of the stock solution with mobile phase.

Five sets of the balsalazide solution were prepared in mobile phase at concentration of 2, 4, 6, 8, and 10 µg/ml. Each of these samples (20 µl) was injected five times into the column and the peak area of the drug was recorded.

Assay of Balsalazide capsules

Twenty capsules of balsalazide each containing 750 mg were accurately weighed the powder. An accurately weighed quantity of powder equivalent to 100 mg of balsalazide was transferred into 100 ml volumetric flask. It is then dissolved completely using distilled water and the volume was made up to the mark with distilled water (1mg/ml).The final concentration of balsalazide was brought 100 µg/ml with distilled water. The solution was then analyzed after dilution by RP-HPLC method. The stock solution was further diluted stepwise with mobile phase in such a way that, various aliquots contain 2 to 10 µg/ml and was filtered through a 0.45 µm membrane filter. All determinations were conducted six times.

RESULTS AND DISCUSSION

The run time of the method was set at 5 min and Balsalazide appeared on the chromatogram at 3.685 min (Fig -1), when the same drug solution was injected 5 times, the retention time of the drug was same. The peak area of balsalazide was calculated and the average of five such determinations was given in Table-1. When the concentration of balsalazide and its respective peak area were subjected to regression analysis by least square method, a high correlation coefficient was observed (r = 0.9998) in the range of 2-10 µg/ ml. the regression of balsalazide concentration over its peak area was found to be Y= 2981 X + 18448 (r^2 = 0.9998) where Y is the peak area and X is the concentration of balsalazide.

The proposed method was also validated for intra-and inter-day variation. When the solutions containing 6 -10 µg/ ml of balsalazide were repeatedly injected on the same day, the coefficient of variation (CV) in the peak area of the drug for five replicate injection was found to be less than 2% also intra-day variation (3and 5 injections) was found to be less than 2% (Table-2) thus the results have shown that the proposed method is highly reproducible. When a known amount of drug solution (6 or 10 µg/ ml) was added to a known concentration of drug solution (10µg/ ml), this was high recovery (99.96 -100.6) of balsalazide (Table-3) indicating that the proposed method is accurate.

The proposed method, developed in the present study has been used to quantify balsalazide in capsule dosage forms. Balsalazide (containing 750 mg of the drug) were analyzed as per the procedure the calibration curve for proposed method was given in (Fig- 2).The average drug content was found to be 99% of the labeled amount (Table-4).

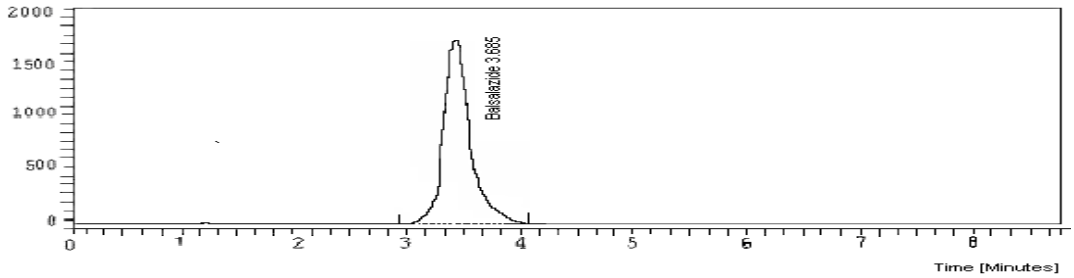

Fig. 1: Typical chromatogram of Balsalazide standard for proposed method

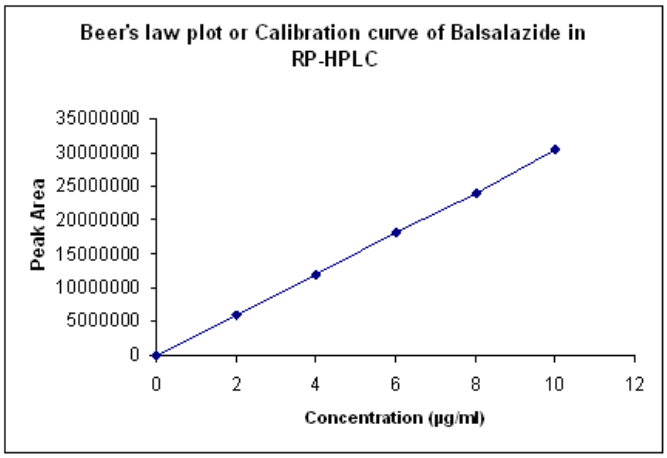

Fig. 2: Calibration curve of Balsalazide (RP-HPLC) for proposed method

Table 1: Calibration of the HPLC method for the estimation of Balsalazide

Concentration of Balsalazide (µg/ml)	Peak area *	C.V. (%)
0.0	0.0	0.0
2.0	6063572	1.21
4.0	12027145	1.98
6.0	18097016	0.95
8.0	23954288	1.54
10.0	30317862	1.23

*Mean of five determinations
Regression equation:
Y= -1121.51 + 0.4717.14 X (r^2 = 0.9998)

Table 2: Inter-and intra-day precision for Balsalazide assay in pharmaceutical dosage forms by the proposed HPLC method

Concentration of Balsalazide (µg/ml)	Conc. of Balsalazide found on			
	Intra-day		Inter-day	
	Mean (n=5)	C.V. (%)	Mean (n=5)	C.V. (%)
6	6.05	1.12	6.12	1.67
10	10.08	1.49	10.15	1.58

Table 3: Recovery of Balsalazide using the proposed HPLC method

Amount of drug added (µg)	Mean (± s.d) amount found (µg) (n=5)	Mean (± s.d) % of recovery (n=5)
8	8.05±0.042	100.6±0.04

Table 4: Mean (± s.d.) amount of Balsalazide in Capsule dosage forms by proposed HPLC method

Tablets	Labeled amount of drug (mg)	Mean (± s.d) amount found (mg) (n=5)	Mean (±s.d) % purity
C_1	750	749.96 ± 0.02	99.96 ± 0.01

ACKNOWLEDGEMENT

The authors wish to express their gratitude to Sun pharmaceuticals Ltd, Dadra for providing the gift sample of pure balsalazide.

REFERENCES

1. Sweetman SC, (Ed. By), "Martindale-The Complete Drug References", Pharmaceutical Press, London (U.K.) 33rd ed., 2002;121
2. O'Neil MJ. Ed., The Merck Index – An Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed., Merck & Co., Inc, 2001;947-948
3. Prakash A and Spencer CM. Review of pharmacology and clinical efficacy in ulcerative colitis. Drugs. 1998;56: 83
4. Naveen kumar GS, Manohara YN and Channabasavaraj KP. International. J Pharma Sci. 2008; 6(2);497-502.
5. Naveen kumar GS, Manohara YN and Karunakar Hegde. Asian J Chem. 2009;21(2):1624-1626.
6. Appala Raju S, Prabakar VK and Kaksay. Asian J Chem. 2005;17(2):1319-1321.