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Estimation of Balsalazide by HPTLC-Densitometry Method in Pharmaceutical Formulations

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Abstract

A simple, precise, rapid, selective, and economic high-performance thin layer chromatography (HPTLC) method has been established for estimation analysis of BAL. HPTLC method was developed using Chloroform: methanol (3.5:2, v/v) as a Mobile Phase and Pre-coated silica gel G60 - F254 aluminum sheet as a SP. Detection wavelength was 361 nm. In HPTLC linear range was 500-3000 ng / band, mean recoveries were found to be 99.99 - 100.04% & R_f of a BAL was found to be 0.61. This HPTLC method is economic, sensitive, and less time consuming than other chromatographic procedures. It is a user-friendly and importance tool for analysis of tablet dosage forms.

Keywords: HPTLC; silica gel G60 - F254; Tablet; Stationary phase (SP)

Introduction

BAL (BAL; 5-[4-carboxyethylcarbamoyl phenylazo] salycylic acid; Figure 1) is a widely used for Ulcerative colitis [1-4].

Literature survey revealed that various analytical methods and pharmacological methods like spectrophotometric [4], Studies of two Novel Sulfasalazine Analogs, Ipsalazide and Balsalazide [5], Sulphasalazine and balsalazide have membrane-stabilizing effects and cytoprotective action on ethanol-treated rat rectocolon [6], A Meta-Analysis of the Efficacy (SSZ) of Sulfasalazine in Comparison with 5-Aminosalicylates (5-ASAs) in the Induction of Improvement and Maintenance of Remission in Patients with Ulcerative Colitis [7], Low dose balsalazide (1.5 g twice daily) and mesalazine (0.5 g three times daily) maintained remission of ulcerative colitis but high dose balsalazide (3.0 g twice daily) was superior in preventing relapses [8] have been reported for the determination of BAL and either individually or combination with some other drugs, but no HPTLC method was reported for estimation estimation of BAL in dosage forms. The review of literature prompted us to develop an accurate, selective and precise estimation method for the estimation of dosage forms.

Experimental

Chemicals and materials

Methanol (A.R. grade), Water (HPLC Grade), Hydrochloric acid (A. R. Grade), Potassium di-hydrogen Phosphate (A. R. Grade), Sodium hydroxide (A. R. Grade), Hydrogen peroxide (A. R. Grade) and Ortho phosphoric acid (A. R. Grade) were used as solvents to prepare the mobile phase.

Chromatographic conditions

The samples were spotted in the form of band width 6 mm with Camag microlitre syringe on precoated silica gel aluminium Plate $60F_{254}$ (20 cm×10 cm with 0.2 mm thickness E. Merck, Germany) using Camag Linomat 5 (Switzerland). A constant application rate of 150 nL/sec was employed and space between two bands was 15.4 mm. Linear ascending development was carried out in twin trough glass chamber using chloroform: methanol: triethylamine (3.5:2:0.4 v/v) as a mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was approximately 80 mm. Subsequent to the development; TLC plates were dried in current of air with the help of an air dryer. Densitometric scanning was performed using Camag TLC scanner 3 in the absorbance mode at 361 nm. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum in the range of 190 - 400 nm.

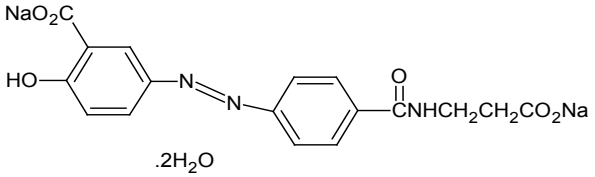

Figure 1: Structure of Dronedarone.

Sample preparation

Intanid capsule having labial claim 750 mg, dissolved methanol to make stock solution having BSZ (0.1 mg/mL) was prepared by removing, as completely as possible, the contents of 20 capsules. For HPTLC analysis, the powder equivalent to 100 mg of BSZ was weighed. The drug from the powder was extracted by methanol. To ensure the complete extraction of the drug, it was sonicated for 20 min and the volume was made up to the 100 mL and diluted further to make concentration of 500 µg/mL for HPTLC. The resulting solution was filtered using Whatmann filter paper 41. Appropriate solution (2 µL containing 1000 ng/spot) was spotted for assay.

Method validation

The developed method was validated for linearity and range, specificity, accuracy, precision, Limit of detection, Limit of quantitation, robustness and solution stability as per ICH guidelines.

Linearity and range: The standard solution (1-6 µl) prepared from

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standard stock solution of 5000 µg/mL was applied on TLC plate with the help of microlitre syringe, using Linomat V sample applicator. The plate was developed and scanned in the above established chromatographic conditions. Peak area was recorded for each concentration of drug; the observations are reported in and calibration curve was plotted as concentration *vs*. peak area.

Specificity: The peak purity of BAL was tested by correlating the spectra of BAL at the peak start (S), peak apex (A) and at the peak end (E) positions. Correlation between these spectra indicates purity of BAL peak. Thus, it can be concluded that no impurities or degradation products were found with the peaks of standard drug solutions.

Accuracy (% Recovery): The accuracy of the method was determined by calculating recoveries of BAL by method of standard additions. Known amount of BAL (80, 100 and 120%) were added to a pre quantified sample solution, and the amount of BAL was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

Method precision (Repeatability): Standard solutions of BAL (500, 1000 and 1500 ng/spot) were prepared and spectrums were recorded. Absorbance was measured at 289 nm using methanol as a blank. The absorbance of the same concentration solution was measured six times and %RSD was calculated.

Intermediate precision (Reproducibility): Variation of results of three different concentrations (500, 1000 and 1500 ng/spot) within the same day (intra- day), variation of results between days (inter- day) were analyzed.

Limits of Detection (LOD) and Limits of quantitation (LOQ): The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using following equation as per ICH guidelines. LOD = 3.3×σ/S; LOQ = 10×σ/S; Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Robustness: Robustness of the method was performed by change in plate activation time, chamber saturation time, volume of mobile phase, development distance.

Solution stability: The sample preparations were analyzed by HPTLC at regular intervals for 24 hrs at room temperature as per test procedure.

Results and Discussion

Method development and optimization of chromatographic conditions

TLC procedure was optimized with a view to develop a TLC densitometry method. Initially, mobile phase consisted of Chloroform: methanol (3.5:2, v/v) gave good resolution with R_f value of 0.61 for BSZ and tailing was observed (Figure 2). A typical peak nature was missing. Finally, the mobile phase consisting of chloroform: methanol: triethylamine (3.5:2:0.4 v/v) gave a sharp and well defined peak at R_f value of 0.61. Well-defined spots were obtained when the chamber was saturated with the mobile phase for 20 min at room temperature.

Validation of the method

Linearity: The linearity was determined at six levels over the range of 500 - 3000 ng/band. The calibration curve for BAL was prepared by plotting area versus concentration. The following equations for straight line were obtained for *BAL*: Linear equation for BAL: Y = 2.9944x + 41.057; Slope = 2.9944, Intercept = 41.057. Coefficient of correlation = 0.999. The linear range, correlation coefficient, detection limit and standard deviation for BAL are by HPLTC method (Table 1, Figure 3).

Specificity: The specificity study was carried out to check the interference from the excipients used in the formulations by preparing synthetic mixture containing both the drugs and excipients. The chromatogram showed peaks for the drug without any interfering peak and the recoveries of the drug were above 99% (Figure 4).

Accuracy: Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 99.99% - 100.04% for BAL (Table 2).

Precision

a) Repeatability: The % RSD < 2 for BAL which indicate that the method is precise.

b) Intra and inter day precision: Variation of results within the same day (intra- day), variation of results between days (inter- day)

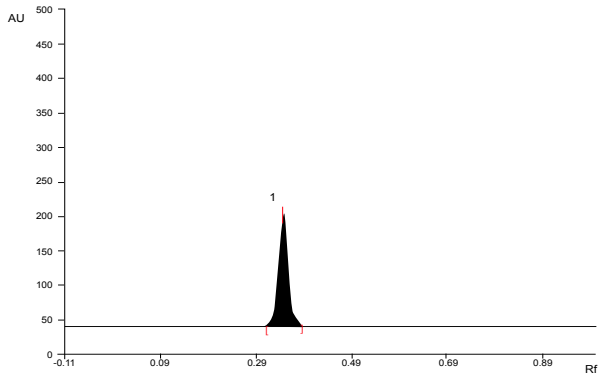

Figure 2: Densitogram of standard BAL, measured at 361 nm, mobile phase Chloroform: methanol (3.5:2, v/v).

Sample	Correlation of center and slope spectra	
	r (s, m)	r (m, e)
BAL	0.992	0.991
BAL formulation	0.994	0.992

Table 1: Peak purity correlation results of BAL in formulation at peak start, middle and end using CAMAG TLC SCANNER III.

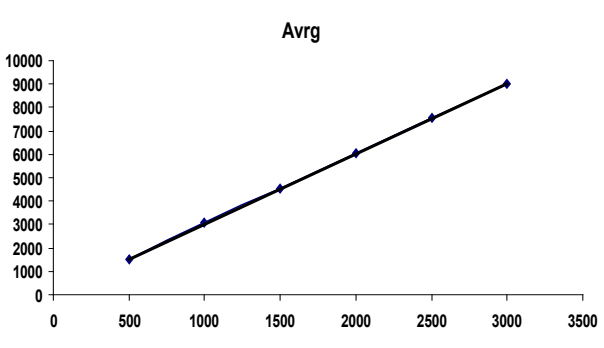

Figure 3: Calibration plot of BAL by the developed HPTLC method.

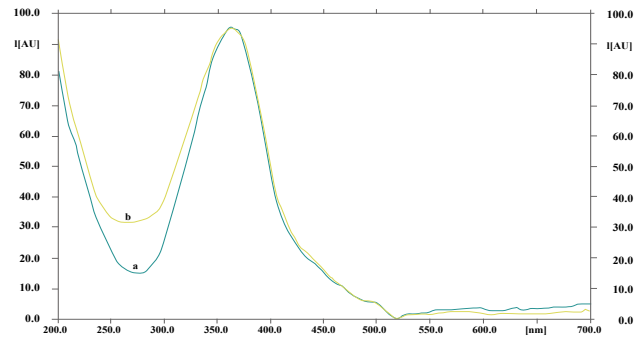

Figure 4: UV-Spectral of BAL (200-400 nm).

Drug	Initial Amount (ng/spot)	Amount added (%)	Amount recovered* (ng/spot)	% Recovered	% R.S.D.
BAL	1000	0	998.75	99.87	0.51
	1000	80	807.45	100.93	1.11
	1000	100	1001.96	100.19	0.74
	1000	120	1202.31	100.23	1.33

*mean of three estimations

Table 2: Results from accuracy study.

were analyzed. The method was found to be precise with % RSD 0.91-1.02 for inter-day study (n=3) and % RSD 0.49-0.63 for inter-day study (n=3). The % RSD < 2 for BAL indicates that the method is precise. (Table 3).

Limits of detection (LOD) and Limits of Quantification (LOQ): Under the experimental conditions used, the lowest amount of drug that could be detected (LOD) for BAL was found to be 0.19 µg/ml. The limit of quantification (LOQ) for BAL was found to be 1.19 µg/ml, with an RSD <2%.

Robustness: Acceptable %RSD values obtained after making small deliberate changes in the developed. Stability indicating HPLC method indicates that the method is robust for the intended purpose (Table 4).

Solution stability: The sample preparations were analyzed by HPTLC system at regular intervals for 24 hrs as per test procedure. The method is also rugged as there was no change in absorbance up to 24 hours of preparation of solution in Methanol.

Method application

The proposed, developed and validated method was successfully applied to analysis of BAL in their marketed formulation. There was no interference of excipients commonly found in tablets as described in specificity studies. The assay results obtained were satisfactory, accurate, and precise as indicated by the good recovery and acceptable standard deviation values (Table 5). The good performance of the method indicates that it can be used for the determination of BAL in pharmaceutical formulation.

Conclusion

This developed and validated method for analysis of BAL in pharmaceutical preparations is very rapid, accurate, and precise. The method was successfully applied for determination of BAL in its pharmaceutical tablet formulation. Moreover it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence this method can be conveniently used for routine quality control analysis of BAL in its pharmaceutical formulation.

Parameter	Data
Linearity range(ng per spot)	500 - 3000
Limit of Detection (ng)	30
Limit of Quantitation (ng)	82
Accuracy (n = 9)	99.54 ± 0.42
Precision (% RSD) Repeatability of application (n = 7)	1.65
Interday (n = 3)	0.49
Intraday (n = 3)	0.78
Specificity	Specific
Robustness	Robust

Table 3: Summary of validation parameters of developed HPTLC method.

Parameter	S.D. of Peak area	% R.S.D.
Mobile phase composition	92.32	0.33
Amount of mobile phase	60.44	0.92
Temperature	71.08	0.62
Relative humidity	81.02	1.28
Plate pretreatment	84.25	1.27
Time from spotting to chromatography	96.51	1.61
Time from chromatography to scanning	88.31	1.29

Table 4: Results from the robustness study of method.

Batch no. C01364704		Average wt = 0.8431 g	
Component	Label claim (mg)	Amount Found (mg) (n = 6)	Amount found (%)
BSZ	750	764.11	101.88
	750	746.25	99.75
	750	749.25	99.87
	750	762.09	101.72
	750	744.82	99.31
	750	749.77	99.97
Mean ± S. D.		752.75 ± 8.72	100.41 ± 1.09
% R.S.D.		1.09	1.09

n = number of determinations

Table 5: Results from analysis of Balsalazide in the combined tablet dosage form Brand name: INTANID (Dr. Reddy's ltd.)

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