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Development and Validation of HPTLC Method for Estimation of Balofloxacin in Bulk Drug and in Tablet Dosage form

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Abstract: Balofloxacin is the fourth generation of a new class of synthetic anti bacterial fluoroquinolone agents. The present work describes a simple, precise and accurate HPTLC method for its estimation as bulk and in tablet dosage form. The chromatographic separation was carried out on pre coated silica gel 60 F 254 aluminum plates using mixture of methanol: butyl alcohol:water:ammonia (6:2:2:0.4v/v) as mobile phase and densitometric evaluation of spots were carried out at 294 nm using camag TLC scanner3 with WINCAT software. The experimental parameters like band size of spot applied, chamber saturation time, solvent front migration, slit width etc were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved in Rf value 0.65. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity, precision, accuracy, and specificity according to ICH guidelines. The proposed method provides a faster and cost effective quality control tool for routine analysis of balofloxacin as bulk drug and in tablet formulation.
Key words: Balofloxacin, HPTLC, densitometric estimation, method development and validation

Introduction:

Balofloxacin chemically (1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7(3 methyl amino-piperidin-1-yl)-4-oxoquinolone-3-carboxylic acid[1.] It has a broad anti bacterial Spectrum, ranging from gram positive bacteria to gram negative bacteria. In literature, various analytical methods, such as RP-HPLC[2-6], UV[7] have been developed for determination of Balofloxacin. However, no HPTLC method is available for estimation of balofloxacin either in bulk or in dosage form.The present study illustrates development and validation of a simple, accurate, economical and reproducible procedure for determination.of balofloxacin by HPTLC as bulk and in tablet dosage form.

Materials and methods

Fixed dose tablets (Balorain 100mg) containing 100mg of balofloxacin procured from local pharmacy store. Silica gel 60 F 254 TLC plates (10x10 cm, layer thickness, 0.2 nm, E-Merck, Germany) were used as stationary phase. All Chemicals and reagents used were of analytical grade and purchased from merck chemicals corporation Ltd, Mumbai. A Camag HPTLC system containing camag linomat IV semi automatic sample

applicator. Hamilton syringe (100µl) camag TLC scanner 3 with WINCAT software version 1.34 camag twin trough chamber (20x10 cm) were used for present study.

Balofloxacin 10mg were weighed accurately dissolved and diluted with methanol to obtain the final concentration of 100µg/ml. Twenty tablets were weighed accurately and ground to fine powder. Weight equivalent to 10 mg of balofloxacin was transferred to conical flask and mixed with methanol. The solution was sonicated for 15 min. the extracts were filtered through whatmann filter paper and residue washed thoroughly with methanol. The extracts and washings were transferred to10ml volumetric flask and volume was made upto 10ml with methanol. Required dilutions were made to get 100 µg/ml.

Various solvent system were tried to separate and resolve spot of balofloxacin from its impurities and other excipients of formulations. The mixture of methanol: Butyl alcohol: water: ammonia (6: 2:2:0.4) could resolve balofloxacin spot with better peak shape (Fig. 1) with Rf value of 0.65 ± 0.01. TLC plates were pre washed with methanol. Activation of plates was done in an oven at 115 c for 10 minutes. The chromatographic conditions maintained were precoated silicagel gel $60F_{254}$ aluminium plates(10x10 cm)as stationary phase, methanol : water : Butyl Alcohol :ammonia as mobile phase and plate saturation time of 15 minutes, wavelength scanning was done at 294nm. A deuterium lamp provided the source of radiation.

3 µl standard solution of balofloxacin was spotted and developed photometric measurements were performed at 294 nm in reflectance mode with camag TLC scanner3 and using WINCAT software version 1.34 incorporation track optimization position. For the preparation of calibration curve aliquots of 1.5-5.5 µl of standard solution of balofloxacin (100µg/ml) were applied on TLC plate using semi automatic spotter. TLC plates were dried, developed and densitometrically analyzed as described earlier.

Results and discussion:

The method was validated as per ICH guidelines[8] in terms of linearity, accuracy, specificity, interday and intra day precision, repeatability of measurement of peak area as well as repeatability of sample application (**Table-1).**The method was found to be linear in the range of-150-550ng/spot (r= 0.999) in five replicates. LOD and LOQ of balofloxacin were found to be 30 and 100 ng/spot. The intraday precision was determined by analysis of standard balofloxacin solution in the concentration range of 250ng/spot and 350 ng/spot for three times on the same day, while inter day precision was determined by analyzing correspondingly standards over a period of one week. The percentage RSD of intraday and interday precision of balofloaxcin the range of 0.5-0.9.The value indicates the method is precious. Repetability of sample application was assessed by spotting 3.5 µl of drug solution six times on TLC plate followed by development of plate and recording the peak area of spots. The percentile RSD peak area values of balofloxacin were found to be 0.32. Repetability measurement of peak area was determined by spotting 3.5µl balofloxacin solution in TLC plate and developed. The separated spot was scanned six times with out changing the position of the plate and the percentile RSD measurement of peak area value of balofloxacin was found to be 0.19.

To confirm the specificity of prepared method, the solution of formula was spotted on the TLC plate which was then developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peak of balofloxacin.

Recovery studies of the drug were carried out for the accuracy and reliability. These studies were carried out at two different levels.

The result of recovery study indicates the proposed method is accurate for estimation of balofloxacin in tablet dosage form. (**Table-2).**

In conclusion the proposed HPTLC method was found to be rapid, specific, precise, and accurate can be used routinely for the estimation of balofloxacin in bulk drug and tablet dosage form.

Table :1 Summary of validation parameters

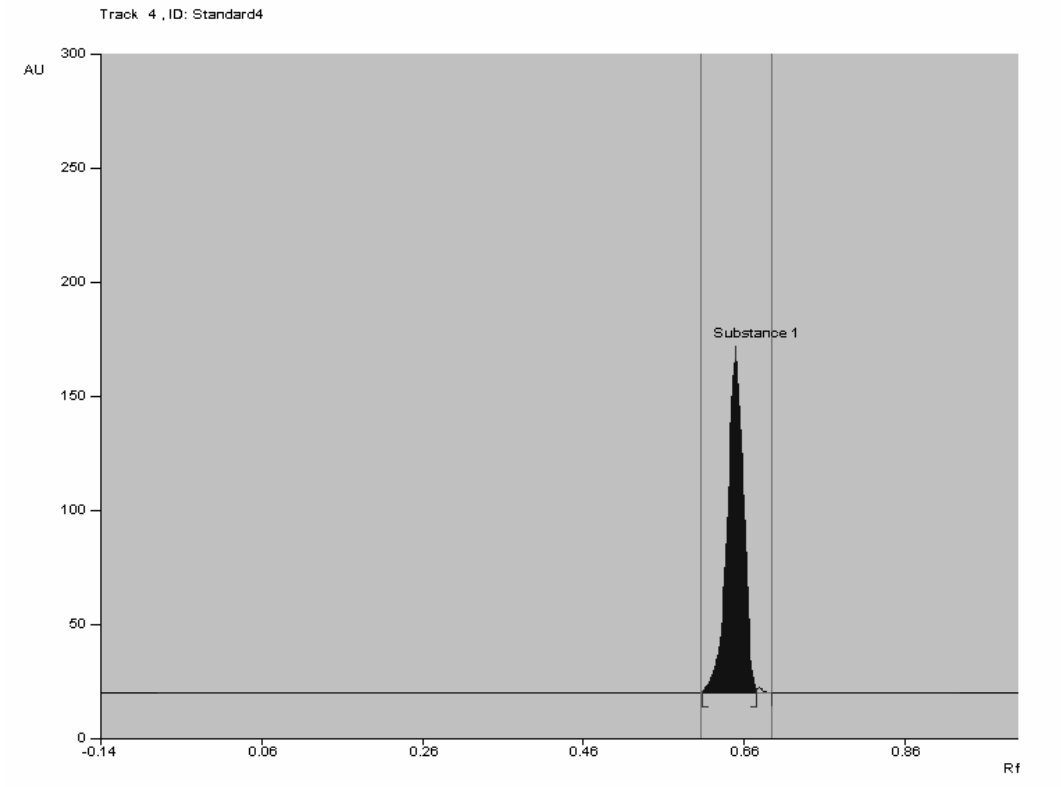
Linearity range (ng/spot)	150-550
r	0.99947
Slope (m)	3.9328
Intercept (c)	639.61
LOD (ng/spot)	30
LOQ(ng/spot)	100
Precision (%RSD) - Intraday - Interday - Repeatability of sample application (n=6) - Repeatability of sample measurement(n=6)	 0.53-0.78 0.52-0.98 0.32 0.19

* Each value is a mean of six observations

Table 2 Recovery studies

Label Claim (mg/tablet)	Amount added (%)	Total amount added (mg)	Amount Recovered (mg)	% Recovery
Balofloxacin (100)	50	50	49.15	98.30
	100	100	98.62	98.62

Fig – 1 HPTLC Chromatogram of balofloxacin standard solution



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