Development and Validation of HPTLC Method for the Estimation of Etoricoxib

N. J. SHAH*, S. J. SHAH, D. M. PATEL AND N. M. PATEL
Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa-383 315, India.

A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the determination of etoricoxib in dosage forms. The stationary phase used was precoated silica gel $60F_{254}$. The mobile phase used was a mixture of chloroform: methanol: toluene (4:2:4 v/v). The detection of spot was carried out at 289 nm. The calibration curve was found to be linear between 100 to 600 ng/spot for etoricoxib. The proposed method can be used to determine the drug content of marketed formulations.

Etoricoxib is a cyclooxyginase-2 (COX-2) selective NSAID used in the treatment of rheumatoid arthritis, osteoarthritis, postoperative dental pain, chronic low back pain, acute gout and primary dismenorrhoea[1]. Chemically it is 5-chloro-2-(6-methyl pyridin-3-yl)-3-(4-methylsulfonylphenyl) pyridine[2]. The drug is available in tablet dosage form and is not yet official in any of the pharmacopoeias. Literature survey revealed HPLC methods[3-5] and RP-HPLC method[6] for the estimation of etoricoxib. The aim of the present investigation was to develop a simple, precise and accurate analytical method for determination of etoricoxib in drug and its various dosage forms.

Etoricoxib working standard was procured as a gift sample from Sun Pharmaceuticals Ltd., Baroda; and silica gel $60F_{254}$ TLC plates (10 × 10 cm, layer thickness 0.2 mm) from E. Merck, Mumbai. All chemicals and reagents used were of analytical grade. Tablets containing 60 mg and 90 mg of etoricoxib were purchased from the local market (Torcoxia-60 - Torrent Pharmaceuticals Ltd., Ahmedabad; and Kingcox-90 - Cadila Pharmaceuticals Ltd., Ahmedabad). A Camag HPTLC system comprising of Camag Linomate V automatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, Camag WinCATS software, Camag twin trough chamber (10 × 10 cm) and ultrasonicator was used during the study.

Standard etoricoxib (10 mg) was weighed accurately and diluted with methanol to obtain a concentration of 100 µg/ml of drug. The content of 20 tablets was ground to a fine powder. Weight equivalent to 25 mg of etoricoxib was transferred to a conical flask and dissolved in methanol. The solution was ultrasonicated for 15 min. The extracts were filtered through Whatman filter paper No. 41 and the residue washed with methanol. The extracts and washings were pooled and transferred to a 250 ml volumetric flask and the volume made up with methanol. Required dilutions were made to get 100 µg/ml of etoricoxib.

TLC plates were pre-washed with methanol. Activation of plates was done in an oven at 50° for 5 min. The chromatographic conditions were precoated silica gel $60F_{254}$ aluminium sheets as stationary phase, chloroform: methanol: toluene (4:2:4 v/v) as mobile phase, chamber and plate saturation time of 30 min; migration distance allowed was 72 mm, and wavelength scanning was done at 289 nm, keeping the slit dimension at 5 × 0.45 mm. A deuterium lamp provided the source of radiation. Four microlitres of standard solution of etoricoxib was applied and developed at constant temperature. Photometric measurements were performed at 289 nm in reflectance mode with Camag TLC scanner 3 using Win CATS software.

Aliquots of 1, 2, 3, 4, 5 and 6 µl of standard solution of etoricoxib were applied on the TLC plate. TLC plate was dried, developed and analyzed photometrically as described earlier. The standard calibration curve was generated using regression analysis with Microsoft excel.

***For correspondence**
E-mail: nehal9175@yahoo.co.in

The developed method was validated in terms of linearity, accuracy, specificity, limit of detection, limit of quantification, intra-day and inter-day precision and repeatability of measurement, as well as repeatability of sample application.

Four microlitres of the filtered solutions of the marketed formulation was spotted on to the same plate, followed by development scanning. The analysis was repeated in triplicate. The spots were resolved into single peak in the chromatogram of drug samples extracted from the marketed formulations. The content of the drug was calculated from the peak areas recorded.

A solvent system that gives dense and compact spot with significant R_f value was desired for quantification of etoricoxib in pharmaceutical formulations. The mobile phase consisting of chloroform: methanol: toluene (4:2:4 v/v) gave R_f value of 0.58 ± 0.03 for etoricoxib. The linear regression data (n = 5, Table 1) showed a good linear relationship over a concentration range of 100-600 ng/spot for etoricoxib. The limit of detection and limit of quantification were found to be 50 ng/spot and 100 ng/spot respectively.

The intra-day precision was determined by analyzing standard solutions in the concentration range of 200-500 ng/spot three times on the same day, while inter-day precision was determined by analyzing corresponding standards for 3 d over a period of 1 w. Repeatability of sample application was assessed by spotting 4 µl of drug solution five times on a TLC plate, followed by development of plate and recording the peak area for five spots. The percent RSD for peak area values of etoricoxib was found to be 0.25. Repeatability of measurement of peak area was determined by spotting 4 µl of etoricoxib solution on a TLC plate and developing the plate. The spot was scanned five times without changing the position of the plate, and percent RSD for measurement of peak area of etoricoxib was 0.44. To confirm the specificity of the proposed method, the solution of the formulation was spotted on the TLC plate, developed and scanned. It was observed that the excipients present in the formulation did not interfere with the peaks of etoricoxib.

Recovery studies of the drugs were carried out for accuracy parameters. These studies were carried out at three levels, i.e., multiple level recovery studies. Sample stock solution from tablet formulation of 100 µg/ml of etoricoxib was prepared. Dilutions were made and recovery studies were performed. Percentage recovery was found to be within limits. The assay values for the marketed formulations were found to be within limits. The low RSD value indicated the suitability of the method for routine analysis of etoricoxib in pharmaceutical dosage forms. The HPTLC technique described is simple, precise, specific and accurate, and the statistical analysis proved that the method is reproducible and selective for the analysis of etoricoxib in bulk drug and tablet formulations.

TABLE 1: VALIDATION PARAMETERS FOR DEVELOPED METHOD

Parameter	Result
Linearity range (ng/spot)	100-600
Correlation coefficient (r)	0.9993
Regression equation (y=mx+c)	15.397
Slope (m)	119.85
Intercept (c)	50 ng/spot
Limit of detection (LOD)	100 ng/spot
Limit of quantification (LOQ)	0.25
Precision (%CV)	0.44
Repeatability of application (n=5)	
Repeatability of measurement (n=5)	

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