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NOTE

HPTLC Method for Determination of Ezetimibe in Tablets

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A simple, sensitive HPTLC method has been developed for the analysis of ezetimibe in its commercial single component tablet formulations (10 mg/tablet). The study employs a silica gel $60GF_{254}$ on aluminium foil and a mobile phase used was a mixture of toluene: acetone (6:4 % v/v). Detection was carried out at 233 nm. The R_f value was 0.52 for ezetimibe. The linearity was observed in the range of 300-2100 ng/spot. The recovery study was carried out by standard addition method and was found to be 99-101 %.The method was validated as per ICH guidelines.

Key Words: Ezetimibe, HPTLC, Validation.

Ezetimibe chemically 1-(4-flurophenyl)-3-[(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azetidione[1] is a selective cholesterol absorption inhibitor[2]. Several analytical methods[3,4] have been developed for the quantitation of ezetimibe.

A pharmaceutical grade of ezetimibe was kindly supplied as a gift sample by Blue Cross Lab. Ltd., Nashik, used without further purification. All chemicals and reagents used were of HPLC grade.

The samples were spotted in the form of bands of width 6 mm with camag 100 µL sample syringe on a precoated silica gel aluminium plate 60F 254 using Camag Linomat V sample applicator. A constant application rate of 0.1 µL/s was employed and space between two bands was 5 mm. The slit dimension was kept at 5 × 0.45 mm and 10 mm/s scanning speed was employed. Mobile phase consists of toluene:acetone (6:4 % v/v); saturation time 20 min. Detection was carried out by using camag TLC scanner 3 at 233 nm. Software used was CATS version 1.3.0.

Calibration curve: A stock solution of 100 mg/mL was prepared in methanol. Different volumes of stock solution were spotted on TLC plate to obtain concentration in the range of 300-2100 ng/spot. The data of peak area *vs.* drug concentration were treated by regression analysis (Table-1).

TABLE-1
LINEARITY STUDY

Parameter	By Area	By Height
Correlation Coefficient (r^2)	0.9994	0.9996
S.D. of slope	2.9154	0.1008
S.D. of Intercept	618.64	44.111

Assay: 20 Tablets (each containing 10 mg ezetimibe) were taken and finely powdered. The powder equivalent to 10 mg of ezetimibe was dissolved in 5 mL of methanol. The solution was centrifuged for 15 min at 600 rpm. The solution was filtered through Whatman filter paper No. 41 and the solution was further diluted to 10 mL to have concentration of ezetimibe equivalent to 100 µg/mL. The percentage label claim was calculated by linear regression line equitation. The method was repeated for 6 times and results are shown in Table-2.

TABLE-2
RESULTS OF ASSAY

Label Claim	10 mg/tablet
Amount found (in %)*	99.3300
S. D.	4.7161
% RSD	0.7900

*Mean of 6 determinations.

Validation of method: The method was validated as per ICH guidelines[5].

Accuracy: It was done by recovery study using standard addition method, known amount of standard ezetimibe was added into pre-analyzed sample and subjected them to the proposed HPTLC method (Table-3). The study was carried out at three different concentration levels.

TABLE-3
RECOVERY STUDIES

Excess drug added to analyte (%)	Recovery* ± % RSD
80	101.19 ± 0.60
100	99.25 ± 0.78
120	99.87 ± 0.50

*Mean of three estimations.

Precision: Intra-day precision was determined by analyzing, the three different concentrations of drug, for three times, in the same day. Inter-day precision was determined by analyzing, the three different concentrations

of the drug daily, for 3 d, in a week. The results were statistically validated by calculating % RSD and found to be lower than 2 %, which shows the high precision of the method.

Repeatability: Repeatability of measurement of peak area was determined by spotting 6 µL of ezetimibe standard solution on TLC plate and developing the plate. The separated spot of ezetimibe was scanned 6 times without changing the position of the plate and % RSD for measurement of peak area was calculated and found to be less than 2 %.

Ruggedness: Ruggedness of the proposed method was determined by analysis of aliquots from homogeneous slot in different laboratories by different analysts using similar operation and environmental conditions.

Limit of detection and limit of quntitation: LOD and LOQ was calculated by the method which was based on the SD of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S) and found to be 56.89 and 172.39 ng/mL, respectively.

Specificity: To confirm the specificity of the proposed method ezetimibe tablet solution was spotted on TLC plate, developed and scanned as described earlier. It was observed that excipients present in formulation did not interfere with peak of ezetimibe. The spectrum of ezetimibe extracted from tablet was also compared with spectrum of standard ezetimibe, which showed good correlation.

The mixture of toluene:acetone (6:4 % v/v) could resolve ezetimibe spot with better peak shape. Combination of toluene and acetone offered optimum migration (R_f = 0.52) and resolution of ezetimibe from other components of formulation matrix. Even pre-saturation of TLC chamber with mobile phase for 20 min assured better reproducibility in migration of ezetimibe and better resolution. Linearity was observed in the range of 300-2100 ng/spot with r^2 = 0.9994. The % recovery was observed in the range of 99-101, shows the accuracy of the proposed method. The low values of % RSD indicate the method is precise and accurate.

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