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NOTE

Spectrophotometric Estimation of Ezetimibe in Tablet Dosage Form

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Two simple, accurate and economical spectrophotometric methods were developed for estimation of ezetimibe in tablet dosage form using standard calibration graph and derivative spectrophotometric method. Both the methods have λ_{max} at 248.1 and 265.9 in 50% methanol, respectively showing linearity in the concentration range of 0–50 μg/mL. The results of analysis have been validated according to ICH guidelines which confirmed that the proposed methods are accurate and precise.

Key Words: Spectrophotometric, Estimation, Ezetimibe.

Ezetimibe, 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)-hydroxyl propyl]-4(S)-(4-hydroxyphenyl)-2-azetidione[1], is a new lipid lowering agent which inhibits the absorption of cholesterol from intestine[2, 3]. Literature survey reveals that there is no spectrophotometric method for estimation of ezetimibe in tablet dosage form. The present work deals with the development of spectrophotometric methods (standard calibration graph method and first derivative spectrophotometric method) and their validation for estimation of ezetimibe in tablet dosage form.

Ezetimibe working standard was obtained as a generous gift from Torrent Pharmaceuticals, Baroda. Ezetimibe tablets were procured from the market [Zetia-Torrent Pharmaceutical, Ezta-Zydus Cadila and Ezedoc-Pinnacle Lupin]. Analysis was performed on UV 1700 series spectrophotometer (Shimadzu) and UV-Vis double beam spectrophotometer 2201 (Systronics) with 1 cm matched couvettes.

In Method A, appropriate amount of ezetimibe was accurately weighed and dissolved in 50% of methanol (Stock A). Standard Stock A solution was diluted to obtain a solution of 100 μg/mL concentration (Stock B) standard solution. Aliquots of the Stock B solution were further diluted to obtain a different concentration solution of ezetimibe. Different concentration solutions were scanned and a calibration curve was plotted at 248.1 nm from mean value. From calibration curve Beers-Lambert law was obeyed in the range of 0–50 μg/mL (Table-1).

In Method B, standard Stock B solution of ezetimibe was scanned over the range 200–300 nm in the spectrum mode and derivatized in all the orders. Finally, the first order was found most suitable to estimate accurate amplitude of derivatized spectrum (Fig. 1). The wavelength selected for measurement is 265.9 nm as at this wavelength ezetimibe exhibited maximun rate of change of absorbance with wavelength against wavelength ($dA/d\lambda$ *vs.* λ)[4]. Different concentration solutions of ezetimibe were prepared from the aliquots of Stock B solution. The rate of change

in absorbance with wavelength against wavelength (dA/dλ *vs.* λ) were measured at 265.9 nm. A calibration curve was plotted between dA/dλ against concentration (Table-1).

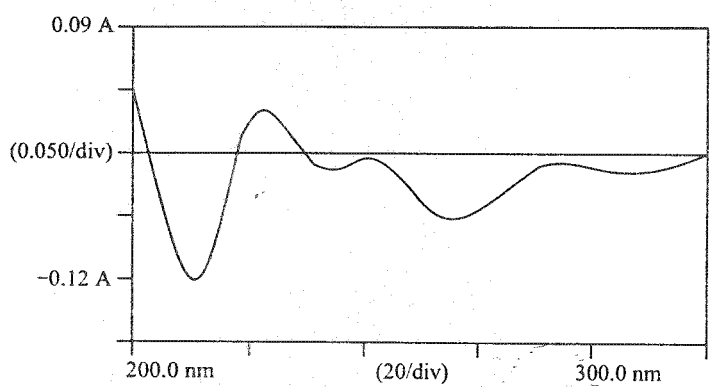

Fig. 1. First derivative spectrum of working standard of ezetimibe

TABLE-1
OPTICAL CHARACTERISTICS

Observations	Method A	Method B
Absorption maxima (nm)	248.1	265.9
Beer's law limit (μg/mL)	0–50	0–50
Regression equation (Y = mx + c):		
Slope (m)	0.0355	0.0016
Intercept (c)	0	0
Correlation coefficient (r)	0.9995	0.9998

Method A: Standard calibration graph method.
Method B: First derivative spectrophotometric method.

As per ICH guideline for "analytical method validation"[5], both methods were validated for linearity, range, accuracy, precision (repeatability and intermediate precision) and robustness. For linearity, a series of dilutions were prepared and response ratios were determined respectively. The range was determined by preparing a series of dilutions from 80–120% of test concentration (30 μg/mL) in six replicates. By recovery studies, known amounts of standard drug were added to the previously analysed tablet sample and the mixtures were analysed by both the proposed methods. The accuracy of the methods was determined. Precision was studied for repeatability and intermediate precision (days, instruments and analysts). Robustness was studied for variation in solvent composition 45, 50 and 55% aqueous methanol (Table-2).

For analysis of tablet sample, twenty tablets were accurately weighed and average weight was determined. The tablets were powdered and powder equivalent to 100 mg of ezetimibe was accurately weighed, transferred to a 100 mL volumetric flask and dissolved in 50% methanol. The solution was filtered through Whatman filter paper no. 41 and diluted to get the concentration in the range of linearity. The sample solutions were scanned similarly as the standard at the selected wavelength in both methods to get the concentration of drug in tablet dosage form. The results of tablet analysis were statistically validated with six replicates (Table-3).

TABLE-2
RESULTS OF VALIDATION PARAMETERS

Validation parameters	Method A			Method B		
	Mean*	SD	RSD	Mean*	SD	RSD
Linearity	100.31	0.121	0.301	100.12	0.187	0.373
Accuracy	100.20	1.050	1.047	99.53	0.727	0.730
Precision repeatability	98.99	0.088	0.894	99.67	0.062	0.623
Intermediate precision:						
Days	99.72	0.049	0.488	99.95	0.022	0.321
Instruments	100.13	0.561	0.112	100.04	0.079	0.263
Analysts	99.07	0.077	0.780	99.81	0.046	0.461
Robustness	99.18	0.040	0.404	99.97	0.044	0.450

*Mean of six replicates. SD = Standard deviation. RSD: Relative standard deviation. Method A: Standard calibration graph method. Method B: First derivative spectrophotometric method.

TABLE-3
ANALYSIS DATA OF TABLET DOSAGE FORM

Tablet formulation	Label claim (mg/tab)	Method	Percentage of label estimated* (mg/tab)	SD	RSD	SEσ
A	10	M_A	9.94	1.339	1.346	0.598
		M_B	10.14	0.506	0.509	0.226
B	10	M_A	9.93	1.126	1.135	0.463
		M_B	9.97	0.599	0.599	0.244
C	10	M_A	9.90	1.183	1.192	0.482
		M_B	10.19	0.601	0.601	0.245

*Mean of six replicates. A: Zetia, 10 mg tablet of Torrent Pharmaceutical; B: Ezta, 10 mg tablets of Zydus Cadila; and C: Ezedoc, 10 mg tablets of Pinnacle Lupin; SD = Standard deviation; RSD = Relative standard deviation; SEσ = Standard error of standard deviation.

Conclusion

Assay precision experiment yielded results that were precise with percentage relative standard deviation less than 2.0%. Percentage recovery in terms of accuracy was found to be in the range of 99.0–101.0% for both methods. Based on the validation study data it can be concluded that the proposed methods are accurate and precise for the analysis of drug. No interference was found from excipients used in tablet dosage form. As compared to HPLC, both methods are rapid and economic, therefore successfully applied for routine analysis of ezetimibe in tablet dosage form.

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