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Review Article

A Review on Analytical Techniques for the Assay of Ezetimibe

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Abstract

Ezetimibe is a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption. It belongs to a group of selective and very effective 2-azetidione cholesterol absorption inhibitors. A brief review of the analytical techniques such as spectrophotometry, liquid chromatography, mass spectrometry so far developed for the estimation of Ezetimibe were summarized in the present study.

Keywords: Ezetimibe; Cholesterol; Methanol; Ethanol

Introduction

Ezetimibe (CAS: 163222-33-1) (Figure 1) is used to treat high blood cholesterol (1-2). Ezetimibe is chemically [(3*R*, 4*S*)-1-(4-fluoro phenyl)-3-[(3*S*)-3-(4-fluoro phenyl)-3-hydroxy propyl]-4-(4-hydroxy phenyl)-2-azetidinone]. It is practically insoluble in water and it is freely soluble in methanol, ethanol and acetone. Ezetimibe ($C_{24}H_{21}F_2NO_3$) reduces the amount of cholesterol absorbed from the food intake which decreases the amount of bad cholesterol (LDL) in blood. It rapidly metabolises via a phase II glucuronide conjugation reaction (3) in the small intestine and liver and the elimination half-life for Ezetimibe and its glucuronide metabolite is approximately 22 hours. Ezetimibe is available with brand names, Ezetrol, Lypqozet, Nexlizet, Roszet, Vytorin, Zetia etc. It was estimated by different analytical techniques such as spectrophotometry (4-8), HPLC (9-18) and mass spectrophotometric techniques (19-22) in tablet formulations as well as biological fluids.

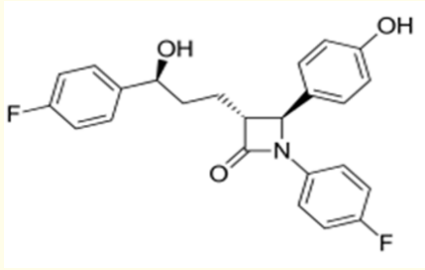

Figure 1: Chemical structure of Ezetimibe.

Metreyi Sharma., *et al*. have developed spectrophotometric method [4] for the estimation of Ezetimibe using methanol (λ_{max} 233 nm) and Beer-Lambert's law was obeyed over the concentration range 6-16 µg/ml. Baby Sudha Lakshmi., *et al*. have developed a visible spectrophotometric method for the assay of Ezetimibe using 1,10-phenanthroline (λ_{max} 510 nm) and hexacyano-ferrate(iii) (λ_{max} 740 nm) in presence of ferric chloride (complex formation) [5]. Kabra*., et al.* developed a spectrophotometric method [6] using ethanol: acetic acid (90:10) for the estimation of Ezetimibe (λ_{max} 252 nm) and Beer-Lambert's law was obeyed over the concentration range 2-20 µg/ml. Ramakrishna had developed two spectrophotometric methods [7] for the estimation of Ezetimibe using

methanol (λ_{max} 233 nm) and sodium hydroxide (λ_{max} 243 nm) and Beer-Lambert's law was obeyed over the concentration range 2-40 and 2-30 µg/ml. Avinash., *et al*. developed a spectrophotometric method [8] using acetonitrile: water (50:50) for the estimation of Ezetimibe (λ_{max} 251 nm) and Beer-Lambert's law was obeyed over the concentration range 5-30 µg/ml. The details of these spectrophotometric methods were shown in Table 1.

Reagent	Linearity (µg/ml)	λ_{max} (nm)	Reference
Methanol	6-16	233	[4]
1,10-Phenanthroline Hexacyano-ferrate (III) in presence of Ferric chloride	-	510 740	[5]
Ethanol: Acetic acid (90:10)	2- 20	252	[6]
Methanol 0.5M NaOH	2-40 2-30	233 243	[7]
Acetonitrile: water (50: 50)	5-30	251	[8]

Table 1: Spectrophotometric methods.

Sistla*., et al.* have developed a RP-HPLC method [9] using a mixture of water (pH 6.8, 0.05%, w/v 1-heptane sulfonic acid) and acetonitrile (30:70, v/v) as mobile phase with flow rate 0.5 mL/min (Detection wavelength 232 nm) methanol for the estimation of Ezetimibe and linearity was observed over the concentration range 0.5-50 µg/ml. Saranjit Singh*., et al.* have developed a RP-HPLC method [10] for the determination of Ezetimibe using a mixture of ammonium acetate buffer (0.02 M, pH adjusted to 7.0 with ammonium hydroxide) and acetonitrile as mobile phase with detection wavelength at 250 nm. Zhiqiang Luo*., et al.* have developed a RP-HPLC method [11] for the separation and determination of eleven potential process related impurities of Ezetimibe using a mixture of acetonitrile: water (pH adjusted to 4.0 with phosphoric acid): methanol at 15:75:10 (v/v/v) as mobile phase A and acetonitrile as mobile phase B (Detection wavelength 210 nm) and were characterized by LC-MS/MS analysis.

Anuradha and Vishal have developed a RP-HPLC method [12] for the isolation and characterization of the alkaline degradant of Ezetimibe using a mixture of 50 mM ammonium acetate buffer (pH 4.5): acetonitrile (50:50, v/v) as the mobile phase (Detection wavelength 242 nm) and were characterized by LC-MS, NMR and IR spectroscopy. Praveen Kumar*., et al.* have developed a RP-HPLC method [13] for the determination of Ezetimibe in tablet dosage form using 0.02 N ortho phosphoric acid: acetonitrile (20:80 v/v) as mobile phase with flow rate of 1 ml/min (Detection wavelength 232 nm) where Ezetimibe was eluted at 3.5 min and linearity was observed over the concentration range 1-10 µg/ml. Baokar., *et al*. have developed a RP-HPLC method [14] for the determination of Ezetimibe in tablet dosage form using a mixture of ammonium acetate buffer and acetonitrile as mobile phase with flow rate of 1.5 ml/min (Detection wavelength 230 nm). Akmar*., et al.* have developed a RP-HPLC method [15] for the estimation of Ezetimibe in pharmaceutical formulations using acetonitrile: 0.02 M potassium dihydrogen orthophosphate buffer (72:28 v/v) as the mobile phase and C8 Kromasil column with flow rate of 1 ml/min (Detection wavelength 232 nm) where Ezetimibe was eluted at 4.24 min and linearity was observed over the concentration range 10-45 µg/ml.

Hossein Danafar developed a RP-HPLC method [16] for the estimation of Ezetimibe in tablets using acetonitrile-ammonium acetate (10 mM, pH 3.0) (75: 25 v/v) as the mobile phase with flow rate of 1 ml/min (Detection wavelength 240 nm) and linearity was observed over the concentration range 10-60 µg/ml. Pandey and Rathanand have developed a RP-HPLC method [17] for the estimation of Ezetimibe in pharmaceutical dosage forms using water: acetonitrile (60:40 v/v) as the mobile phase and Luna phenomenex column with flow rate of 1.5 ml/min (Detection wavelength 225 nm). Hanshyam Panjwani., *et al*. have developed a RP-HPLC method [18] for the estimation of Ezetimibe in pharmaceutical dosage forms using acetonitrile: 10 mM Na_2HPO_4 pH 7.0 (55:45, v/v) as the mobile phase and Betasil C18 column with flow rate of 1 ml/min (Detection wavelength 233 nm) and linearity was observed over the concentration range 4-24 µg/ml. The details of these liquid chromatographic methods were summarized in Table 2.

Hossein Danafar., *et al*. [19] Oliveira*., et al.* [20] Nuran Özaltin., *et al*. [21] Shuijun Li.,., *et al*. [22] have developed mass spectrophotometric methods for the determination of Ezetimibe in human plasma and some of the parameters were discussed in Table 3.

Mobile phase (v/v)	λ (nm)	Column	Linearity (µg/mL)	Reference
Water (pH 6.8, 0.05% 1-heptane sulfonic acid): Acetonitrile (30:70)	232	Kromasil C18	0.5 - 50	[9]
Ammonium acetate buffer (0.02 M, pH adjusted to 7.0 with ammonium hydroxide): Acetonitrile	250	C8	5-500	[10]
Mobile phase A: Acetonitrile-Buffer (pH adjusted to 4.0 with phosphoric acid): Methanol (15:75:10) Mobile phase B: Acetonitrile	210	Phenomenex Luna	-	[11]
Ammonium acetate buffer (pH 4.5, 50 mm): Acetonitrile (50:50)	242	Waters Symmetry C8	-	[12]
0.02N ortho phosphoric acid: Acetonitrile (20:80)	232	Zorbax SB C18	1-10	[13]
Ammonium Acetate: Acetonitrile	230	ODS-3V	10-50	[14]
Acetonitrile: 0.02 M potassium dihydrogen orthophosphate buffer (72:25)	232	C8 Kromasil	----	[15]
Acetonitrile: Ammonium acetate (10 mM, pH 3.0), 75:25	240	-	10-60.0	[16]
Water: Acetonitrile (60:40)	225	C18 Luna Phenomenex	----	[17]
18 Acetonitrile: 10 Mm Na_2 HPO_4 (pH 7.0) (55:45)	233	Betasil C_{18}	4-24	[18]

Table 2: Liquid chromatographic methods.

Mobile phase (v/v)	Linearity (ng/mL)	Method	Reference
Acetonitrile: Ammonium acetate (10 mM, pH 3.0) (75:25)	0.05-30	LC-APCI-MS	[19]
Acetonitrile: Water (85:15)	0.25-20	LC-MS-MS	[20]
Trimethylsilyl ether	0.015- 0.25	GC-MS	[21]
Acetonitrile: Ammonium acetate (5mM)	0.02 -20	LC-MS/MS	[22]

Table 3: Mass spectrometric methods.

Conclusion

The present study represents a detailed review of the analytical methods so far developed for the estimation of Ezetimibe in pharmaceutical dosage forms as well as human plasma.

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