REVIEW ARTICLE

In-Depth Investigation of Analytical Methods for the Determination of Bempedoic Acid and Ezetimibe in Biological Fluid and Pharmaceutical Dosage Form: A Review

Anisha I. Kadiwala[1]*, Pinkal H. Patel[1], Gunosindhu Chakraborthy[1]

[1]*Department of Pharmaceutical Quality Assurance,Faculty of Pharmacy, Parul Institute of Pharmacy and Research, Parul University, Post Limda, Vadodara, Gujarat, India*

Received: 07th March, 2021; Revised: 27th March, 2021; Accepted: 17th May, 2021; Available Online: 25th June, 2021

ABSTRACT

Bempedoic acid is a new agent which reduces cholesterol synthesis. It is also influencedby fatty acid synthesis, low-density lipoprotein cholesterol (LDL-C). In early trials, it was well tolerated without major side effects. Alone or in many combinations with Ezetimibe, Bempedoic acid lowers levels of non–high-density lipoprotein cholesterol. The Bempedoic acid and Ezetimibe fixed-dose combination significantly lowered low-density lipoprotein cholesterol versus placebo or other oral monotherapies and had a favorable safety profile when added to maximally tolerated statin therapy in patients with hypercholesterolemia and high Cardiovascular Read more at http://acronymsandslang.com/meaning-of/medicine-and-science/CV.html (CV). As a result, fixed-dose combinations of hypolipidemic agents may provide an attractive option for hypercholesterolemia's effective and safe management. Controlled clinical studies successfully showed a consistent relationship between LDL-C and cardiovascular risk reduction, such that lipid-lowering therapy became a cornerstone in CV risk reduction.

Keywords: Analytical method, Bempedoic acid, Ezetimibe, HPLC, UV

International Journal of Pharmaceutical Quality Assurance (2021); DOI: 10.25258/ijpqa.12.2.07

How to cite this article: Kadiwala AI, Patel PH, Chakraborthy G. Formulation of Generic In-Depth Investigation of Analytical Methods for the Determination of Bempedoic Acid and Ezetimibe in Biological Fluid and Pharmaceutical Dosage Form: A Review. International Journal of Pharmaceutical Quality Assurance. 2021;12(2):45-51.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Low-density Lipoprotein (LDL) known as bad cholesterol. High LDL level leads to a build-up of cholesterol in our arteries. Cholesterol levels are measured in mg/dL.

LDL level as per age group:

- Age 19 or younger:
- Age 20 or younger (Men):less than 100 mg/dL.
- Age 20 or younger (Women):

These are the cholesterol level, based on your age and gender.[1] Bempedoic acid (BA) is a small molecule of adenosine triphosphate-citrate lyase inhibitor indicated to treat adults with hypercholesterolemia (Fig. 1). This could reduce LDL-C levels and is well tolerated.[2] BA requires activation by a specific enzyme acyl-CoA synthetase, which is primarily restricted to the liver. Therefore, it is believed that, unlike statins, myotoxicity is unlikely to occur with Bempedoic acid because it does not inhibit cholesterol biosynthesis in skeletal muscle due to the absence of acyl-CoA synthetase in these cells. The effect of Bempedoic acid is additive,not redundantto that of statins, because the target of Bempedoic acid, Anterior Cruciate Ligament (ACL), is a distinct regulatory checkpoint on the cholesterol biosynthesis pathway HMG-CoA reductase, the primary target of statins. Inability to tolerate statins because of muscle symptoms contributes to uncontrolled cholesterol levels and insufficient cardiovascular risk reduction. Bempedoic acid, a prodrug activated by a hepatic enzyme that does not present in skeletal muscle, inhibits ATP-citrate lyase.[3]

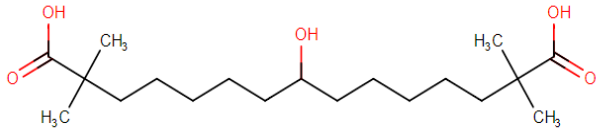

Figure 1: Chemical structure of Bempedoic acid[3]

Preparation

Bempedoic acid (Fig. 2) was prepared by condensation of 1,5-dibromopentane with ethyl isobutyratethrough LDA in Tetrahydrofuran in DMPU −78°C to give ethyl 7-bromo-2,2-dimethyl heptanoate, which is dimerized with tosyl methyl isocyanide in the presence of NaH and Bu_4NI in DMSO,

*Author for Correspondence: annukadi2305@gmail.com

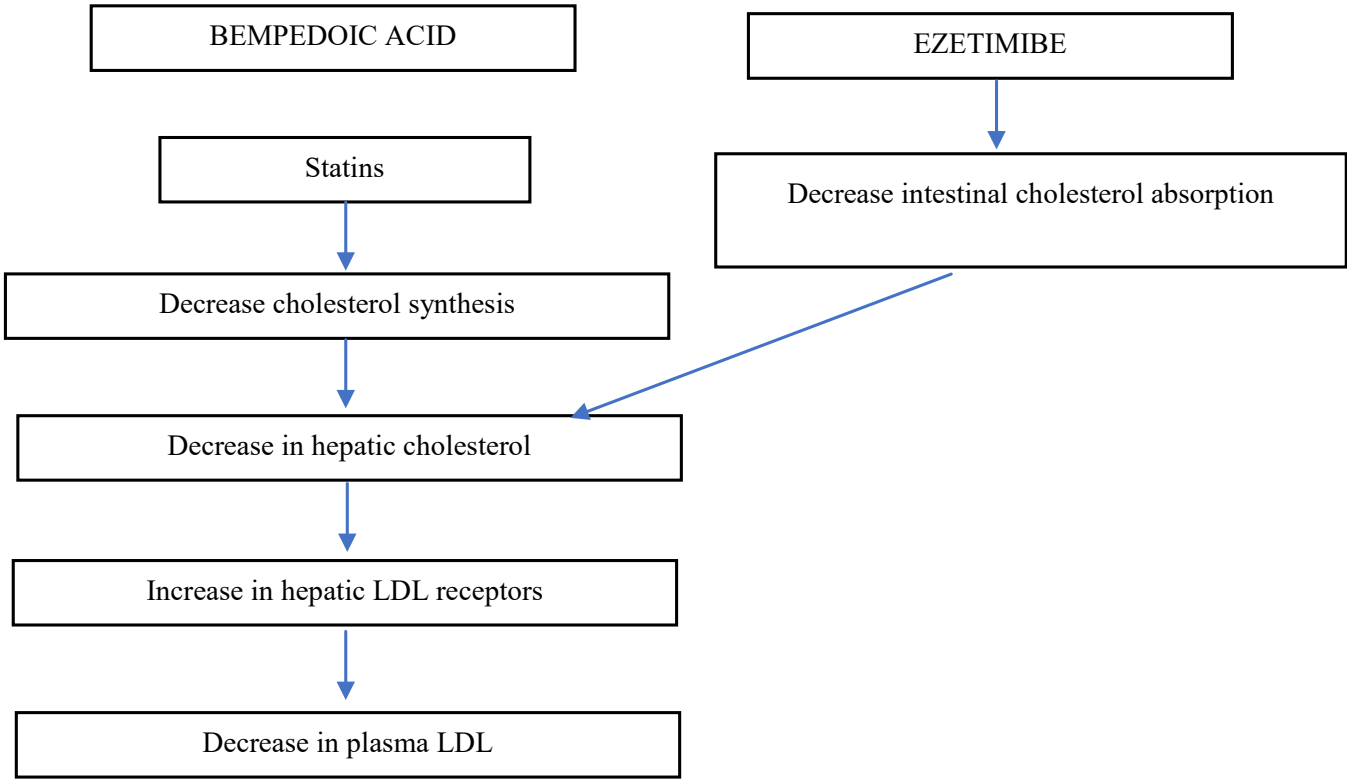

Figure 2: Mechanism of action for bemedoic acid[4]

which provide diethyl 8-isocyano-2, 2,14,14-tetramethyl-8-tosylpentadecanedioate. The reaction of intermediate with aqueous HCl in CH_2Cl_2 affords 2,2,14,14-tetramethyl-8-oxopentadecanedioic acid diethyl ester, which is hydrolyzed with aqueous KOH in refluxing $EtOH/H_2O$ to yield dicarboxylic acid. Ketone is finally reduced using $NaBH_4$ in MeOH.[5]

(1) Method for determination of Bempedoic acid by High-Performance Liquid Chromatograph (HPLC) method (Table 1).

1. A 15 µL aliquot from the extraction procedure described above was injected into the HPLC system utilizing an Alltima C8 (5µ, 250 × 4.6 mm ID) HPLC column running 15–40% acetonitrile in 25 mM K_2HPO_4 (pH 7.0) gradient before UV detection at 254 nm on a G1314A photodiode array detector (PDA).Bempedoic acid concentrations were determined by comparing the sample peak area to the peak area of the Bempedoic acid calibration standard.[6]
2. A 15 µL aliquot of the aqueous layer (top, approximately 0.8 ml) from the extraction procedure described above was injected into the HPLC system utilizing an Alltima C8 (5µ, 250 × 4.6 mm ID) HPLC column running a 15–40% acetonitrile in 25 mM potassium hydrogen phosphate (pH 7.0) gradient before being UV detection at 254 nm on a G1314A detector. Bempedoic acid concentrations were determined by comparing the sample peak area to the peak area of a Bempedoic acid calibration standard.[7]

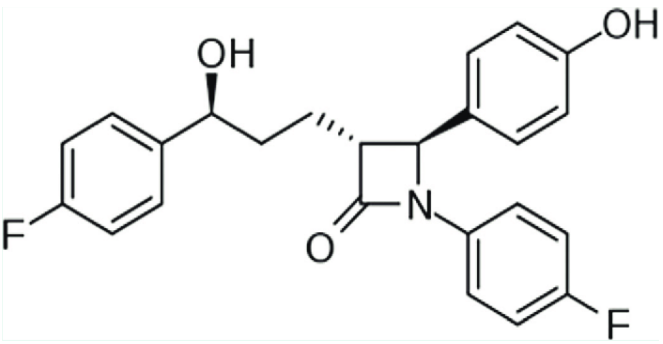

Figure 3: Chemical Structure of Ezetimibe

Ezetimibe

Ezetimibe is a drug that selectively inhibits intestinal cholesterol absorption (Fig. 3). In trials demonstrated an additional reduction in LDL-C levels of 12–19% when Ezetimibe was taken in combination with a statin. It is a drug that selectively inhibits intestinal cholesterol absorption. Ezetimibe is metabolized within the small intestine and the liver; it is then excreted back into the gastrointestinal tract *via* bile, where it can again inhibit cholesterol absorption. This pathway gives Ezetimibe a long half-life, approximately 22 hours. It isfinally excreted predominantly *via* feces. In addition to reducing gastrointestinal cholesterol absorption[8] (Table 2).

(2) Methods for determination of Ezetimibe Single and combination with other drugs by UV-Spectroscopy and Chromatography and other techniques (Table 1).

Table1: Methods for determination of Ezetimibe single and combination with other drugs by UV Spectroscopy, chromatography and other techniques.

Sr. No.	*Method*	*Description*	*Ref.no.*
1	Stability indicating RP-HPLC method for simultaneous determination of Simvastatin and Ezetimibe from tablet dosage form	Stationary phase: C18 ODS column and isocratic elution Mobile phase:acetonitrile: phosphate buffer (pH 4.5,0.01M)in the ratioof 65:35 v/v Flow rate: 1.0 mL/min. Detection: 232 nm Retentiontime: Ezetemibe:10 minutes Simvastatin:10–20 minutes	9
2	Reverse-phase high-performance liquid chromatography method for estimation of Ezetimibe in bulk and pharmaceutical formulations	Stationary phase: C8 column Mobile phase: acetonitrile:0.02 M potassium dihydrogen orthophosphate buffer (72:28 v/v) Flow rate: 1 mL/min Detection: 232 nm Retention time: 4.24 minutes	10
3	Spectrophotometric method for the determination of Ezetimibe in pharmaceutical formulations	Wavelength: 234 nm Solvent: ethanol Range: 5–20 µg/mL	11
4	Development and validation of stability-indicating assay method by UPLC for a fixed dose combination of Atorvastatin and Ezetimibe	Stationary phase: Kromasil eternity C18 column (2.5 µm, 2.1 × 50 mm) Mobile phase:acetonitrile and ammonium acetate buffer (pH 6.70; 0.01M) Flow rate: 0.2 mL/min Detection: 245nm Retention time: Ezetimibe:1.254 minutes Atorvastin:0.675 minutes	12
5	Method development and validation for simultaneous determination of Ezetimibe and Simvastatin in combined pharmaceutical dosage form by RP-HPLC method	Stationary phase: Chromosil C-18 column(250 × 4.6 mm) Mobile phase: methanol: acetonitrile: 0.1%Orthophosphoric acid 75:20:05 (v/v/v) Flow rate: 0.5–1.5 mL/min Detection: 243nm Retention time: Ezetimibe:3.30 Simvastatin:6.17	13
6	A novel reverse phase liquid chromatographic method development and validation for the simultaneous of Atorvastatin, Ezetimibe and Fenofibrate in bulk and tablet dosage form	Stationary phase: C-18 (250mm x 4.6 mm) Mobile phase:methanol: water (70:30% v/v) Flow rate: 1 mL/min Detection: 250 nm Retention time: Atorvastatin -2.103 minutes Ezetimibe- 3.660 minutes Fenofibrate-5.987 minutes	14
7	Stability-indicating RP-HPLC method development for determination of Ezetimibe in tablet dosage form	Stationary phase: Zorbax SB C18 (250 mm x 4.6 mm) Mobile phase:0.02N ortho phosphoric acid: acetonitrile (20:80 v/v) Flow rate: 1 mL/min Detection: 232 nm Retention time: approx. 3.5 minutes	15
8	Method development and validation for simultaneous estimation of Atorvastatin and Ezetimibe in pharmaceutical dosage form by HPLC	Stationary phase:C18(4.6 x 250mm) Mobile phase: methanol and acetonitrile (40:10:50) Flow rate: 1.2 mL/min Detection: 233 nm Retention time: Ezetimibe:4.462 minutes Atorvastatin:3.355	16
9	RP-HPLC method for simultaneous estimation of Rosuvastatin and Ezetimibe from their combination tablet dosage form	Stationary phase:C18 (250 × 4.6 mm) Mobile phase: acetonitrile: water:0.02 M phosphate buffer pH 8 (40:10:50 v/v) Flow rate: 1 mL/min Detection: Rosuvastatin 253 nm Ezetimibe 230 nm Retention time: Rosuvastatin: 2.75 minutes Ezetimibe: 4.69 minutes	17

Table1: (Continued)

Sr.No.	*Method*	*Description*	*Ref.no.*
10	Simultaneous quantification of related substances of Ezetimibe and Simvastatin in combined dosage form using a novel stability-indicating liquid chromatographic method	Stationary phase: InertsilODS-3 (150 × 4.6 mm, 5.0 µm) column Mobile phase: solution A contains 0.1% orthophosphoric acid solution in water, solution B contains 0.1% orthophosphoric acid solution in acetonitrile Flow rate: 2.0 mL/min Detection: 238 nm Retention time: Ezetimibe: 4.61 minutes Simvastatin:3.72 minutes	18
11	Development and validation of a reversed-phase HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms	Stationary phase: Kromasil 100 C18 column Mobile phase: water (pH 6.8, 0.05%, w/v 1-heptane sulfonic acid) and acetonitrile (30:70, v/v) Flow rate: 0.5 mL/min Detection:232 nm Retention time: 5.97 minutes	19
12	Simultaneous estimation of Atorvastatin and Ezetimibe in combined formulation by RP-HPLC	Stationary phase: phenomenex Gemini C-18 (250 × 4.6 mm) Mobile phase: acetonitrile: ammonium acetate buffer pH 3.0 (50:50, v/v) Flow rate: 1.2 mL/min Detection: 247 nm Retention time: Atorvastatin -3.0 minutes Ezetimibe- 5.2 minutes	20
13	A simple and validated RP-HPLC method for the simultaneous determination of Ezetimibe and Fenofibrate in bulk and pharmaceutical dosage forms	Stationary phase: Inertsil ODS (250×4.6 mm×5µ) Mobile phase: Acetonitrile: Water in the ratio of (80:10:10 % v/v/v) Flow rate: 1 mL/min Detection: 251 nm Retention time: Ezetimibe: 3.23 minutes Fenofibrate:6.48minutes	21
14	Simultaneous estimation of Simvastatin and ezetimibe in bulk drug and tablet formulation by High-performance liquid chromatography and High-performance thin-layer chromatography (HP-TLC)	Stationary phase: Hypersil C-18 column (250 mm × 4.6 mm) Mobile phase: acetonitrile: Water (80:20, v/v) Flow rate: 1 mL/min Detection:235 nm Retention time:Ezetimibe:4.14 ± 0.001 minutes Simvastatin:9.43 ± 0.004 minutes	22
15	Simultaneous RP-HPLC method for estimation of ezetimibe and Simvastatin in bulk and dosage forms	Stationary phase: C18 column (symmetry, 4.6 mm × 25 cm) Mobile phase: methanol: water (95:05 v/v) Flow rate: 0.8 mL/minutes Detection:248 nm Retention time:Ezetimibe: 3.2 minutes Simvastatin: 4.9 min	23
16	Simultaneous RP-HPLC method for estimation of ezetimibe and Fenofibrate in synthetic mixture	Stationary phase:C18 column (Kromosil, 4.6mm × 25 cm, 5µm) Mobile phase: acetonitrile: 0.05 M ammonium acetate buffer (85:15 v/v) Flow rate: 1.3 mL/min Detection:253 nm Retention time:Ezetimibe: 2.41^{+} 0.011 minutes Fenofibrate: 6.03+ 0.023 minutes	24
17	RP-HPLC method for simultaneous estimation of atorvastatin calcium ezetimibe in pharmaceutical formulation.	Stationary phase: Hypersil BDS (250 mm × 4.6 mm) Mobile phase:Acetonitrile: water: Methanol (350:550:100 v/v/v) Flow rate:2 mL/min Detection:250 nm Retention time:Ezetimibe: 21.712 minutes Atorvastatin:10.414 minutes	25

Table1: (Continued)

Sr.No.	*Method*	*Description*	*Ref.no.*
18	A validated reverse Phase HPLC method for the Simultaneous estimation of Atorvastatin calcium and ezetimibe in pharmaceutical dosage forms	Stationary phase: Phenomenex C18 (250 x 4.6mm) Mobile phase: Acetonitrile and Buffer (0.1% v/v orthophosphoric acid, pH adjusted to 6 with Triethylamine) (60:40) Flow rate: 1 mL/min Detection:232 nm Retention time:Ezetimibe: 3.7 minutes Atorvastatin: 6.1 minutes	26
19	Simultaneous spectrophotometric estimation of Ezetimibe and Atorvastatin in pharmaceutical dosage form	Wavelength: Ezetimibe: 232.5 nm Atorvastatin: 246.5 nm Solvent: methanol Range: 5–30 µg/mL	27
20	RP-HPLC method for simultaneous estimation of simvastatin and Ezetimibe in bulk drug and its combined dosage form.	Stationary phase: Luna C18 column Mobile phase: methanol: water: acetonitrile (75: 18.75: 6.25 % v/v/v) Flow rate: 1.8 mL/min Detection:231 nm Retention time: Ezetimibe: 13.5 + 0.5 minutes Simvastatin:4.02 + 0.3 minutes	28
21	Development and validation of UV spectrophotometric method for the simultaneous estimation of Rosuvastatin and Ezetimibe in pharmaceutical dosage form	Wavelength: Ezetimibe:229 nm Rosuvastatin: 223 nm Solvent: distilled water Range:4–32 µg/mL	29
22	Development and validation of RP-HPLC method for simultaneous estimation of Ezetimibe and Glimepiride in tablet dosage form	Stationary phase: C18 Phenomenex column (250 × 4.6 mm,5µ) Mobile phase: methanol: 20 mM potassium phosphate buffer (pH 3.00) (80: 20 % v/v) Flow rate: 1 mL/min Detection:230 nm Retention time: Ezetimibe: 4.183 minutes Glimepiride:5.292 minutes	30
23	New simple and economical spectrophotometric methods for estimation of ezetimibe in bulk drug and pharmaceutical dosage forms	**UV method:1** Wavelength: Ezetimibe: 233nm Solvent: methanol Range: 2-40 µg/ml **UV method:2** Wavelength: Ezetimibe: 233nm Solvent: 0.5 M NaOH Range:2-30 µg/ml	31
24	A simple and sensitive RP-HPLC method for simultaneous estimation of atorvastatin calcium, Ezetimibe and Fenofibrate in combined tablet dosage form	Stationary phase: C18 HS column (250 × 4.6 mm) Mobile phase: methanol: acetonitrile: 0.02M Ammonium acetate buffer pH adjusted to 10 (60:30:10, v/v/v) Flow rate: 1 mL/min Detection:230 nm Retention time: Ezetimibe: 3.427 ± 0.0049 minutes Fenofibrate: 6.680 ± 0.0015 minutes Atorvastatin Calcium :2.120 ± 0.078 minutes	32
25	Simultaneous assessment of Atorvastatin-Ezetimibe combination in tablets by An Ion-pair RP-HPLC	Stationary phase: C18 Phenomenex column (250 × 4.6 mm,5µ) Mobile phase: 35% of 10^{-3} M cetrimide and 65% acetonitrile Flow rate: 1.5 mL/min Detection:230 nm Retention time: Ezetimibe:3.8minutes Atorvastatin :3.2 minutes	33

Table 2: Marketed formulation of Bempedoic acid and Ezetimibe

Sr.no.	*Brand name*	*Company name*	*Formulation*	*Dose*
1	Nexlizet	Esperion therapeutics	Tablet	Bempedoic acid (180 mg) Ezetimibe (20 mg)

CONCLUSION

This review describes the reported Spectroscopic and Chromatographic methods developedBempedoic acid and Ezetimibe. As per this review, it was concluded that forBempedoic acid and Ezetimibe, different Spectroscopic and chromatographic methods are available for single-single drugs. It was observed that still, any combination method of Bempedoic acid and Ezetimibe is not available. Thus, all methods were simple, accurate, economical, precise, and reproducible. Nearly all Methods were of RP-HPLC and UV absorbance detection because these methods provided with best available reliability, repeatability, analysis time, and sensitivity.

REFERENCES

1. https://medlineplus.gov/cholestrollevelswhatyouneedtoknow.html
2. https://www.tandfonline.com/doi/full/10.1080/14779072.2020.1782744?scroll=top&needAccess=true
3. Mancini GB, Baker S, Bergeron J, Fitchett D, Frohlich J, Diagnosis, prevention, and management of statin adverse effects and intolerance: Canadian Consensus Working Group update, 2016;32: 35-65
4. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins Cell. 2015;161(1):161-172.
5. Pathy K, Bempedoic acid a small molecule drug process and synthesis, innovation and/or advantages, development status and/or regulatory status,2019;3(1):222-227.
6. Filippov S, pinkoskySL, etc el. ETC-1002 regulates immune response, leukocyte homing, and adipose tissue inflammation via LKB1-dependent activation of macrophage AMPK. J Lipid Res. 2013;54(8):2095-2108.
7. Pinkosky SL, Filippov S, Srivastava RAK, Hanselman JC, Bradshaw CD, Hurley TR, et al. AMP-activated protein kinase and ATP-citrate lyase are two distinct molecular targets for ETC-1002, a novel small molecule regulator of lipid and carbohydrate metabolism. J Lipid Res. 2013;54(1):134-151.
8. Daniel Hammersley, MarkSigny, Ezetimibe: an update on its clinical usefulness in specific patient groups 2017;8(1):4-11
9. Dixit RP, Barhate CR, Padhye SG, Viswanathan CL, nagarsenker MS. stability indicating RP-HPLC method for simultaneous determination of Simvastatin and Ezetimibe from tablet dosage form. indian J pharm science, 2010; 72(2): 204-210.
10. Akmar SK, Kothapalli L, Thomas A, Jangam S, Deshp AD, reverse phase high performance liquid chromatography method for estimation of Ezetimibe in bulk and pharmaceutical formulations. Indian Journal of Pharmaceutical Sciences,2007,69 (5): 695-697
11. Narasimharaju BC, Devalarao G, Ramanjaneyulu S. spectro-photometric method for the determination of Ezetimibe in pharmaceutical formulations. biomedical and pharmacology journal,2008;1(2):413-416.
12. Goel A, Baboota S, Sahni JK, Srinivas KS, Gupta RS, Gupta A, development, and validation of stability-indicating assay method by UPLC for a mixed dose combination of Atorvastatin and Ezetimibe, chromatography Science, 2013;51(3):222-228
13. Palani Shanmugasundaram, Kamarapu, method development and validation for Simultaneous estimation of Atorvastatin and Ezetimibe in pharmaceutical dosage form by HPLC. Asian journal of pharmaceutical and clinical research, 2018;4(11): 54-61.
14. Praveen Kumar, S. Vidyadhara, T.E.G.K. Murthy, B. Venkateswara Rao and V. Nikhila, A novel RP-liquid chromatography development method and validation for the dosage for the simultaneous estimation ofAtorvastatin, Ezetimibe and Fenofibrate in bulk and tablet dosage form. International journal of pharmaceutical sciences and research,2016;7(10)4145-4151.
15. Dixit RP, Barhate CR, Padhye SG, Viswanathan CL, Nagarsenker MS. stability indicating RP-HPLC method for simultaneous determination of Simvastatin and Ezetimibe from tablet dosage form.Indian J pharm Science2010;72(2)201-210.
16. S.K. Raul, A.B. Aravelli, D. Jhansi method development and validation for simultaneous estimation of Atorvastatin and Ezetimibe in pharmaceutical dosage form by HPLC.World journal of pharmaceutical sciences, 2014;11(16)54-61.
17. Mohammed Ishaq, Beludari, Karanam Vanitha, Prakash, RP-HPLC method for simultaneous estimation of Rosuvastatin and Ezetimibe from their combination tablet dosage form.International journal of chemical and analytical science,2013;4(4)205-209.
18. Desai PR, Mehta PJ, Ojha SK, Chokshi AB. simultaneous quantification of related substances of Ezetimibe and Simvastatin in combined dosage formusing a novel stability-indicating liquid chromatographic method, Acta Chromatographical. 2018;30(2):85-94.
19. Sistla R, Tata VSSK, Kashyap YV, Chandrasekar D, Diwan PV. development and validation of a reversed-phase HPLC method for the determination of Ezetimibe in pharmaceutical dosage forms.Journal of pharmaceutical and biomedical analysis. 2005;39(3,4):225-517.
20. Ganesh M, Hemalatha P, SakthimanigandanK,Lee SG. simultaneous Estimation of Atorvastatin and Ezetimibe in combined formulation by RP-HPLC. Asian J chemistry, 2012;24(4)1867-1871.
21. Yamini Mangalagiri, Siva Sreelakshmi Mamidala, Sathish Kumar Konidala. A simple and validated RP-HPLC method for the simultaneous determination of Ezetimibe and Fenofibrate in bulk and pharmaceutical dosage forms. Asian J. Pharm. Ana.2015; 5(2) 93-99.
22. Chhalotiya UK, Bhatt KK, Captain AD. Simultaneous Estimation of Simvastatin and Ezetimibe in Bulk Drug and Tablet Formulation by High-Performance Liquid Chromatography and High-Performance Thin-Layer Chromatography. Research J. Pharm. and Tech. 2010; 3(2):507-511.
23. R Siva Kumar, MR Santhanakrishnan, P Kumar Nallasivan, R Venkatanarayanan. Simultaneous RP-HPLC Method for Estimation of Ezetimibe and Simvastatin in Bulk and Dosage Forms. Research J. Pharm. and Tech. 2008; 1(3):211-214.
24. H.I. Pawar, Lata Kothapalli, Asha Thomas, R.K Nanda, Shivaji Mare. Simultaneous RP-HPLC Method for Estimation of Ezetimibe and Fenofibrate in Synthetic mixture. Research J. Pharm. and Tech.2008; 1(1):25-28.
25. MB Mundlik, CK Gadewar, NA Chandekar, NM Mahajan, PD Telgote, AV Chandewar. RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium Ezetimibe in Pharmaceutical Formulation. Asian J. Research Chem.2010; 3(2):485-490.
26. P Nagaraju, Z Vishnu Vardhan, JVLNSeshagiri Rao. A Validated Reverse Phase HPLC Method for the Simultaneous Estimation of Atorvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Forms. Asian J. Research Chem.2010;3(1):225-228.
27. Godse VP., Deodhar MN, Bhosale AV, Sonawane RA, Sakpal PS, Borkar DD, Bafana YS. Simultaneous Spectrophoto-

metric Estimation of Ezetimibe and Atorvastatin in Pharmaceutical Dosage Form. Asian J. Research Chem.2009;2(1):86-89.

28. Nilesh Jain, Ruchi Jain, Hemant Swami, Deepak Kumar Jain. RP-HPLC Method for Simultaneous Estimation of Simvastatin and Ezetimibe in Bulk Drug and its Combined Dosage Form. Asian J. Research Chem.2008; 1(1):29-31.
29. Sireesha. D, M. Laksmi Monika, Vasudha Bakshi. Development and Validation of UV Spectrophotometric Method for The Simultaneous Estimation of Rosuvastatin and Ezetimibe in Pharmaceutical Dosage Form. Asian J. Pharm. Ana. 2017; 7(3): 135-140.
30. Ashwini Parmar, Sandeep Sonawane, Santosh Chhajed, Sanjay Kshirsagar. Development and validation of RP-HPLC method for simultaneous estimation of Ezetimibe and glimepiride in tablet dosage form. Asian J. Pharm. Ana. 2017; 7(3): 185-188.
31. Ramakrishna Kommana. New Simple and Economical Spectrophotometric Methods for Estimation of Ezetimibe in Bulk Drug and Pharmaceutical Dosage Forms. Asian J. Research Chem.2011;4(2):250-253.
32. Abhijit D. Dhiware, Padmanabh B. Deshpande, Santosh V. Gandhi, Sujith Nair, Suvarna Vanjari. A Simple and Sensitive RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium, Ezetimibe and Fenofibrate in Combined Tablet Dosage Form. Research J. Pharm. and Tech.2013;6(10):1085-1088.
33. Saleh Trefi. Simultaneous assessment of Atorvastatin-Ezetimibe combination in tablets by An Ion-pair RP-HPLC. Research J. Pharm. and Tech. 2020; 13(9):4195-4199.