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Analytical Method Development & Validation For The Simultaneous Determination Of Olmesartan Medoxomil And Azelnidipine In Bulk And It's Pharmaceutical Formulation By Rp-Hplc

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ABSTRACT: -

A sensitive high-performance liquid chromatographic (HPLC) method were developed and validated for the estimation of Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL) in bulk and pharmaceutical formulations. The chromatographic separation was achieved by RP- HPLC using a mixture of Acetonitrile: Phosphate Buffer (60:40) pH 4.3 as the mobile phase, a C18 column & at 256 nm with flow rate 1ml/min. The linear and reproducible calibration curve over the range was 2–20 µg/ml for AZEL & 5-50 µg/ml. for OLME. The retention time of OLME & AZEL was found to be 3.122 & 5.342 respectively. These methods were tested and validated for various parameters according to ICH guidelines. The proposed methods were successfully applied for the determination of Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL) in pharmaceutical formulations. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation <2%), while being simple, cheap and less time consuming. The method showed adequate precision with a relative standard deviation (RSD) smaller than 3%. The accuracy was analyzed by adding a standard drug and good recovery values were obtained for all drug concentration used. The analytical procedure is reliable and offers not only advantages in terms of speed but also met the regulatory requirements for specificity, Linearity, LOD, LOQ, Precision, accuracy

KEYWORDS: Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL), Spectrophotometer, HPLC etc.

1. INTRODUCTION

Hypertension, also known as high blood pressure, is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure usually does not cause symptoms itself. It is, however, a major risk factor for stroke, coronary artery disease, heart failure, atrial fibrillation, peripheral arterial disease, vision loss, chronic kidney disease, and dementia. Hypertension is a major cause of premature death worldwide

Olmesartan belongs to the angiotensin II receptor blocker (ARB) family of drugs. ARBs selectively bind to angiotensin receptor 1 (AT1) and prevent the protein angiotensin II from binding and exerting its hypertensive effects. As the principal presser agent of the renin-angiotensin system, Angiotensin II causes vasoconstriction, stimulation of synthesis and release of aldosterone, cardiac stimulation and renal reabsorption of sodium. Olmesartan blocks the vasoconstrictor effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in vascular smooth muscle. Chemically it is (5-methyl-2-oxo-2*H*-1,3-dioxol-4-yl) methyl 4-(2-hydroxypropan- 2-yl)-2-propyl-1- ({4-[2-(2*H*-1,2,3,4-tetrazol-5 yl) phenyl] phenyl}methyl)-1*H*-imidazole-5-carboxylate.(Figure 01).

Azelnidipine used to treat high blood pressure. It belongs to a group of medicines that block the calcium channels in the blood vessel. It works by relaxing the blood vessels in patients with high blood pressure. It inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. Azelnidipine inhibits trans-membrane Ca2+ influx through the voltage-dependent channels of smooth muscles in vascular walls. Chemically it is 3-(1-benzhydrylazetidin-3-y1)-5-isopropy1-2- amino-1,4-dihydro-6-methyl-4-(3-nitrophenyepyridine-3,5 dicarboxylate). (Figure 02)

High-performance liquid chromatographic (HPLC) is the most frequently applied technique in the determination of drugs in biological fluids and dosage forms. We believe that the availability of this new method, with increased simplicity, sensitivity and selectivity, will be very useful for the determination of Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL) in raw material and pharmaceutical formulation. This gradient HPLC method uses a simple mobile phase and does not require complicated sample preparation. The aim of this study was to develop a simple, rapid and reproducible reversed-phase HPLC method.

2. EXPERIMENTAL

2.1 Materials

OLME & AZEL was supplied by Pure& Cure Health Pvt Ltd. & Mg lab India and was used without further purification. Sodium hydroxide was purchased from Molychem (Mumbai). Hydrochloric acid and hydrogen peroxide was procured from LOBA Chemie Pvt. Ltd. (Mumbai). HPLC grade methanol and acetonitrile was purchased from S. D. Fine-chem Ltd. (Mumbai) whereas HPLC grade water was purchased from Merck Ltd. All other chemicals were of analytical reagent grade. The marketed formulation ie Olmezest-AZ 20 (manufactured Sun Pharma Lab. Ltd India.) were purchased from Local market.

2.2 Chemical structure :

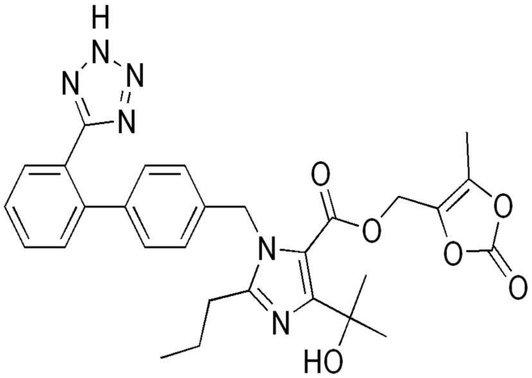

Figure 01 :- Olmesartan

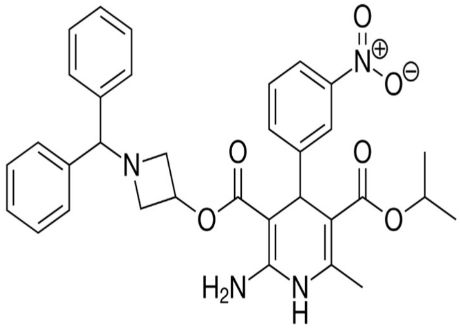

Figure 02 :- Azelnidipine

2.3 Instrumentation

The HPLC system consisting of Thermo Separation Quaternary Gradient HPLC pump Spectra System P4000 with PDA detector of Spectra System, manual rheodyne injection system, the software was a Data ace software version 6.1. The chromatographic separation was performed using Grace C_{18} (250mm × 4.6 mm i.d., 5mm particle size) Separation was achieved using a mobile phase consisting of Acetonitrile: Phosphate Buffer (60:40) pH 4.3 at a flow rate of 1ml/min and UV detection at 256 nm. The column was maintained at ambient temperature with injection volume of 20 µl. The mobile phase was filtered through 0.45 µm Chrom Tech Nylon-66 filter and degassed in ultrasonic bath prior to use. A blank chromatogram was recorded before the studies. Quantization of result was performed using peak area counts.

2.4 Standard preparation

OLME Standard Stock Solution:

Accurately weighed quantity 10 mg of OLME was dissolved in methanol and volume was made up to 100 ml mark (100 µg/ml). The stock standard solution was diluted further with mobile phase to get various concentrations.

AZEL Standard Stock Solution:

Accurately weighed quantity 10 mg of AZEL was dissolved in methanol and volume was made up to 100 ml mark (100 µg/ml). The stock standard solution was diluted further with mobile phase to get various concentrations.

2.5 System suitability test:

System suitability is a pharmacopoeial requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard solutions.

2.6 Application of proposed method for estimation of OLME & AZEL in laboratory sample

Three different laboratory mixtures of OLME & AZEL were prepared by appropriately weighing the quantities of drug samples so as to get the concentration of 20 & 8µg/ml respectively. The peak area of standard laboratory sample and Test laboratory sample was compared to obtain the concentration.

2.7 Application of proposed method for estimation of OLEM & AZEL in formulation:

For the estimation of drug from commercial formulation twenty tablets were weighed accurately. The average weight was determined, finely powdered and powder equivalent to 10 mg of drug was transferred. The test and sample solution prepared, the solution was filtered through Whatman filter paper no. 41. Further dilution was made to get final concentration of 20 & 8µg/ml of OLME & AZEL. Equal volume of standard and sample solution was injected separately after equilibrium of stationary phase. The chromatograms were recorded and response i.e. peak area were measured.

2.8 Method Validation

- **Specificity (Selectivity)**

Specificity was measured as ability of the proposed method to obtain well separated peak for OLME & AZEL without any interference from component of matrix. The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix. Mean retention time for –

OLME – 3.122
AZEL – 5.342

- **Accuracy and precision**

It was ascertained on the basis of recovery studies performed by standard addition method. The results of recovery studies and statistical data are recorded in Table No. 3 Precision of an analytical method is expressed as S.D or R.S.D of series of measurements. It was ascertained by replicate estimation of the drugs by proposed method.

- **Ruggedness:**

The studies of ruggedness were carried out under two different conditions-

a) Days
b) Analyst.

a) Interday (Different days):

Same procedure was performed as under marketed formulation analysis on different days. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 4

b) Different analyst:

The sample solution was prepared by two different analysts and same procedure was followed as described earlier. The % label claim was calculated as done in marketed formulation estimation.

3 RESULT & DISCUSSION:

3.1 Preparation of Calibration Curve: -

The mobile phase was allowed to equilibrate with the stationary phase until steady baseline was obtained. The series of concentration from 5-50µg/ml & 2-20 µg/ml of both drug solutions were injected and peak area was recorded. The graph plotted as the concentration of the drug Vs peak area depicted in Figure 03& 04

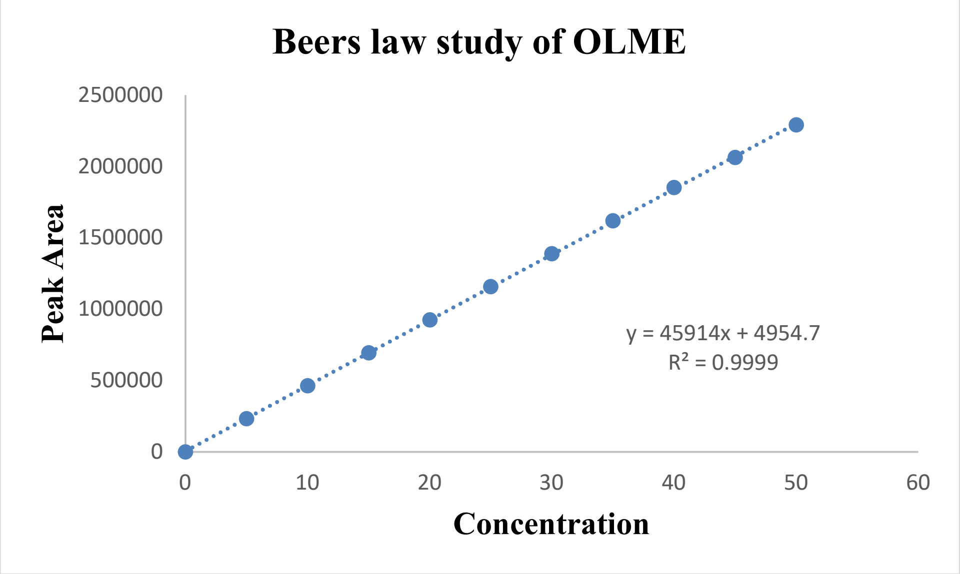

Figure 03: Standard calibration curve for OLME

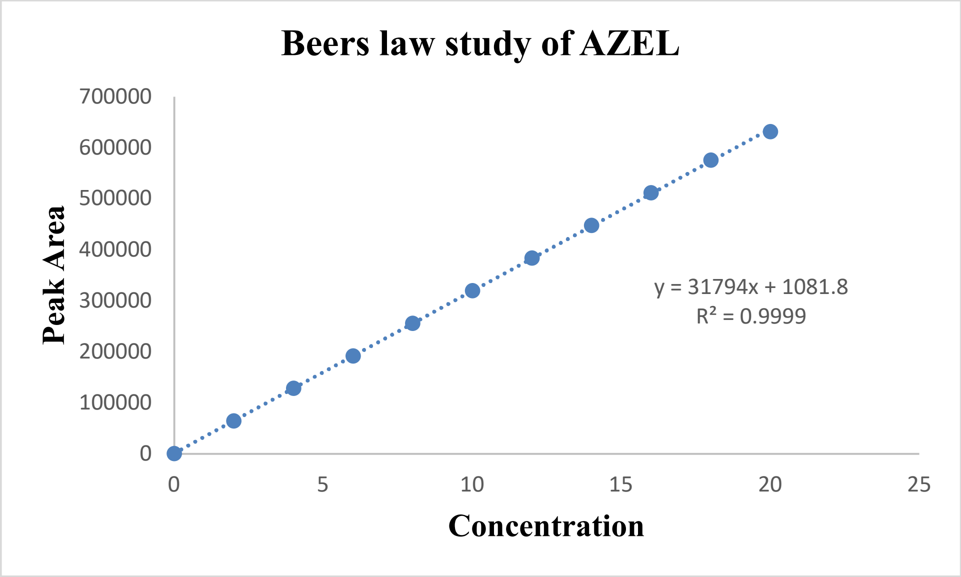

Figure 04: Standard calibration curve for AZEL

3.2 System suitability test:

Filtered mobile phase was allowed to equilibrate with stationary phase until steady baseline was obtained. A 20 µL std. drug solution was injected which was made in five replicates and the system suitability parameters were recorded as shown in Table No. 1

Table No1 . : Summary of system suitability test results

Sr.no.	Parameter	OLME	AZEL
1.	Retention time (min)	3.112	5.342
2.	Asymmetry	2.8252	1.1456
3.	Efficiency	418392.9	51908.11

***Results are mean of five replicates**

3.3 Application of proposed method for estimation of OLME & AZEL in laboratory sample & Marketed formulation

The proposed methods applied for the estimation of the drugs in standard laboratory mixture has yielded very encouraging results and thus it was extended for the estimation of drug in marketed tablet formulation. Recovery studies were performed by adding a known amount of standard drug to preanalysed sample and contents were reanalyzed by proposed methods. The peak area of standard laboratory Sample and Test laboratory sample was compared to obtain the concentration. The % Label claim of marketed formulation was calculated

Table No. 2: Summary of laboratory mixture and marketed formulation analysis by RP-HPLC Method

Sr. no.	Sample	Statistical data	% Estimation % Recovery		% Label claim	
			OLME	AZEL	OLME	AZEL
1.	Standard Laboratory	**Mean**	99.80	99.87	-	-

	mixture	**S.D.**	0.265	0.252	-	-
		C.V.	0.003	0.003	-	-
2.	Marketed formulation	**Mean**	100.37	100.06	100.33	100.33
		S.D.	0.548	0.557	0.929	1.012
		C.V.	0.005	0.006	0.009	0.010

3.4 Validation

Validation is normally done to assure the reliability of the proposed method and was performed as per the ICH guidelines for the following criteria.

- **Accuracy**

Accuracy of method is ascertained by recovery studies performed at different levels of concentrations. Mean % recovery were found to be within 98-102%.

- **Precision**

The methods were found to be precise with ±S.D. of 0.008 and 0.004 for the estimation of OLME and AZEL respectively. Result are shown in table 3.

Table No 3: Summary of validation parameters for the proposed method

Validation Parameters	**OLME**	**AZEL**
Linearity µg mL-1	5-50	2-20
Accuracy mean	100.37	100.06
Precision (% RSD)	0.008	0.004

- **Ruggedness**

The studies of ruggedness were carried out under two different conditions-
Different days and different analyst. The % label claim was calculated. Data obtained for day 1, day 2, and day 3 is shown in Table No. 4

- **Specificity:**

Specificity was measured as ability of the proposed method to obtain well separated peak for OLME and AZEL without any interference from component of matrix.
Mean retention time for –
OLME – 3.122
AZEL – 5.342
The values obtained were very close to that in standard laboratory mixture indicates no interference from the component of matrix.
Typical chromatogram is shown in the Figure 05

Table No 4: Summary of Ruggedness by RP-HPLC method

Parameter	Statistical data	% Estimation	
		OLME	AZEL
Interday	Mean	100.63	100.43
	S.D.	0.321	0.723
	C.V.	0.003	0.007
Intraday	Mean	100.87	100.10
	S.D.	0.321	0.436
	C.V.	0.003	0.004
Different analyst	Mean	100.36	99.88
	S.D.	0.7956	0.2588
	C.V.	0.0078	0.0025

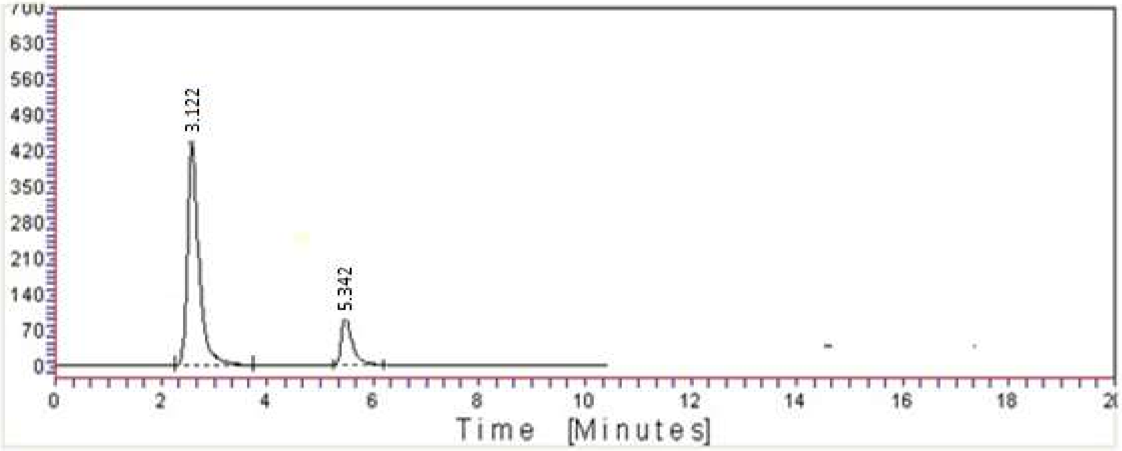

Figure 05: Chromatogram obtained by formulation of OLME and AZEL

- **Linearity and range:**

According to USP tablet powder equivalent to 80, 90, 100, 110, 120 % of label claim was taken and dissolved & diluted appropriately with mobile phase to obtain a concentration in the range of 80%-120% of the test concentration. The chromatograms of the resulting solutions were recorded. The plot showing linearity and range study for OLME and AZEL is shown in the Figure 06 and 07.

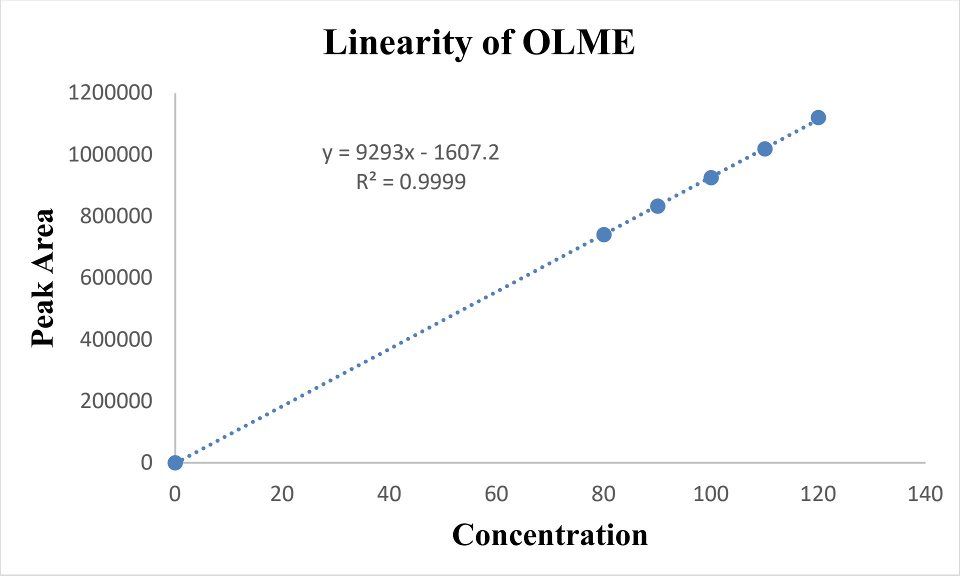

Figure 06 : -Plot of linearity and range study for OLME

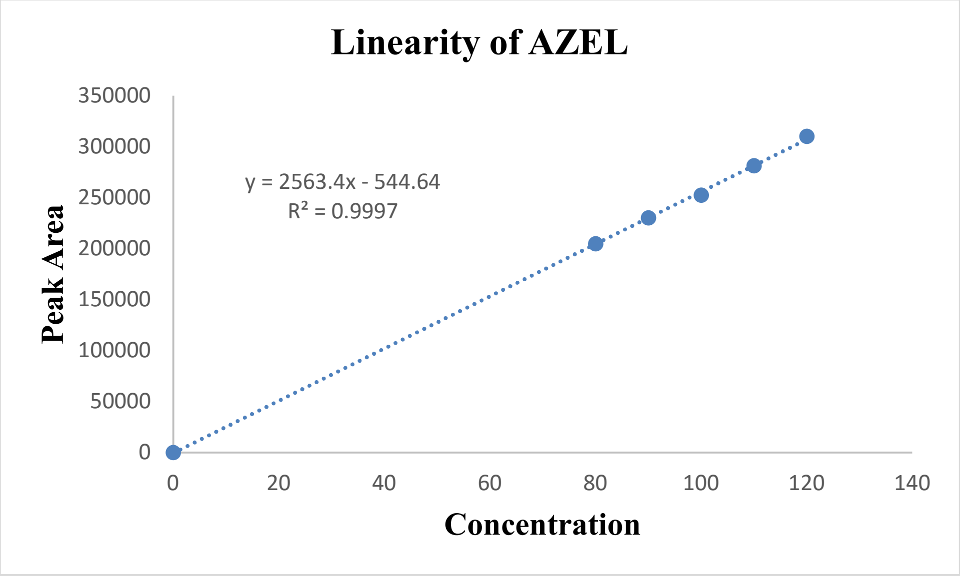

Figure 07: -Plot of linearity and range study for AZEL

4. CONCLUSION

A simple, specific, accurate and precise RP- HPLC method has been developed and validated as per ICH guideline for simultaneous estimation of Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL) in their combined dosage form. Validation parameters like Linearity, Accuracy, Precision, Robustness, System suitability, Specificity were tested. Observation of all these parameters leads to the point that developed RP-HPLC method is linear, accurate, precise, specific and robust. It can be successfully adopted for routine quality control analysis of Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL) in Combined dosage form without any interference from common excipients and impurity. This method can now transfer to utilize for routine laboratory analysis and assay of Olmesartan Medoxomil (OLME) & Azelnidipine (AZEL) in their combined dosage form.

5 REFERENCES

1. Sethi. P.D., HPLC, Quantitative analysis of pharmaceutical formulation, CBS Publisher and Distributor, New Delhi, preface, 101
2. Beckett AS., Stenlake JB., “Practical Pharmaceutical chemistry”, fourth edition, volume 2, CBS Publisher and Distributor, New Delhi, 1997; 1-85.
3. M. Sudheer1, A. B. N. Nageswara Rao, D. Hari Hara Theja, M. Siva Prakash, Development of Stability Indicating RP-HPLC Method for Simultaneous Determination of Azithromycin and Ambroxol HCl (SR) in the Tablet Formulation. Der Pharmacia Lettre, 2012, 4 (3):803-810
4. ICH, Stability Testing of New Drug Substances and Products, International Conference on Harmonization, IFPMA, Geneva, 2003
5. Indian Pharmacopoeia, Government of india ministry of health & family welfare, published by the Indian Pharmacopoeia commission, Ghaziabad, 2014; vol. III: 2774.
6. Soni IJ, Panchal HJ, “Development and Validation of Dual Wavelength UV Spectrophotometric Method for simultaneous estimation of Cilnidipine and Olmesartan Medoxomil in Tablet dosage form.” Ind. J. Pharma. and Bio. Res. 2014, 2, 76-81.
7. Jayaprakash R, Natesan S. Optimization of stability indicating RP-HPLC method for the estimation of an anti-cancer drug Sorafenib Tosylate in pure and pharmaceutical dosage form. Int. J. Of Pharmacy and Analytical Research, 2017; 6(1): 141-152.
8. ICH guidelines, Q1A (R2): Stability Testing of New Drug Substances and Products, International Council for Harmonisation Geneva. ⟨http://www.ich.org/products/ guidelines /quality/ article/ quality-guidelines/html), 2003
9. Heinz WJ, Kahle K, Beyersdorf A, Schirmer D, Lenker U, Keller D, Langmann P, Klinker H. Highperformance liquid chromatographic method for the determination of sorafenib in human serum and peritoneal Xuid. Cancer Chemother Pharmacol, 2011; 68: 239–245.
10. Kalaichelvi R, Jayachandran E. Quantitative Estimation of Sorafenib Tosylate Its Pure Form and in Its Tablet Formulation by RP-HPLC Method. Journal of Chemistry, 2013: 1-3.
11. Damle MC, Kale S. Development and Validation of Stability-Indicating HPTLC Method for Estimation of Sorafenib tosylate. European Journal of Biomedical and Pharmaceutical Sciences, 2017; 4(6): 432-438.
12. ICH guidelines, Q2 (R1): Validation of Analytical Procedures: Text and Methodology, International Conference on Harmonization Geneva. ⟨http://www.ich.org/products/ guidelines/quality/article/quality guidelines/html), 2005
13. Michael W. Dong,” Modern HPLC for practicing scientist” A John Wiley & Sons, Inc., Publication, Page no. 194,217.
14. Jeffery GH., Basset J., Mendham J., Denney RC., “Vogel’s textbook of quantitative analysis”, 5th Edn., Longman Scientific and Technical, 1999: Page no. 10-11.
15. Skoog, DA., West DM., Holler FJ., “Analytical chemistry –An Introduction”, 6th Edn., Saunder college publishing, 1994: Page no 3.

16. Sharma BK., “Instrumental methods of chemical analysis”, 23rd Edn., Goel publishing House, Meerut, 2004: Page no7-8.
17. Beckett AS., Stenlake JB., “Practical Pharmaceutical chemistry”, 4th Edn, vol 2, CBS Publisher and Distributor, New Delhi, 1997: Page no 1-85.
18. Christianah MA., Pui-Kai L., “Analytical Profile of Drug Substances” Edi. By Klaus Florey, Page no 124-141.
19. Beckett, A.H., Stenlake. J.B., “Practical Pharmaceutical chemistry”, 4th Edn., Part –II, CBS Publishers and Distributors, New Delhi, Page no 278-282.
20. Ahuja S., Scypinski S., “Handbook of modern pharmaceutical analysis”, Vol.3, Academic press,2001: Page no 349.
21. Christen GD., Analytical Chemistry, fifth ed., John Wiley & Sons, Inc.2001: Page no 505
22. Illet and Ripper, “Selectivity optimization in HPLC”, In; Brown, R., Phyllis, Eligrushka, Advances in chromatography, 39, Marcel Dekker, Inc.,1998: Page no 264-65.
23. Sethi PD., PLC-Quantitative analysis of pharmaceutical formulations”, CBS publishers and Distributors, New Delhi, 2001: Page no 11.
24. Sethi PD., HPLC-Quantitative analysis of pharmaceutical formulations”, CBS publishers and Distributors, New Delhi,200 Page no 1-5.