



New Analytical Method Development and Validation for Estimation of Dolutegravir Sodium in Synthetic Mixture by Using RP-HPLC

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ABSTRACT:

Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phase and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and % RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form.

Keywords: Chromatography, Alumina, Treatment, Instrument, Spectrophotometer.

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1. Introduction

Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phase and a mobile phase [1,2]. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed selectively in the mobile phase [3,4]. Retention of the solutes by the stationary phase may be achieved by one or a combination of mechanisms [5]. Certain substances, such as alumina or silica gel, interact with the solutes primarily by adsorption, either physical adsorption, in which the binding forces are weak and easily reversible, or chemisorption's, where strong bonding to the surface can occur [6,7]. Another important mechanism of retention

is partition, which occurs when the solute dissolves in the stationary phase, usually a liquid coated as a thin layer on the surface of an inert material or chemically bonded to it [8-11].

Dolutegravir Sodium

Dolutegravir is a HIV-1 integrase inhibitor that blocks the strand transfer step of the integration of the viral genome into the host cell [12,13]. The effect of this drug has no homology in human host cells which gives it an excellent tolerability and minimal toxicity. Dolutegravir, in combination with rilpivirine, was approved as part of the first complete treatment regimen with only two drugs for the treatment of adults with HIV-1 named Juluca [14-17].

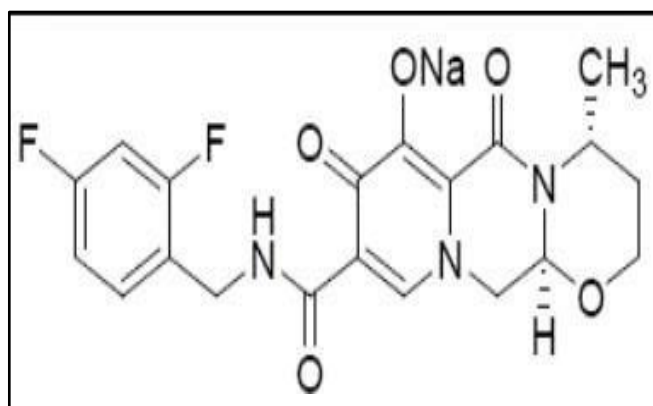


Figure 1: Structure of Dolutegravir Sodium

2. Experimental work and Results

Instrumentation

Waters



Figure 2: HPLC (waters)

The present work has been done on waters HPLC. It has 715 binary pumps, Rheodyne injector with a 20-microlitre loop, U.V. Vis. detector, Thermo C-18 column (4.6 x 250mm, 5 μ particle size) with data ace software. The hardware and software specifications of the instrument are given below [18,19].

Lab India (3000 +)

The present work has been done on Lab India (3000 +) series spectrophotometer. It has a double beam-double detector. In this the sample beam and the reference beam enter different detectors respectively.

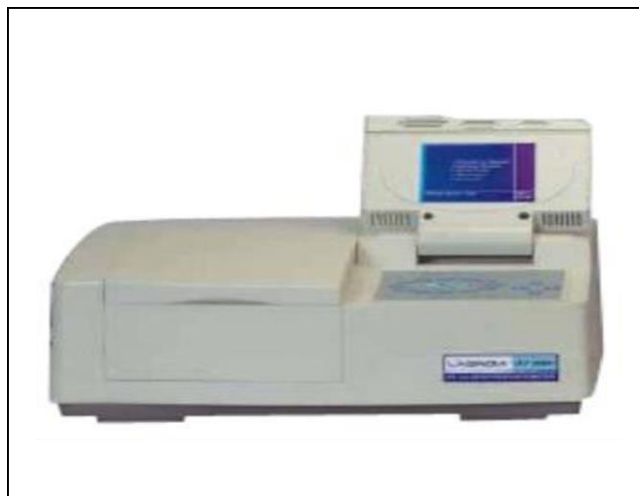


Figure 3: UV Spectrophotometer (Lab India 3000 +)

3. Methodology

The proposed research work entitled “**New analytical Method Development and Validation for Estimation of Dolutegravir Sodium in synthetic mixture by using RP-HPLC**”. In the present work a simple, selective, rapid, precise and economical UV spectrophotometric and reverse phase HPLC method have been developed and validated for the estimation of Dolutegravir in marketed formulation [20].

Method- RP-HPLC

Table 1: Chemical reagent used

Chemicals/Reagents	Grade	Company
Acetonitrile	HPLC	Merck
Methanol	HPLC	Merck
Water	HPLC	Milli-Q

Table 2: Working standard/API

Working standard	Grade	Potency	Batch No.	Company
Dolutegravir sodium	API	99.9%	1711104328	Aurobindo

Table 3: Commercial formulations

Name	Company
Company name	In-House Formulation
Strength	Physical Mixture Eq. to 50mg

Table 4: Instrument specification

HPLC	Waters
Pump	515 Binary pump
Injector	Rheodyne injector with a 20-microlitre loop
Detector	U.V. Vis. detector
Software	Data ace software

Column	Thermo C-18 column (4.6 x 250mm, 5 μ particle size)
Balance	Citizen (Cx-265)
Millipore	Mili- Q
Sonicator	PCI (Mumbai)

Identification and Characterization of drugs

Solubility

Solubility of drug was observed by dissolving it in different solvents.

Table 5: Solubility of drug in different solvents

Solvent	Solubility
	Dolutegravir Sodium
Water	Slightly soluble
0.1N Hcl	Insoluble
0.1N NaoH	Sparingly soluble
Methanol	Freely Soluble
Acetonitrile	Soluble
Ethanol	Sparingly Soluble

1. **Melting point-** Melting point of the Dolutegravir 187-189°C was found through Melting point apparatus.

2. Determination of λ max of Drug

Standard solution (10 μ g/ml) of pure drug was prepared. The pure drug solution was scanned on UV spectrophotometer to determined λ max.

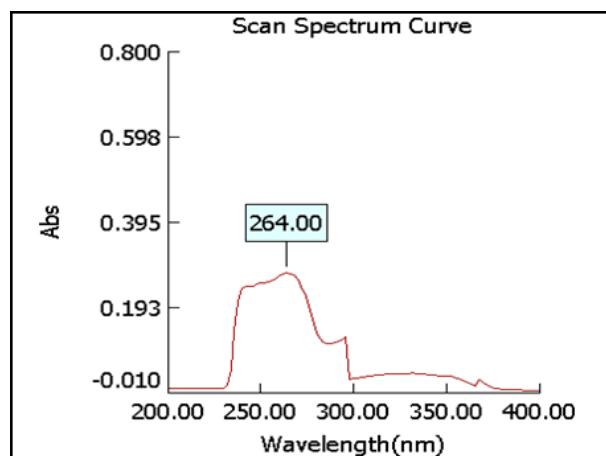


Figure 4: Determination of λ max of Dolutegravir Sodium

Selection of Mobile Phase

Initially to estimate Dolutegravir Sodium number of mobile phase in different ratio were tried. A results was shown in Table 6. Taking into consideration the system suitability parameter like (Retention time) Rt, tailing factor, No. of theoretical plates and height equivalent to a theoretical plate (HETP), the mobile phase found to be most suitable for analysis was Methanol: Acetonitrile in the ratio of 50:50 v/v. The mobile phase was filtered through 0.45 μ filter paper to remove particulate matter and then degassed by sonication. Flow rate employed

for analysis was 1.0 ml/min[21].

Procedure for preparation of mobile phase

100 ml of methanol and 100ml of acetonitrile was filtered through 0.45 filter paper.

Table 6: Mobile phase selection

Mobile Phase	Ratio	Retention Time
		Remark
Methanol : water	50 : 50 v/v	Poor resolution
Acetonitrile : Methanol	50: 50 v/v	Most Suitable

Selection of diluent

Diluent used for preparation of sample were compatible with mobile phase and no any significant effect on retention and resolution of analyte. After various trials methanol was used as diluents [22].

Selection of separation variable

Table 7: Separation variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5
Bonded Phase	Octa decylsilane (C ₁₈)
Mobile Phase	
Methanol	50 ml
Acetonitrile	50 ml
Diluent	Methanol
Flow rate	1.0 ml/min
Temperature	Ambient
Sample Size	20 l
Detection wavelength	264 mm
Retention time	
Dolutegravir sodium	4.274 ± 0.3 min.

Preparation of standard stock solution Accurately weighed 10 mg of Dolutegravir was transferred into 50 ml volumetric flasks and dissolved in 10 ml of Methanol, then volume was made up to 50 ml with acetonitrile and vortex it to get complete dissolution of drug. Stand it aside for few minute, Concentration of Dolutegravir was 200 µg/ml. (Stock- A)

- 1. Preparation of Sub Stock Solution** 5 ml of solution was taken from stock-A of Dolutegravir transferred into 10 ml volumetric flask separately and diluted up to 10 ml with diluent (Methanol) to give concentration of 100 µg/ml (Stock-B)[23].
- 2. Preparation of Different Solution** 0.5ml, 1.0 ml, 1.5ml, 2.0ml and 2.5ml of Stock-B was taken separately in 10 ml volumetric flask and volume was made up to 10ml with Methanol. This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml, 25µg/ml for drug.

Linearity and Calibration Graph

To establish the linearity of analytical method, a series of dilution ranging from 5-25

µg/ml was prepared. All the solution was filtered through 0.2µm membrane filter and injected, chromatograms were recorded at 264nm and it was repeated for three times. A calibration graph was plotted between the mean peak area and respective concentration and regression equation was derived[24,25].

Table 8: Linearity of Dolutegravir Sodium

Standard Concentration µg/ml	Area under Curve (AUC)			Mean
	Rep-1	Rep-2	Rep-3	
0	0	0	0	0
5	233.456	239.987	244.458	239.300
10	458.987	462.458	459.789	460.411
15	658.895	665.478	670.145	664.839
20	855.478	862.458	873.145	863.694
25	1082.658	1085.458	1084.789	1084.302

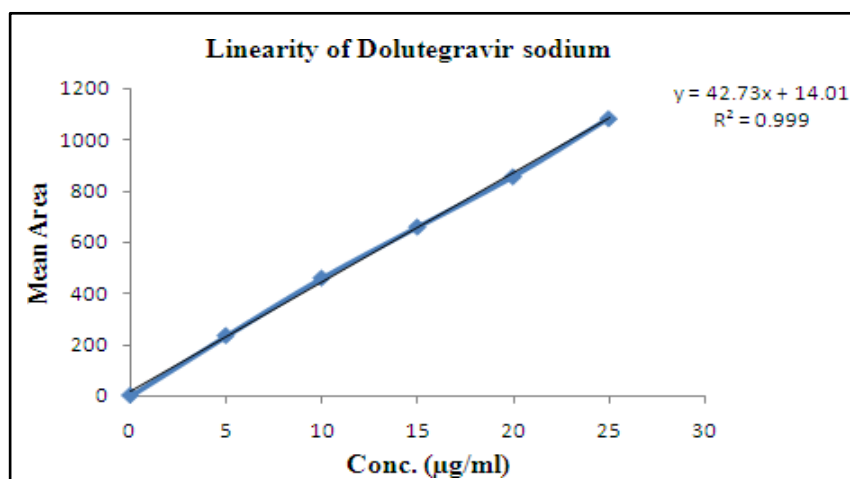


Figure 5: Calibration Curve of Dolutegravir Sodium

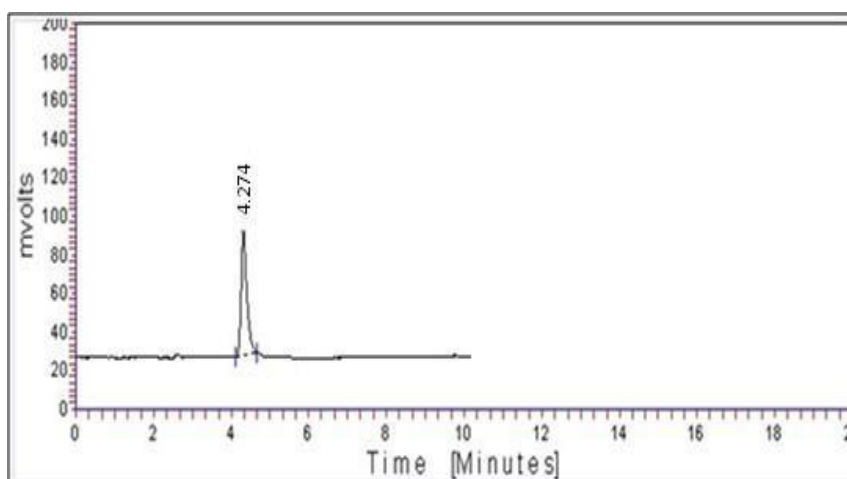


Figure 6: Chromatogram of Dolutegravir Sodium

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.0 ml/min. After complete saturation of column, three replicates of working standard of Dolutegravir Sodium (10 µg/ml) was injected separately. Peak report and column performance

report were recorded for all chromatogram [26].

Table 9: System suitability parameters of Dolutegravir Sodium

System suitabilityParameter	RT	AUC	HETP	Tailingfactor
Rep-1	4.125	458.987	0.145	1.35
Rep-2	4.369	462.458	0.214	1.36
Rep-3	4.321	459.789	0.147	1.12
Mean	4.274	465.582	0.199	1.305
S.D.	0.084	6.512	0.069	0.113
% R.S.D.	1.977	1.399	34.646	8.625

Validation of Developed Method

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25 µg/ ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. The regression equation and correlation coefficient of curve are given and the standard calibration curve of the drug is shown in figure [27]. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration (Table given below 10)

Table 10: Response ration data for linearity of Dolutegravir Sodium

Replicates	Concentration(µg/ml)	Mean AUC	Response Ratio
Rep-1	5	236.73317	47.346
Rep-2	10	465.5825	46.558
Rep-3	15	668.48867	44.565
Mean			46.156

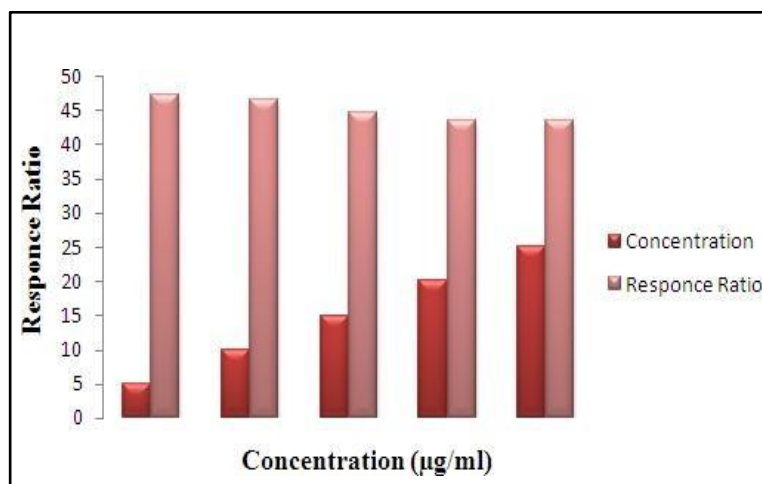


Figure 7: Response Ratio Curve of Dolutegravir Sodium

Specificity

Specificity of the method was carried out to assess unequivocally the analyte presence of the components that might be expected to be present, such as impurities, degradation products and

% RSD	0.296
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Table 12: Recovery study of Dolutegravir Sodium (100% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found. (µg/ml)			% conc. Found			Mean
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	% conc.
5	5	5.01	5.02	4.96	100.2	100.4	99.2	99.933
5	5	5.02	4.95	4.95	100.4	99	99.0	99.466
5	5	4.98	4.96	4.96	99.6	99.2	99.2	99.333
MEAN								99.719
SD								0.091
% RSD								0.092

Table 13: Recovery study of Dolutegravir Sodium (120% Level)

Conc. of sample (µg/ml)	Amt. Added (µg/ml)	Conc. Found. (µg/ml)			% conc. Found			Mean
		Rep-1	Rep-2	Rep-3	Rep-1	Rep-2	Rep-3	% conc.
5	6	5.99	6.01	5.98	99.83	100.17	99.67	99.89
5	6	5.98	6.02	5.99	99.67	100.33	99.83	99.94
5	6	5.45	6.01	5.98	90.83	100.17	99.67	96.89
MEAN								98.907
SD								1.748
% RSD								1.768

Precision

Repeatability

The repeatability was performed for five replicate at five concentrations in linearity range 5, 10, 15, 20 and 25 µg/ml for Dolutegravir Sodium indicates the precision under the same operating condition over short interval time [29]. Results of repeatability are reported in table respectively 14.

Table 14: Repeatability of Dolutegravir Sodium

CONC.REP.	CONCENTRATION FOUND (µg/ml)					MEAN
	5	10	15	20	25	
Replicate-1	4.95	9.95	14.65	20.05	24.98	
Replicate-2	4.92	9.98	15.05	19.95	25.02	
Replicate-3	4.98	9.85	14.95	20.03	25.06	
MEAN	4.953	9.975	14.933	19.978	25.005	
% MEAN	99.067	99.750	99.556	99.892	100.020	99.657
SD	0.029	0.077	0.156	0.079	0.038	0.076
% RSD	0.029	0.077	0.157	0.079	0.038	0.076

Intermediate Precision**a) Day to Day Precision**

Intermediate precision was also performed within laboratory variation on different days in five replicate at five concentrations. Results of day-to-day intermediate precision for Dolutegravir Sodium reported in table respectively 15.

Table 15: Day-to-Day variation of Dolutegravir Sodium

CONC.REP.	CONCENTRATION FOUND ($\mu\text{g/ml}$)					MEAN
	5	10	15	20	25	
Replicate-1	4.98	10.45	14.89	20.05	25.12	
Replicate-2	4.87	10.21	15.02	20.05	24.89	
Replicate-3	5.02	9.98	14.78	20.02	24.98	
MEAN	4.970	10.113	14.910	19.910	24.957	
% MEAN	99.400	101.133	99.400	99.550	99.827	99.862
SD	0.056	0.198	0.113	0.256	0.125	0.150
% RSD	0.056	0.196	0.114	0.257	0.125	0.150

Robustness

As per ICH norms, small, but deliberate variations in concentration of the mobile phase were made to check the method's capacity to remain unaffected. The ratio of mobile phase was change from, methanol: acetonitrile (50:50 % v/v), to (45:55 % v/v). Results of robustness are reported in table 16.

Table 16: Robustness of Dolutegravir Sodium

CONC. REP.	CONCENTRATION FOUND ($\mu\text{g/ml}$)					MEAN
	5	10	15	20	25	
Replicate-1	5.05	9.98	15.01	19.85	24.95	
Replicate-2	4.99	10.05	15.02	20.05	24.89	
Replicate-3	4.85	10.05	14.98	19.86	24.78	
MEAN	4.975	9.963	14.947	19.958	24.947	
% MEAN	99.500	99.633	99.644	99.792	99.787	99.671
SD	0.073	0.157	0.101	0.090	0.106	0.106
% RSD	0.074	0.158	0.102	0.090	0.106	0.106

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Table 17: LOD and LOQ

Name	LOD ($\mu\text{g/ml}$)	LOQ ($\mu\text{g/ml}$)
Dolutegravir Sodium	1.08	3.27

Analysis of Tablet Sample

Twenty tablets were taken and their average weight was determined. They are crushed to fine powder; amount equal to 10 mg of Dolutegravir Sodium was taken in 100-ml volumetric flask. The volume is made up to the mark by mobile phase and filtered by what Mann filter paper (no.41) and the filtrate was used to prepare samples of different concentration. Results of tablet analysis are reported in table 18.

Table 18: Analysis of tablet sample

S. No.	Parameter	Dolutegravir Sodium
1.	Mean	99.95
2.	S. D.	0.123
3.	% RSD	0.256

4. Discussion

The stability indicating RP-HPLC method was developed for estimation of Dolutegravir Sodium in bulk and capsule dosage form by isocratically using Methanol: Acetonitrile in the ratio of 50:50 v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5 μ particle size) column as stationary phase and chromatogram was recorded at 264 nm. Then developed method was validated by using various parameters [30].

System suitability

The system suitability parameter was carried out to verify that the analytical system was working properly and could give accurate and precise result. The six replicates of reference standard, 10 μ g/ml of Dolutegravir Sodium were injected separately and chromatogram was recorded [31]. The result of system suitability parameter is reported in table 19.

Table 19: Results of system suitability parameters

Parameters	Dolutegravir Sodium
HETP	0.199 $\pm$ 0.069
Tailing Factor	1.305 $\pm$ 0.113
Retention time	4.274 $\pm$ 0.084

Linearity

The linearity of analytical method was carried out to check its ability to elicit test results that are proportional to the concentration of analyte in sample within a given range. Different levels of standard solutions were prepared and injected into the HPLC and the chromatogram was recorded. The results of linearity are reported in table 20.

Table 20: Results of linearity of Dolutegravir Sodium

Parameter	Dolutegravir Sodium
Concentration (μ g/ml)	5-25
Correlation Coefficient (r^2)*	0.999
Slope (m)*	42.73
Intercept (c)*	14.01

*value of five replicate

Specificity

Specificity of the method was determined and the peaks of diluent, mobile phase and excipient of tablets did not interfere with standard peaks Dolutegravir Sodium.

Accuracy

The validity and reliability of proposed methods were assessed by recovery studies. The recovery of added standards (80%, 100% and 120%) was found at three replicate and three

concentrations level. The value of % means just close to 100, SD and % RSD are less than 2 indicate the accuracy of method. Result of recovery study shown in table.

Table 21: Results of recovery study

% LEVEL	% MEAN
	Dolutegravir Sodium
80%	98.778
100%	99.719
120%	98.907

* Value of three replicate and three concentrations.

Precision

Precision was determined by repeatability and Intermediate precision of drug. Repeatability result indicates the precision under the same operating condition over short interval time. The intermediate precision study is expressed within laboratory variation on different days and analyst to analyst variation by different analyst. The value of SD and %RSD is less than 2 indicate the precision of method. Result of precision shown in table 7.4.

Table 22: Results of Precision

Parameter	% MEAN
	Dolutegravir Sodium
Repeatability	99.657
Intermediate precision	
Day to day precision	99.862

* Value of five replicate and five concentrations

5. Conclusion

The proposed methods were found to be linear in the range of 5 -25 µg/ml with correlation coefficient close to one. Precision was determined by repeatability, Intermediate precision and reproducibility of the drugs. The robustness of developed method was checked by changing in the deliberate variation in solvent. The result obtained shows the developed methods to be Cost effective, Rapid (Short retention time), Simple, Accurate (the value of SD and %RSD less than 2), Precise and can be successfully employed in the routine analysis of these drugs in bulk drug as well as in tablet dosage form. The Cost effectiveness, Simplicity, Rapidly and Reproducibility of the proposed method completely fulfill the objective of this research work.

6. References

1. MR Hutchings; S Athanasiadou; I Kyriazakis; I J Gordon. Proc NutrSoc.2003, 62(2), 361.
2. E Cindy; M Houghton. Wild Health: How Animals Keep Themselves Well and What We Can Learn from Them, 2002.
3. BS Sekhon. J Pharm Educ Res., 2011, 2(2), 55-56.
4. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines, World Health Organization, Geneva, 2000; 2001; 1.
5. K Swatantra. Archives of Applied Science Research, 2010, 2(1), 225-226.
6. Pharmaceutical Applications with HPLC, Agilent library, 2000.
7. Narottam Pal, Avanapu Srinivasa Rao And Pigilli Ravi kumar, Simultaneous, HPLC

- Method Development and Validation for Estimation of Lamivudine, Abacavir and Dolutegravir in Combined Dosage Form with their Stability Studies, Asian Journal of Chemistry; Vol. 28, No. 2 (2016), 273-276.
8. Girija B. Bhavar, sanjay S. Pekamwar, Kiran B. Aher, ravindra S. Thorat, sanjay R. Chaudhari, High-Performance Liquid Chromatographic and High- Performance Thin-Layer Chromatographic Method for the Quantitative Estimation of Dolutegravir Sodium in Bulk Drug and Pharmaceutical Dosage Form, Sci Pharm. 2016; 84: 305–320.
 9. Bhavar Girija Balasaheb, Aher Kiran Balasaheb, Thorat Ravindra Subhash, Kakadsachin Jijabapu, Pekamwar, Sanjay Sudhakar, Development and Validation of UV Spectrophotometric Method for Estimation of Dolutegravir Sodium in Tablet Dosage Form, Malaysian Journal of Analytical Sciences, Vol 19 No 6 (2015): 1156 – 1163.
 10. Nagasarapu Mallikarjuna Rao, Dannana Gowri Sankar, Development and validation of stability-indicating HPLC method for simultaneous determination of Lamivudine, Tenofovir, and Dolutegravir in bulk and their tablet dosage form, Future Journal of Pharmaceutical Sciences 1 (2015) 73- 77.
 11. Rajkumar Prava, Ganapathy Seru, Vamsi Krishna Pujala and Surendra Babu Lagu, RP-HPLC method development and validation for the simultaneous determination of lamivudine, abacavir and dolutegravir in pharmaceutical dosage forms, World J Pharm Sci 2017; 5(5): 168-181.
 12. Satyadev T. N. V. S. S., Bhargavi Ch. and B. Syam Sundar, Development and validation of high performance liquid chromatographic method for the determination of Dolutegravir in human plasma, Der Pharmacia Sinica, 2015, 6(4):65-72.
 13. Kalpana Nekkala, V. Shanmukh Kumar, D. Ramachandran, Development and Validation for the Simultaneous Estimation of Lamivudine, Tenofovir Disoproxil and Dolutegravir In Drug Product by RP-HPLC, J. Pharm. Sci. & Res. Vol. 9(9), 2017, 1505-1510.
 14. Talari Kalpana, Dr. Tiruveedula, Raja Rajeswari, Ramana Reddy Ganji, Development and Validation of Analytical Method for Determination of Dolutegravir Sodium, Lamivudine and Tenofovir Disoproxil Fumarate Using Reverse Phase High Performance Liquid Chromatography, Der Pharma Chemica, 2017, 9(8):117-127.
 15. Gadapa Nirupa and Upendra M Tripathi, RP-HPLC Analytical Method Development and Validation for Simultaneous Estimation of two Drugs Nitazoxanide, Ofloxacin and its Pharmaceutical Dosage Forms, Int.J. Chem Tech Res.2012, 4(2).
 16. Sani A. Ali, Chijioke C. Mmuo, Rafat O. Abdulraheem, Sikirat S. Abdulkareem, Emmanuel T. Alemika, Musa A. Sani and Mohammed Ilyas, High Performance Liquid Chromatography (HPLC) Method Development and Validation Indicating Assay for Ciprofloxacin Hydrochloride, Journal of Applied Pharmaceutical Science 01 (08); 2011: 239-243.
 17. T.A. Phazna Devi, Aravind Setti, S. Srikanth, Sivaramaiah Nallapeta, Smita C. Pawar, J. Venkateshwara Rao, Method development and validation of paracetamol drug by RP-HPLC, J Med Allied Sci 2013;3(1):08-14.
 18. Carolina Passarelli Gonçalves, Flavio Sussumu Yasuda, Maria Aparecida dos Santos, Romulo Dragani Reis, Maria Isabel Savino, William Ribeiro, Luciane Reche, Ivair Donizete Gonçalves, Luis Carlos Marques and Maria Cristina Marcucci, Development and Validation of an HPLC-DAD Method for the Determination of Coumarin in Syrups with Guaco and Critical Analysis of Drug Labels, American Journal of Phytomedicine and Clinical Therapeutics, 2017 Vol. 5 No. 3:19.
 19. R. Kayesh, A. Rahman, M. Z. Sultan, M. G. Uddin, F. Aktar, and M. A. Rashid, Development and Validation of a RP-HPLC Method for the Quantification of Omeprazole in Pharmaceutical Dosage Form, J. Sci. Res. 2013;5 (2): 335-342.
 20. Jahnvi Bandla, S. Ganapaty, Stability indicating RP-HPLC method development and

- validation for the simultaneous determination of Sofosbuvir and Velpatasvir in tablet dosage forms, *Indian J. Pharm. Biol. Res.* 2017; 5(4):10-16.
21. Mallesh Kurakula, Tariq R Sobahi, AM El-Helw and Magdy Y Abdelaal, Development and Validation of a RP-HPLC Method for Assay of Atorvastatin and its Application in Dissolution Studies on Thermosensitive Hydrogel-Based Nanocrystals, *Tropical Journal of Pharmaceutical Research.* 2014; 13 (10): 1681-1687
 22. J. Mamatha and N. Devanna, Simultaneous RP-HPLC Method Development and It's Validation For Estimation of Sofosbuvir and Velpatasvir In Their Combined Dosage Form, *Rasayan J. Chem.* 2018; 11(1): 392- 400.
 23. Mounika Arrabelli, Yeshwanth Reddy Musukula and Raghuram Reddy Adidala, Method Development and Validation of Zidovudine by RP-HPLC, *IJRPC.* 2014, 4(3), 606-610.
 24. Akiful haque, S. Hasan Amrohi, Mahesh Nasare, Prashanth Kumar.K, Pradeep Kumar. T, Nivedita. G, Prakash V Diwan, Analytical method development and validation for the estimation of Naproxen using RP-HPLC, *IOSR Journal of Pharmacy*, 2012; 2(4):19-24.
 25. P. R. Solanki, S. Prachia and S. D. Boobb, RP - HPLC method for estimation of paracetamol from pharmaceutical formulation febrinil, *Sci. Revs. Chem. Commun.* 2(3), 2012: 232-236.
 26. Gunjan Rao, Anju Goyal, An Overview on Analytical Method Development and Validation by Using HPLC, *The Pharmaceutical and Chemical Journal*, 2016, 3(2):280-289.
 27. K. V. Lalitha, Golla Murali Mohan J. Ravindra Reddy, K. Vinod Kumar, A. Alikeya, RP-HPLC Method Development and Validation for the Simultaneous Estimation of Paracetamol and Flupirtine Maleate in Pharmaceutical Dosage, *Journal of Scientific and Innovative Research* 2013; 2 (3): 634-641
 28. Yashpalsinh N Girase, Srinivasrao V, DiptiSoni1. Development and Validation of Stability Indicating RP-HPLC Method for Rivaroxaban and Its Impurities. *SOJ Biochem.* 2018; 4(1):1-6.
 29. Sufiyan Ahmad, Sharma Deepika, Patil Amol, Warude Kapil, Md. Rageeb Md. Usman, Novel RP-HPLC Method Development and Validation of Meloxicam Suppository, *Indian Journal of Pharmaceutical Education and Research.* 2017; 51(4)
 30. Anjaneyulu. N, Nagakishore. R, Nagaganesh. M, Muralikrishna. K, Nithya. A, Sai lathadevi. B, Saikiran Goud. M and Sridevi. N Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Lamivudine and Tenofovir Disproxil Fumerate in Combined Dosage Form. *Asian Journal of Biomedical and Pharmaceutical Sciences.* 2013; 3(23): 7-11.
 31. Bhatt KK, Emanuel Michael Patelia and Ishani Amin, Development of a Validated Stability-Indicating RP-HPLC Method for Dronedarone Hydrochloride in Pharmaceutical Formulation. *J Anal Bioanal Techniques* 2013, 4:1