Section A -Research paper

Analytical Method Development And Validation Of Amlodipine And Celecoxib 10/200mg Tablets As Per Ich Guidelines

Logeshwari T, Marina Juliet A, Deepak M, Vijey Aanandhi M*

Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India. Pin code-600117

Corresponding Author

M.Vijey Aanandhi

Department of Pharmaceutical Chemistry and Analysis,School of Pharmaceutical Sciences,
Vels Institute of Science, Technology and Advanced Studies (VISTAS),Pallavaram.Chennai-600 117.
Mobile: 9840959519
Email: mvaanandhi@gmail.com

ABSTRACT

Objective: A simple, novel, sensitive, rapid high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for quantitative determination of Amlodipine and Celecoxib in combined dosage.

Methods: The chromatographic development was carried out on RP-HPLC. The column used Agilent (Eclipse XDB-phenyl 250 x 4.6 x 5µm) or equivalent with mobile phase consisting of500mL of Acetonitrile, 500mL of milli-Q water and 2mL of Trifluoroacetic acid. The flow rate was 1.5 mL/min and the effluents were monitored at 238 nm.

Results: The retention time was found to be Amlodipine about 3.0 minutes and Celecoxib about 8 minutes. The method was validated as per International Conference on Harmonization Guideline with respect to linearity, accuracy, precision, and robustness. The calibration curve was found to be linear over a range of 1–10 µg/mL with a Amlodipine and Celecoxib regression coefficient were found to be 0.9998 and 0.9999 respectively. The method has proved high sensitivity and specificity.

Conclusion: The results of the study showed that the proposed RP-HPLC method was simple, rapid, precise and accurate which is useful for the routine determination of Amlodipine and Celecoxib in combined dosage in its pharmaceutical dosage form.

Keywords: Amlodipine & Celecoxib, Combined dosage form, Rapid high-performance liquid chromatographic, Validation.

INTRODUCTION

Amlodipine is chemically named as 3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate it is a synthetic dihydropyridine and calcium channel blockers blocks the movement of extracellular calcium ions into peripheral and myocardial smooth muscle thereby it is used for the treatment of hypertension and Angina Pectoris[(1)] . Many Analytical methods reported that amlodipine with alone or combination with other API from biological fluids and formulation in RP-HPLC, HPTLC, LCMS/MS, electrochemical methods and Capillary electrophoresis[(2-6)] .Celecoxib is chemically named as 4-[5-(4-methylphenyl)-3-(trifluoromethyl) pyrazol-1-yl] benzene sulfonamide it is a selective COX-2 inhibitor it inhibits selective COX-2 inhibitor enzyme relieve from the pain, thereby it is used for the treatment of osteoarthritis and rheumatoid arthritis [(7)] .Many analytical methods reported with Celecoxib or in combination with other API in RP-HPLC, LC/MS, spectrofluorometry, spectrophotometry, and capillary electrophoresis[(8-13)] .

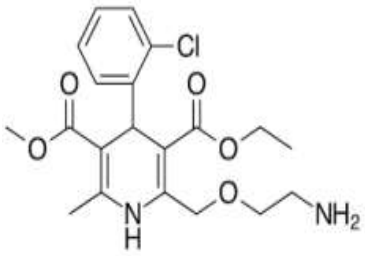
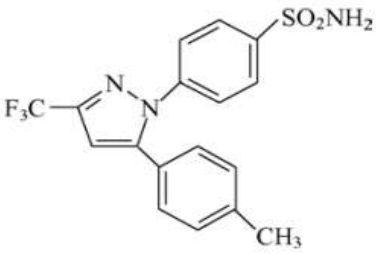
Description	Amlodipine	Celecoxib
Molecular formula	$C_{26}H_{31}ClN_2O_8S$	$C_{17}H_{14}F_3N_3O_2S$
Molecular weight	567.1	381.4
solubility	slightly soluble in water	Poorly soluble in water
Chemical structure		

Table No: 1 Description of Amlodipine and Celecoxib

The patient who have both hypertension and osteoarthritis sometimes they face difficulties in taking both tablet, to overcome this problem combination of amlodipine and Celecoxib in single tablet were administered[(12)] . Literature survey reveals that only few analytical methods developed for the estimation of Amlodipine and Celecoxib in combination with UV spectroscopic methods. The purpose of the study is to develop and validate the method as per ICH guidelines.

EXPERIMENTS

Chemicals & Reagents

Pure samples of Amlodipine (99.98%) and Celecoxib (100%) Working standard procured from(Hetero drugs limited).Acetonitrile and TFA used a HPLC grade or Equivalent were Purchased from Central drug house Private LTD . Methanol used was of HPLC grade and were purchased from Advent Chembio PVT.LTD. 0.45µm Nylon filters [Thermo Fisher QNN9797919] are used.

Instruments Required

Equipment and Instruments used in this validation has been calibrated and maintained and will be used within their calibrated period. The weighing operations for this study were performed on the Mettler XS 205 Analytical balance. The Samples were ultrasonically processed using an Ultrasonicator from CHEM54/ Chemlabs. Chromatographic separation was performed using HPLC with PDA detector Aligent Technologies (Waters 2695) by using the Column Agilent (Eclipse XDB-phenyl 250 x 4.6 x 5µm) or equivalent Chromatograms were recorded using the Empower version 3 software (Agilent Technologies) installed on a personal computer. The mobile phase was pumped at the rate of 1.5 mL/minute and the absorption of analytes was detected at 238 nm. The experiments were carried out in an air-conditioned laboratory maintained at 25°C±2°C. The amount of standard and sample solutions injected into the HPLC instrument for the analysis was set at 20 µL with a retention time of Amlodipine about 3.0 minutes and Celecoxib about 8 minutes.

Materials required

Volumetric flasks, glass beakers, measuring cylinders, pipettes, 0.45µm Nylon syringe Filters.

Preparation of Mobile phase

Measured 500mL of Acetonitrile, 500mL of milli-Q water and 2mL of Trifluoroacetic acid, mix well sonicated for 10 minutes.

Preparation of Diluent

Milli-Q water: Methanol in the ratio of 100:400 (% v/v).

Preparation of Standard Solution

Weigh accurately about 27.7 mg of Amlodipine besylate (equivalent to 20 mg Amlodipine) and 20.0 mg of Celecoxib working/reference standard and transfer it into a 200 mL volumetric flask, add about 140 mL of diluent, sonicate to dissolve the contents and dilute to the volume with diluent. (100 µg/mL of Amlodipine and 100 µg/mL of Celecoxib)

Preparation of Sample Solution

Preparation of Sample stock solution (Amlodipine)

Weigh 10 tablets and calculate the average weight. Crush the tablets into fine powder by using mortar and pestle and. Accurately weigh and transfer the tablet powder equivalent to 10 mg of Amlodipine and 200 mg of Celecoxib (about 500.0 mg of crushed powder) into a 100 mL volumetric flask, add about 70 mL of diluent and mechanically shaken at 200 RPM for 30 minutes, sonicate for 30 minutes with intermediate shaking, allow it to attain room temperature, then dilute to volume up to the mark with diluent and shake well. Filter the above sample solution through 0.45 µm PVDF syringe filter. (100 µg/mL of Amlodipine)

Preparation of Sample Solution (Celecoxib)

Pipette out 5 mL of above sample stock solution was transferred into 100 mL volumetric flask and dilute to the volume with diluent and mix well. (100 µg/mL of Celecoxib)

RESULTS AND DISCUSSION

Primarily numerous trials for optimization of method was performed using different phase composition, different organic solvent, different ratio of organic to buffer, different stationary phase and different internal standard chromatographic settings to achieve the finest peak resolution of Amlodipine and Celecoxib.

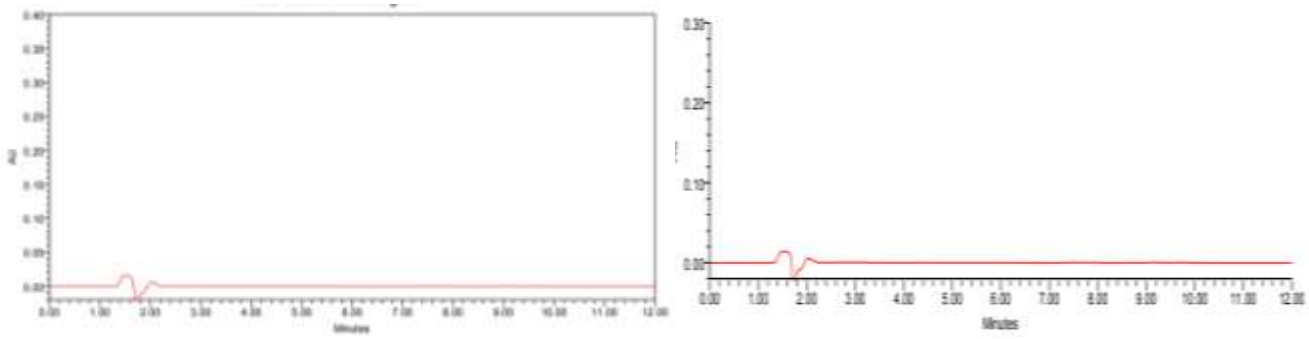
Analytical method validation

The analytical method was optimized and validated in accordance with the current ICH guidelines ([ICH] Q2 [Rl]) and to accomplish the vision of various parameters.

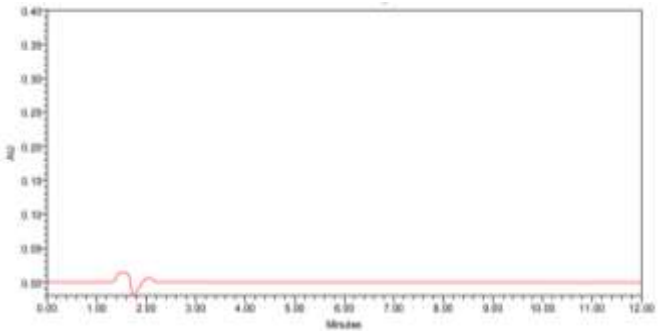
1. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present**.** For specificity evaluation: Blank solution, Placebo solution, Sample solution and standard solution were injected into the HPLC system

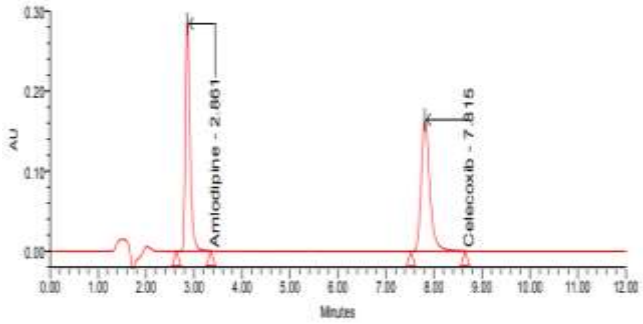
Sample Information: Blank solution



Sample Information: Placebo solution



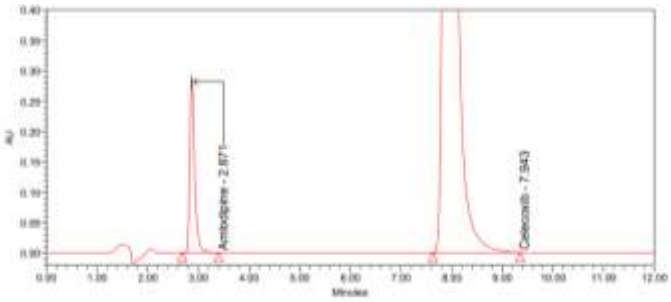
Sample information: STD solution



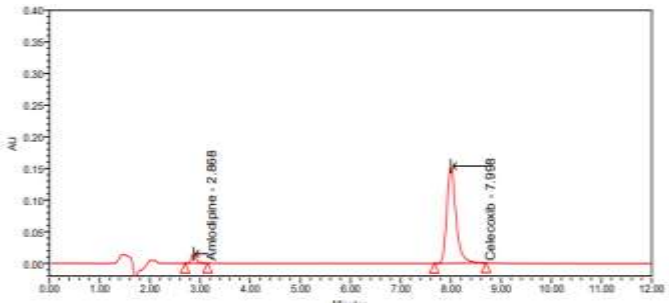
Peak	RT	Area
Amlodipine	2.8	1906397
Celecoxib	7.9	2043630

Table No: 2 Peak table (detector at 238nm)

Sample Information: Sample solution



Sample Information: sample solution



Peak	RT	Area
Amlodipine	2.8	1860334
Celecoxib	7.9	2019786

Table No: 3 Peak table (detector at 238nm)

2. Accuracy (Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted true value or an accepted reference value and the Actual value found. The recovery of an analyte spiked sample into the mixture of the sample to be examined is typically used to evaluate accuracy studies. I have employed three different concentrations of solution for my accuracy studies, including 5 mg/mL, 10 mg/mL, and 15 mg/mL. Each concentration was injected for these, and the mean percent recovery was computed below which has been depicted in the Table No.4 & 5

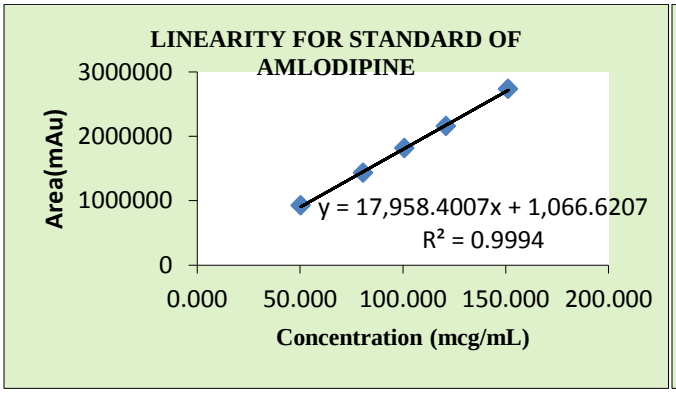
Accuracy % level	Recovery in %	Mean Recovery in %	% RSD
50%	99.57	99.15	0.883
50%	98.14		
50%	99.73		
100%	99.02	99.46	0.561
100%	100.09		
100%	99.28		
150%	100.78	100.39	0.661
150%	100.76		
150%	99.62		

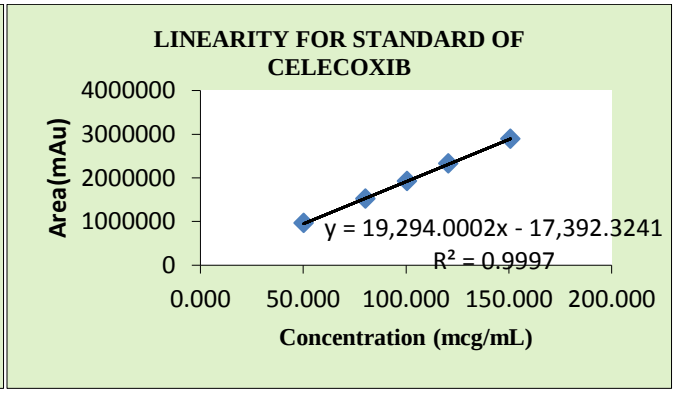
Accuracy % level	Recovery in %	Mean Recovery in %	% RSD
50%	99.87	100.77	0.908
50%	101.70		
50%	100.74		
100%	99.06	100.77	0.813
100%	99.93		
100%	98.32		
150%	100.50	99.65	1.016
150%	98.53		
150%	99.92		

Table No: 4 & 5 Recovery result of Amlodipine and Celecoxib

3. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. For the evolution of the linearity of the analytical method, standard dilutions of Amlodipine and Celecoxib in a concentration range of 50.4142 µg/ml to 150.1792 µg/ml for Amlodipine and Celecoxib prepared as per the test procedure of methodology and analyzed on the HPLC system. Results were tabulated in Table No. and the linearity plot was depicted in Figure No.





4. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility

A. Method precision (Repeatability): The precision can be determined by using sample with RP-HPLC method. Samples solution of six replicate injection containing Amlodipine 10mg and Celecoxib 200mg injected for determining the % RSD which less than 2.0%.

No.of Injections	Amount added in mg	Result in %
1 (10mg)	9.6	96.00
2 (10mg)	9.6	96.00
3 (10mg)	9.9	99.00
4 (10mg)	9.8	98.00
5 (10mg)	9.7	97.00
6 (10mg)	9.8	98.00
Mean	**9.700**	**97.00**
Std Dev	**0.121**	**1.25**
%RSD	**1.25**	**1.25**

No.of Injections	Amount added in mg	Result in %
1 (200mg)	197.400	96.00
2 (200mg)	199.100	96.00
3 (200mg)	197.600	99.00
4 (200mg)	200.700	98.00
5 (200mg)	200.500	97.00
6 (200mg)	197.400	98.00
Mean	**198.800**	**99.40**
Std Dev	**1.546**	**0.773**
%RSD	**0.78**	**0.78**

Table No: 6 & 7 Repeatability Result of Amlodipine and Celecoxib

B. System precision: The resolution between the peaks of Amlodipine and Celecoxib should not less than 2.0 from system suitability solution. The tailing factor for Amlodipine and Celecoxib peaks in the standard preparation should not be more than 2.0.The relative standard deviation for the average area of Amlodipine and Celecoxib peak five replicate injections of standard preparation should not be more than 2.0%

Name of the parameter	Amlodipine	Celecoxib
Retention time (RT)	2.8	7.8
Area (%RSD NMT2.0)	0.8	0.7
Tailing factor (NMT 1.8)	1.4	1.3
Theoretical plates (NLT 2000)	4656	10067

Table No: 8 Result of system precision of Amlodipine and Celecoxib

C.Intermediate precision: % Assay of individual and average value should be 90.0% to 110.0% of labeled amount of Amlodipine and Celecoxib.The %RSD for the assay results of six sample preparations should not be more than 2.0.The %RSD for the combined assay results of 12 replicate preparations between method precision and intermediate precision should not be more than 2.0%

No. of Sample	% Assay(AMLO)	% Assay(CELE)
1	96.00	98.70
2	96.00	99.60
3	99.00	98.80
4	98.00	100.40
5	97.00	100.30
6	98.00	98.70
Mean	**97.00**	**99.40**
% RSD	**1.210**	**0.773**

Table No: 9 Intermediate precision result of Amlodipine of Celecoxib

5. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Mobile Phase	**% Recovery**	**% RSD**
450:550:2 (Actual)	101.4	
500:500:2	100.4	0.78%
550:500:2	101.2	
Flow rate		
1.5ml/min (Actual)	101.4	
1.3ml/min	100.4	0.98%
1.7ml/min	101.2	
Wavelength		
238 nm (Actual)	100.4	
236nm	99.0	0.79%
238nm	99.6	
Column Temperature		
25^0 C (Actual)	100.7	
20^0 C	100.0	0.83%
30^0 C	99.2	

Table No: 10 Robustness result of Amlodipine of Celecoxib

6. Solution stability

The % Recovery between area of stability standard solution and initial standard solution should be within 98.0 % to 102.0%.

Intervals	Area	% of Recovery
Initial	1906397	99.57
2Hrs	1946397	98.14
12Hrs	1928524	99.73
16Hrs	1918313	99.02
20Hrs	1918790	100.09
24Hrs	2015679	98.6
30Hrs	2015876	98.6

Intervals	Area	% of Recovery
Initial	2019786	98.70
2Hrs	2040432	99.60
12Hrs	2013649	98.80
16Hrs	2047590	100.40
20Hrs	2053573	100.30
24Hrs	2015471	98.70
30Hrs	2015467	98.69

Table No: 11 & 12 Result of Solution stability of Amlodipine & Celecoxib (STD & sample solution)

7. Filter validation: The % Difference between the average % assay value of the centrifuged preparations and the % assay value of each filtered preparation should not be more than ± 2.0%

Filter Details	**% Results**	**% Difference**	**%RSD**
Centrifuged (Unfiltered)	100.40	-	0.912%
0.45µ Nylon Filter 1 ml discarded	99.60	-0.8	
0.45µ Nylon Filter 2 ml discarded	98.80	-0.6	
0.45µ Nylon Filter 4 ml discarded	98.70	-0.5	

Table No: 13 Filter validation result of Amlodipine of Celecoxib

DISCUSSION

The present work is based on the quantification of Amlodipine and Celecoxib combined dosage by using RP-HPLC with PDA detector. The physical and chemical parameters were chosen based on Amlodipine and Celecoxib. Based on the system suitability the stationary phase was chosen. XBD-Eclipse Phenyl Agilent Zorbax was used for separation of analytes for evaluating the parameters. The optimization of mobile phase done on the different preliminary trials. Mobile phase containing 500mL of Acetonitrile, 500mL of milli-Q water and 2mL of Trifluoroacetic acid were found to ideal combination for the system suitability parameters. The Mobile phase ratio, flow rate, Wavelength, column temperature was done with different preliminary trials for evaluation of chromatographic peak resolution with minimum solvent consumption. The filter validation studies is the critical part of Qc tests samples were filtered by using syringe filters and filtrate were collected and analyzed by HPLC for the quantification of API. Centrifuged

samples were used as controls for recovery of 100% and to calculate the analyte binding in a syringe filter. The solution stability studies were also done by storing the sample and standard solution in the proper room temperature for determining the stability of samples and standard solution. The developed methods were validated as per ICH guidelines which specific, simple and reliable which successfully applied for the quantification of amlodipine and Celecoxib without any excipient interference.

REFERENCES

1. Kumar S, R B, Analytical method development and validation of Amlodipine besylate in tablet dosage form Journal of Drug Delivery and Therapeutics. 2019; 9 (4- A):463-466
2. Attimarad M, Venugopala KN, Aldhubiab BE, Nair AB, SreeHarsha N, Pottathil S, Akrawi SH. Development of UV spectrophotometric procedures for determination of amlodipine and Celecoxib in formulation: use of scaling factor to improve the sensitivity. Journal of Spectroscopy; 2019:1-0.
3. N. Usharani, K. Divya, and V. V. S. Ashrtiha, "Development and validation of UV-derivative spectroscopic and RP-HPLC methods for the determination of amlodipine besylate and valsartan in tablet dosage form and comparison of the developed methods by student's T-test," Indian Journal of Pharmaceutical Education and Research, 2017: 51(4):776–782.
4. J. J. Pandya, M. Sanyal, and P. S. Shrivastav, "Simultaneous densitometric analysis of amlodipine, hydrochlorothiazide, lisinopril, and valsartan by HPTLC in pharmaceutical formulations and human plasma," Journal of Liquid Chromatography & Related Technologies;2017;40(9);467–478
5. L. Wang, W. Liu, Z. Zhang, and Y. Tian, "Validated LC-MS/ MS method for the determination of amlodipine enantiomers in rat plasma and its application to a stereo selective pharmacokinetic study," Journal of Pharmaceutical and Biomedical Analysis;2018; 158; 74–81.
6. M. Attimarad, N. Sreeharsha, B. E. Al-Dhubaib, A. B. Nair, and K. N. Venugopala, "Capillary electrophoresis: MEKC assay method for simultaneous determination of olmesartan medoxomil, Amlodipine besylate and hydrochlorothiazide in tablets," Indian Journal of Pharmaceutical Education and Research, 2016;50(2); 188–195.
7. Nagamani P, Manjunath SY, Hem ant Kumar T, Development and Validation of RP-HPLC Method for Estimation of Amlodipine Besylate and Celecoxib in Pharmaceutical Formulation, Journal of Drug Delivery and Therapeutics. 2020; 10(6):31-36
8. A. A. Gouda, M. I. Kotb El-Sayed, A. S. Amin, and R. El Sheikh, "Spectrophotometric and spectrofluorometric methods for the determination of non-steroidal anti-inflammatory drugs: a review," Arabian Journal of Chemistry,2013;6(2); 145–163
9. O. S. S. Chandana and R. Ravichandrababu, "Stability indicating HPLC method for Celecoxib related substances in solid dosage forms," International Journal of Research in Pharmaceutical Sciences,2017; 7(1);10–18.
10. M Manasa, VM Aanandhi ,Stability indicating method development and validation of semaglutide by RP-HPLC in pharmaceutical substance and pharmaceutical product 2021:14(3): 1385-1389
11. M. K. Srinivasu, D. Sreenivas Rao, and G. O. Reddy, "Determination of Celecoxib, a COX-2 inhibitor, in pharmaceutical dosage forms by MEKC," Journal of Pharmaceutical and Biomedical Analysis, 2002;28(3-4), 493–500.

12. P. Shanmugasundaram G.Haritha, Vijey Aanandhi M Novel method Development for Metformin, Ivabradine, Metoprolol and Ertugliflozin and its Validation in API and Pharmaceutical Dosage Form by RP-HPLC method 2021:14(4) 2055-2061

13. K.S. Syed ali abtheen, R. Maheswari, P. Shanmugasundaram and M. Vijeyaanandhi* Simultaneous Estimation of Chlorhexidine Gluconate, Metronidazole, Lignocaine Hydrochloride and Triamcinolone Acetonide in Combined Dosage Form by RP-HPLC 2008 20(2): 1130-1136

14. Mandale TR, Kondawar MS, Kadam SD. Development and validation of analytical method for simultaneous estimation of amlodipine besylate and Celecoxib in pure and combined dosage form. Research Journal of Pharmacy and Technology. 2020; 13(9):4280-4.