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Qbd Approach Of Analytical Method Development And Validation Of Camylofin And Mefenamic Acid In Bulk And Pharmaceutical Dosage Form

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Abstract

A simple, Accurate, precise method was developed for the estimation of the Camylofin and Mefenamic acid in bulk and pharmaceutical dosage form. The chromatogram was run through HSS 100 x 2.1 mm, 1.8. Mobile phase containing 0.1N Phosphate buffer: Methanol taken in the ratio 55:45 (% v/v) was pumped through the column at a flow rate of 0.2 ml/min. Temperature was maintained at 30°C. The optimized wavelength selected was ACQUITY TUV detector ChA 220.0 nm. Retention time of Camylofin was found to be 1.455 min Mefenamic acid was found to be 1.099 min. %RSD of the Camylofin and Mefenamic acid was found to be 0.9 and 0.9. %Recovery was obtained as 99.97% for Camylofin and 100.44 for Mefenamic acid. LOD and LOQ values obtained from regression equations of Camylofin were 0.03 and 0.08 respectively, and the Regression equation is y = 37722x + 1092.5. LOD and LOQ values obtained from regression equations of Mefenamic acid were 0.08 and 0.23 respectively, and the Regression equation is y = 30948x +6750.5. The chromatographic run time was decreased and method was rugged and robust, so the method developed was simple and economical and can be adopted in regular Quality control test in Industries.

Key Words: Camylofin, Mefenamic acid, System suitability Method development, RP-UPLC

1. Introduction

The majority of medications in multi-component dosage forms can be effectively analyzed using the UPLC system due to its numerous advantages, such as rapidity, specificity, reliability, accuracy, precision, and ease of automation.
The utilization of UPLC methodology eliminates the need for repetitive extraction and isolation procedures. UPLC encompasses various modes of separation, including Size Exclusion Chromatography, Reversed Phase Ion Chromatography, Affinity Chromatography, Normal Phase Mode, and Inverted Phase Mode. The quality of a drug significantly impacts its efficacy and safety. To ensure that customers have access to secure and

effective medicinal compounds, it is imperative to prioritize quality assurance and control in pharmaceutical and chemical formulations.
Therefore, when assessing the suitability of a chemical for medical use, it is essential to analyze both the pure substance and its pharmaceutical formulations. The quality of the methodologies employed in data generation directly impacts the quality of the analytical findings (1). Establishing robust and dependable analytical protocols is imperative to ensure that pharmaceuticals and their formulations meet regulatory standards. Various challenges arise in developing these protocols depending on the nature and properties of the compound. In addition to attaining selectivity, speed, cost-effectiveness, simplicity, sensitivity, reproducibility, and accuracy in results are key considerations. These aspects offer researchers an opportunity to address obstacles related to implementing novel analytical techniques within the chemical and pharmaceutical sectors.
Various physico-chemical methods are employed for the examination of physical occurrences resulting from chemical reactions. Notable among these techniques are optical methods (such as Refractometry, Polarimetry, emission and fluorescence analyses), photometry (encompassing photocolorimetry, spectrophotometry including UV-Visible and IR spectroscopy, and nepheloturbidimetry), and chromatographic methods (including column, paper, thin layer, gas-liquid, and high-performance liquid chromatography). Modern approaches like nuclear magnetic resonance (NMR) and paramagnetic resonance (PMR) are gaining prominence. The integration of mass spectrometry (MS) with gas chromatography stands out as a highly effective analytical tool. Chemical methodologies consist of gravimetric and volumetric procedures relying on complex formation; acid-base, precipitation, and redox reactions. Titrations in non-aqueous environments alongside complexometry have found application in pharmaceutical analysis. The pharmaceutical industry continues to witness a steady influx of new medicinal compounds [1-2].

Ultra-Performance Liquid Chromatography (UPLC) is a burgeoning field within analytical separation science that upholds the practicality and foundational principles of traditional UPLC while enhancing the overall interconnected attributes of velocity, sensitivity, and resolution. By utilizing fine particles, UPLC can push the boundaries of speed and peak capacity to new thresholds, thus leading to what is known as Ultra Performance Liquid Chromatography. Through the strategic implementation of chromatographic principles, UPLC maximizes the potential of separations by employing columns packed with smaller particles and/or higher flow rates, resulting in heightened speed, sensitivity, and superior resolution [2-6].
Camylofin is a smooth muscle relaxant that exhibits both anticholinergic properties and direct smooth muscle effects. The anticholinergic mechanism occurs through the inhibition of acetylcholine binding to muscarinic receptors, albeit with moderate potency. Direct smooth muscle relaxation is induced by the inhibition of phosphodiesterase type IV, resulting in elevated cyclic AMP levels and consequent reduction in cytosolic calcium concentration. Consequently, Camylofin exerts a comprehensive effect in alleviating smooth muscle spasms. Primarily employed in the treatment of stomach discomfort in infants and children, it is commonly administered alongside paracetamol for addressing abdominal pain and fever symptoms.[7]

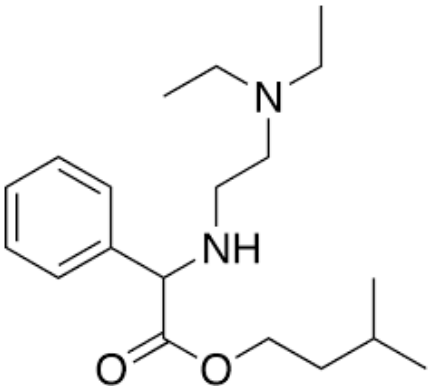

Figure1: Chemical structure of Camylofin

Mefenamic acid is classified as an aminobenzoic acid, wherein anthranilic acid has one of its hydrogen atoms on the nitrogen atom substituted by a 2,3-dimethylphenyl group. Despite being categorized as a non-steroidal anti-inflammatory drug, its anti-inflammatory effects are deemed to be relatively minor. This compound is utilized for alleviating mild to moderate pain conditions such as headaches, dental pain, osteoarthritis, and rheumatoid arthritis. Its functionalities include serving as an analgesic, antirheumatic medication, non-

steroidal anti-inflammatory agent, antipyretic, inhibitor of EC 1.14.99.1 (prostaglandin-endoperoxide synthase), environmental pollutant, and xenobiotic. Mefenamic acid is characterized as an aminobenzoic acid and a secondary amino compound. Its chemical denomination is 2-[(2,3-dimethylphenyl)amino]benzoic acid with a molecular formula of $C_{15}H_{15}NO_2$ and a molecular weight of 241.28 g/mol.[8].

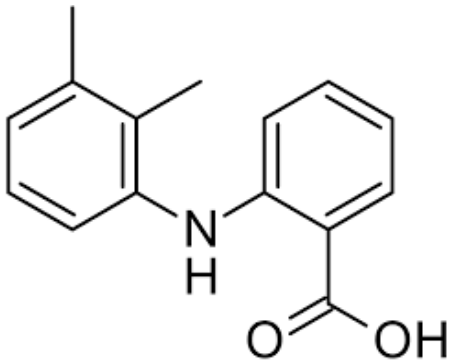

Figure2: Chemical Structure of Mefenamic acid

In this article, we have delved into the capacity of Ultra-Performance Liquid Chromatography (UPLC) to enhance the analysis of samples encountered within the realm of pharmaceutical development and manufacturing. The focus has been on assessing UPLC's potential to streamline analysis durations while upholding the integrity and excellence of analytical data produced, in comparison to conventional liquid chromatography methods.

Experimental work: [9-12]

2. Materials and Methods:

Chemicals and reagents

Pure Camylofin and Mefenamic acid were acquired from Spectrum Pharma Lab in Hyderabad. Hydrochloric acid of analytical reagent (AR) grade (HCl) and sodium hydroxide of AR grade (NaOH) were sourced from Rankem in India. Hydrogen Peroxide (H_2O_2) was procured from Qualigens. Acetic acid of AR grade was purchased from Fisher Scientific in India and S.D. Fine Chem Ltd., respectively. Potassium dihydrogen orthophosphate and orthophosphoric acid were obtained from S.D. Fine Chem Ltd. and Merck India Pvt Ltd., respectively. Ultra Performance Liquid Chromatography (UPLC) grade Acetonitrile (ACN) and Methanol (MeOH) were acquired from Fisher Scientific. The UPLC grade water used throughout the analysis was obtained from the Merck Milli-Q water purification unit.

Apparatus and Equipment

Ultra performance liquid chromatography (UPLC) investigations were conducted using the WATERS UPLC 2965 SYSTEM, equipped with a Photo diode array detector (PDA) set at 220 nm for ultraviolet detection. Various columns were employed in the study, namely Agilent C18 (150×4.6 mm, 5 µm), Discovery C18 (150*4.6mm, 5 µm), Zodiac (150*4.6mm, 5 µm), BDS (150*4.6mm, 5 µm), and Phenomenex (150*4.6mm, 5 µm). Design Expert® (version 11.0.0) modeling software from Stat-Ease Inc., based in Minneapolis, MN, USA, was utilized to generate contour plots and three-dimensional representations.

A pH meter (Eutech Instruments pH Tutor, pH meter, India) was employed to verify the pH levels of all solutions.

Additionally, other essential equipment included a sonicator (ePEI ultrasonic generator), analytical balance (Mettler Toledo), vortex meter (IKA Vortex), and Hot Air oven (Yorco Scientific).

Preparation of buffer

0.01N Potassium dihydrogen orthophosphate

Accurately weighed 1.36 gm of Potassium dihydrogen Ortho phosphate in a 1000 mL of Volumetric flask add about 900 mL of milli-Q water added and degas to sonicate and finally make up the volume with water then added 1 mL of Triethylamine then pH adjusted to 3.0 with diluted Orthophosphoric acid solution.

0.1% Orthophosphoric acid buffer
1 mL of Orthophosphoric acid solution in a 1000 mL of volumetric flask add about 100 mL of milli-Q water and final volume make up to 1000 mL with Milli-Q water

Initial UPLC runs of Camylofin and Mefenamic acid
Initial UPLC runs of Camylofin of 10 µg/mL and Mefenamic acid of 10 µg/mL concentrations were performed using

- Different buffer viz, Potassium dihydrogen orthophosphate and Ortho phosphoric acid.
- Different organic modifier viz, Acetonitrile and methanol
- Different columns such as Symmetry C18 (150×4.6 mm, 5 µm), Agilent C18 (150×4.6 mm, 5 µm), Discovery C18(150*4.6mm,5 µm), Zodiac (150*4.6mm,5 µm), BDS (150*4.6mm,5 µm) and Phenomenex (150*4.6mm,5 µm) column.

Preparation of Drug solutions:
Standard stock solutions: Accurately weighed and transferred 25 mg of Mefenamic acid and 5 mg of Camylofin working Standards into two different 50 ml clean and dry volumetric flasks, add 10 mL of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (500 µg/mL Mefenamic acid and 100 µg/mL Camylofin).

Standard working solution (100% solution): 1 mL from the above two stock solutions were taken into a 10 mL volumetric flask and made up to 10 mL (50µg/mL Mefenamic acid and 10µg/mL Camylofin).
Sample stock solutions: Weighed sample powder equivalent to 1 tablet (250 mg and 50 mg Mefenamic acid and Camylofin) was transferred into a 500 mL volumetric flask, 50 mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.
(500 µg/mL of Mefenamic acid and 100 µg/mL of Camylofin).

Sample working solution: From the filtered solution 1 mL was pippeted out into a 10 mL volumetric flask and made up to 10 mL with diluent. (50 µg/mL Mefenamic acid and 10 µg/mL Camylofin).

Methodology for Validation Parameters: [13-16]
System suitability parameters:
The system suitability parameters were determined by preparing standard solutions of Camylofin (50ppm and 10ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.
The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:
Standard stock solutions:
Accurately weighed and transferred 25 mg of Mefenamic acid and 5 mg of Camylofin working Standards into two different 50 ml clean and dry volumetric flasks, add 10 mL of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (500 µg/mL Mefenamic acid and 100 µg/mL Camylofin).

Standard working solution (100% solution): 1 mL from each stock solution was pipette out and taken into a 10 mL volumetric flask and made up with diluent.

Sample stock solutions: Weighed sample powder equivalent to 1 tablet (250 mg and 50 mg Mefenamic acid and Camylofin) was transferred into a 500 mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (500 µg/ml of Mefenamic Acid and 100 µg/ml of Camylofin).

Sample working solution: From the filtered solution 1 mL was pipette out into a 10 mL volumetric flask and made up to 10 mL with diluent. (50 µg/mL Mefenamic acid and 10 µg/mL Camylofin).

Linearity:
Standard stock solutions: Accurately weighed and transferred 25 mg of Mefenamic acid and 5 mg of Camylofin working Standards into two different 50 ml clean and dry volumetric flasks, add 10 mL of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (500 µg/mL Mefenamic acid and 100 µg/mL Camylofin).
25% Standard solution: 0.25 mL each from two standard stock solutions was pipette out and made up to 10 mL and mix well. (12.5µg/mL of Mefenamic acid and 2.5µg/mL of Camylofin)
50% Standard solution: 0.5 mL each from two standard stock solutions was pipette out and made up to 10 mL and mix well. (25 µg/mL of Mefenamic acid and 5 µg/mL of Camylofin)
75% Standard solution: 0.75 mL each from two standard stock solutions was pipette out and made up to 10 mL and mix well. (37.5 µg/mL of Mefenamic acid and 7.5 µg/mL of Camylofin)
100% Standard solution: 1.0 mL each from two standard stock solutions was pipette out and made up to 10 mL and mix well. (50 µg/mL of Mefenamic acid and 10 µg/mL of Camylofin)
125% Standard solution: 1.25 mL each from two standard stock solutions was pipette out and made up to 10 mL and mix well. (62.5 µg/mL of Mefenamic acid and 12.5 µg/mL of Camylofin)
150% Standard solution: 1.5 mL each from two standard stock solutions was pipette out and made up to 10 mL and mix well. (75 µg/mL of Mefenamic acid and 15 µg/mL of Camylofin)

Accuracy:
Standard stock solutions: Accurately weighed and transferred 25 mg of Mefenamic acid and 5 mg of Camylofin working Standards into two different 50 ml clean and dry volumetric flasks, add 10 mL of diluent, sonicated for 10 minutes and make up to the final volume with diluents. (500 µg/mL Mefenamic acid and 100 µg/mL Camylofin).
50% Spiked solution: 0.5 mL of sample stock solution was taken into a 10 mL volumetric flask, to that 1.0 mL from each standard stock solution was pipette out, and made up to the mark with diluent.
100% Spiked Solution: 1.0 mL of sample stock solution was taken into a 10 mL volumetric flask, to that 1.0 mL from each standard stock solution was pipette out, and made up to the mark with diluent.
150% Spiked Solution: 1.5 mL of sample stock solution was taken into a 10 mL volumetric flask, to that 1.0 mL from each standard stock solution was pipette out, and made up to the mark with diluent.

Acceptance Criteria:
The % Recovery for each level should be between 98.0 to 102
Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.
Robustness conditions like Flow minus (0.1ml/min), Flow plus (0.3ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25 mL Standard stock solutions were pipette out and transferred to two separate 10 mL volumetric flasks and made up with diluent. From the above solutions 0.25 mL Camylofin, 0.25 mL Mefenamic acid solutions respectively were transferred to 10 mL volumetric flasks and made up with the same diluent.

LOQ sample Preparation: 0.25 mL standard stock solutions were pipette out and transferred to two separate 10 mL volumetric flask and made up with diluent. From the above solutions 0.9 mL Camylofin and 0.69 mL Mefenamic acid solutions respectively were transferred to 10 mL volumetric flasks and made up with the same diluent.

Method validation
The final optimized chromatographic analytical method was validated as per the International Conference on Harmonization (ICH) Q2 (R2) guidelines for system suitability, Linearity, Accuracy, Precision, Limit of detection, Limit of quantitation and Robustness. Standard stock solution was prepared by dissolving 25mg of Mefenamic acid and 5 mg Camylofin in 50 mL of diluents to a final concentration of 500µg/mL and 100µg/mL. Then 1 mL stock solution is transferred into 10 mL volumetric flask and made up to the volume, mix. The final concentration of Mefenamic acid is 50 µg/mL and Camylofin is 10µg/mL.

Linearity
Standard calibration curves were generated with seven different concentrations including the LOQ by making serial volume to volume dilution of stock solution I over the range of 25-150 µg/mL. Linear calibration curves were generated between peak area and drug concentration. The linearity was examined using linear regression curve.

Accuracy
The accuracy of developed analytical method was analysed by developed method, accuracy experiments were carried out using standard addition method. Three different level concentrations (50%, 100%, and 150%) of standards were added to pre-analysed samples in triplicate. The percentage accuracy of Camylofin at each level and each triplicate were calculated and the mean of percentage accuracy (n=9) and the relative standard deviation was determined.

Precision
The precision of the developed analytical method was determined by repeatability (intraday) and intermediate precision (inter-day). Repeatability defines the use of the analytical procedure within a laboratory over a short period of time that was examined by assaying the samples during the same day. Intermediate precision was evaluated by comparing the assays on different days. SD and %RSD were determined.

Limits of detection and quantitation
Limits of detection (LOD) and limit of quantitation (LOQ) were determined from the signal-to-noise ratio. The detection limit was referred to as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was referred to as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten.

Robustness
The Robustness is one of the validation parameter, it measure of method capacity to remain unaffected by small, deliberate changes in chromatographic conditions was studied by testing the influence of small changes in the organic content of mobile phase (±10%), flow rate (±10%) and Temperature (±10%).

Degradation studies: [17]
Oxidation:
Each 1 mL of stock solution of Camylofin and 1 mL of stock solution of Mefenamic acid to two volumetric flasks, individually added 1 mL of 10% Hydrogen peroxide (H_2O_2) and mixed. The solutions were kept for 30 min at 60^0c. For UPLC study, the resultant solution was diluted to get final concentration of Mefenamic acid is 50 µg/mL and Camylofin is 10µg/mL and 10 µL was injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:
Each 1 mL of stock solution of Camylofin, and 1 mL of stock solution of Mefenamic acid to volumetric flask, added separately 1 mL of 2N Hydrochloric acid and refluxed for 30 mins at 60 ^{o}c. The resultant solution was diluted to obtain Mefenamic acid is 50 µg/mL and Camylofin is 10µg/mL and 10 µL solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:
Each 1 mL of stock solution of Camylofin, and 1 mL of stock solution of Mefenamic acid to volumetric flask added separately 1 mL of 2N sodium hydroxide and refluxed for 30 mins at 60 ^{o}C. The resultant solution was diluted to obtain Mefenamic acid is 50 µg/mL and Camylofin and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:
The standard drug solution of Camylofin and Mefenamic acid was placed in oven at 105°C for 1 hr to study dry heat degradation. For UPLC study, the resultant solution was diluted to 50 µg/mL & 10 µg/mL solution and inject each solution 10 µL into the chromatographic system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:
The drug's photochemical stability was investigated by subjecting solutions of Camylofin and Mefenamic acid at concentrations of 500 µg/mL and 100 µg/mL to UV light. The solutions were exposed to UV light in a chamber for one hour or an equivalent of 200-Watt hours/m2 in a photo stability chamber. Subsequently, for the UPLC analysis, the resulting solution was diluted to obtain Mefenamic acid is 50 µg/mL and Camylofin is 10µg/mL. Inject 10 µL of each solution into the chromatographic system, and chromatograms were generated to evaluate the sample's stability.

Neutral Degradation Studies: Stress testing under neutral conditions was studied by refluxingthedruginwaterfor1hrs at a temperature of 60º C. For UPLC study, the resultant solution was diluted to 50 µg/ml & 10 µg/ml solution and 10 µL were injected into the system and the chromatograms were recorded to assess the stability of the sample.

3. Results and discussion:

Optimized UPLC method for Camylofin and Mefenamic acid:
Chromatographic conditions:
Column dimensions: HSS 100 x 2.1 mm, 1.8 µ.
Mobile Phase: 0.1N Phosphate buffer: Methanol (55:45 v/v)
Flow rate: 0.2 mL/min
Temperature: 30 ºC
UV detector with wave length: ACQUITY TUV detector ChA 220.0 nm

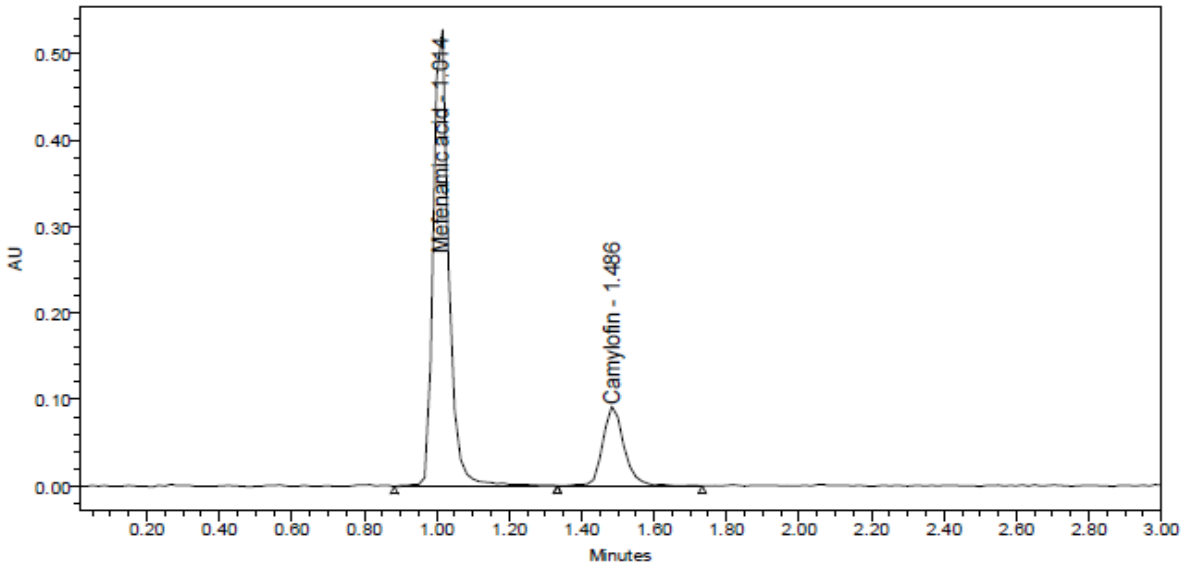

Figure 3 : Optimised Standard chromatogram for Camylofin and Mefenamic acid

Table 1 : System suitability for Camylofin and Mefenamic acid

	Peak Name	RT	Area	USP Plate Count	USP Resolution	USP Tailing
1	Mefenamic acid	1.014	1582377	2388.3		1.1
2	Camylofin	1.486	382613	3560.9	6.1	1.4

Here system suitability values obtained for the Camylofin and Mefenamic acid found to be within the limitations therefore the following Parameters were used for the method validation as per the ICH guidelines.

Method validation:
Specificity:

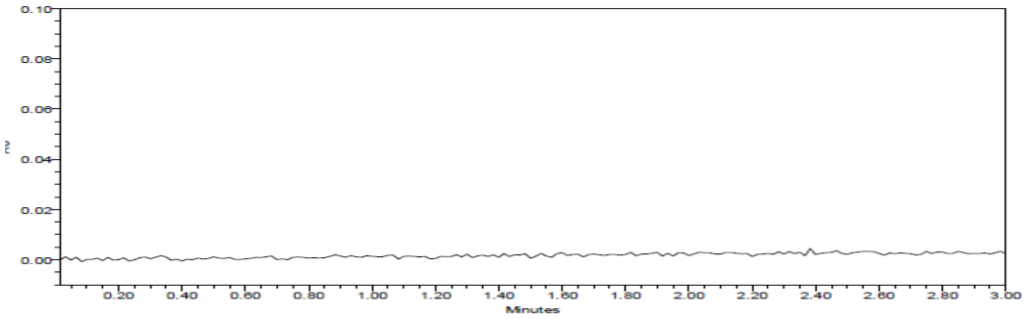

Figure4 blank Chromatogram

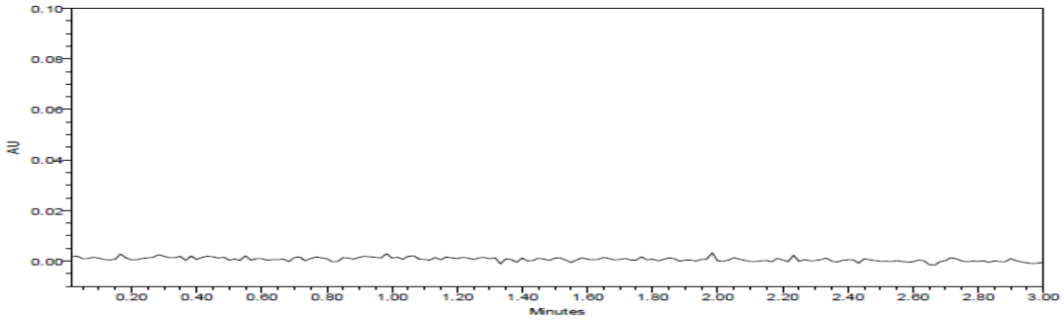

Figure5 Placebo Chromatogram

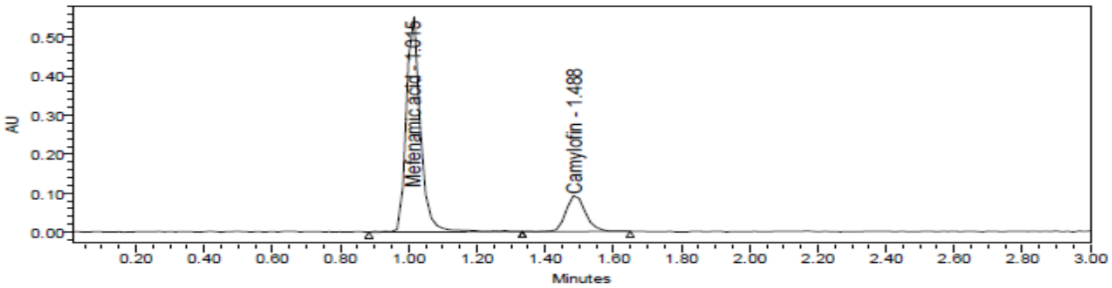

Figure6: Standard Chromatogram of Camylofin and Mefenamic acid

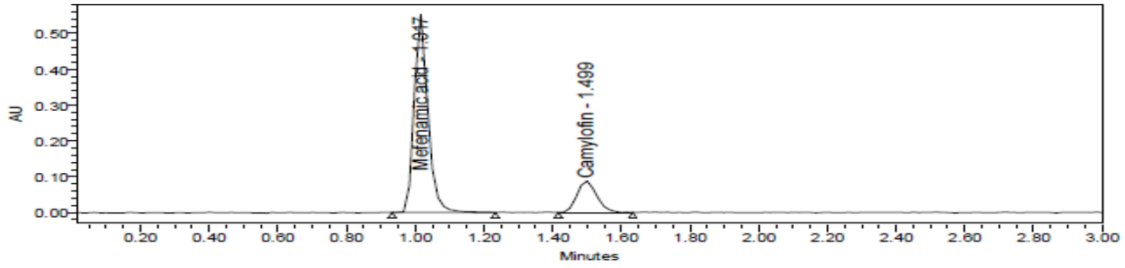

Figure7 Sample Chromatogram of Camylofin and Mefenamic acid

Discussion: Retention time of Camylofin and Mefenamic acid was 1.488 min and 1.015min. We did not find and interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific.

Precision:
System Precision: Six times injected one working sample solution of 50 ppm and 10 ppm and tabulated peak areas as below table 2 and calculated the %RSD, found to be 0.9% RSD of Mefenamic acid and 0.9% of Camylofin.

Table 2 System precision data

S.No	Peak Area of Mefenamic acid	Peak Area of Camylofin
1	1554803	383094
2	1569031	380533
3	1566154	373827
4	1561828	377080
5	1582377	378613
6	1592286	381910
AVG	1574335	379176
STDEV	13829.8	3405.6
%RSD	0.9	0.9

Method precision: Six working sample solutions of 50 ppm and 10 ppm are injected in to chromatographic system and tabulated peak areas as below table 3 and calculated the %RSD, found to be 0.7% RSD of Mefenamic acid and 0.5% of Camylofin.

Table 3 Method precision data

S. No	Peak Area of Mefenamic acid	Peak Area of Camylofin
1	1555298	380797
2	1567364	378626
3	1567952	378771
4	1567630	377907
5	1587748	378084
6	1564501	382365
AVG	1568416	379425
STDEV	10612.0	1771.7
%RSD	0.7	0.5

Intermediate precision: Six working sample solutions of 50 ppm and 10 ppm are injected on the next day of the preparation of samples and tabulated peak areas as below table 4 and calculated the %RSD, found to be 0.6% RSD of Mefenamic acid and 0.5% of Camylofin.

Table 4 Intermediate precision data

S.No	Peak Area of Mefenamic acid	Peak Area of Camylofin
1	1541071	377477
2	1551346	377363
3	1542150	377516
4	1536862	381738
5	1551156	379456
6	1560929	378506
Average	1547252	378676
STDEV	8845.9	1704.1
%RSD	0.6	0.5

LINEARITY:

Demonstrated the linearity of assay method, inject 6 standard solutions with concentrations of about 2.5ppm to 15ppm of Camylofin & 12.5ppm to 75ppm of Mefenamic acid. Plot a graph to concentration versus peak area. Slope obtained was y = 30948x +6750.5 (Mefenamic acid) and y = 37722x + 1092.5 (Camylofin) and both drugs correlation co-efficient were found to be 0.999.

Table 5 Linearity Concentration and Response

Mefenamic acid		**Camylofin**	
Conc (µg/mL)	**Peak area**	**Conc (µg/mL)**	**Peak area**
0	0	0	0
12.5	384125	2.5	94546
25	764645	5	189633
37.5	1208841	7.5	286288
50	1577417	10	378165
62.5	1920948	12.5	477165
75	2315127	15	562257

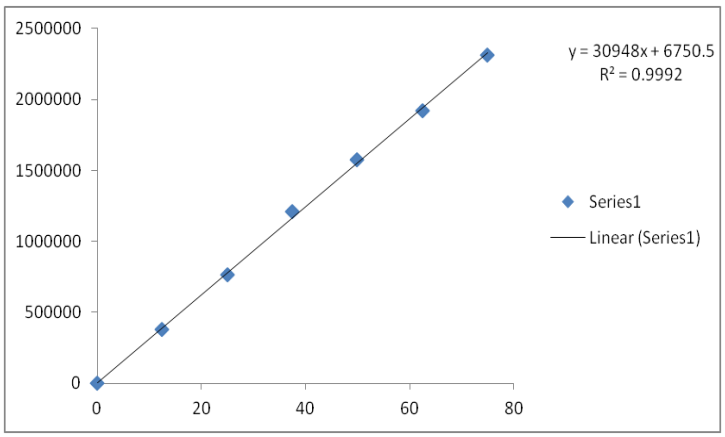

Figure 8 Linearity Plot of Mefenamic Acid

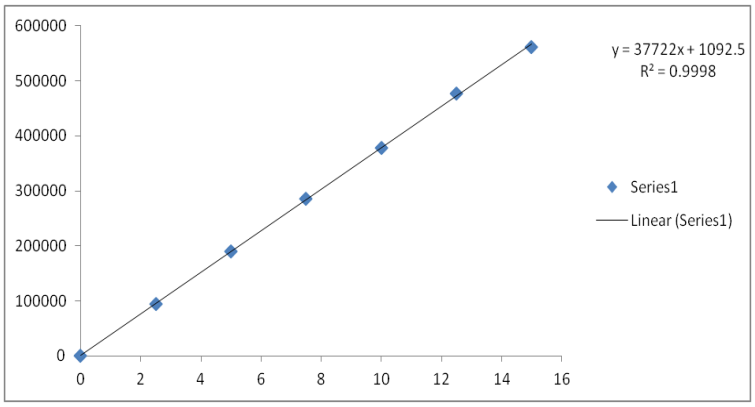

Figure 9 Linearity Plot of Camylofin

Accuracy:

Three concentrations of 50%, 100%, 150% are injected in a triplicate manner and %Recovery was calculated as 100.44% of Mefenamic acid and 99.77% of Camylofin and chromatograms were shown in fig 6.11-6.13.

Table 6 Accuracy data of Mefenamic Acid

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	25	24.95	99.82	100.44%
	25.02	100.11	99.84	
	24.97	99.88	99.86	
100%	50	50.31	100.64	
	50	50.62	101.25	
	50	49.82	99.65	
150%	75	76.03	101.38	
	75	75.48	100.64	
	75	75.42	100.57	

Table 7Accuracy data of Camylofin

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	5	5.07	101.30	**99.77%**
	5	4.91	98.21	
	5	4.95	99.07	
100%	10	9.99	99.94	
	10	10.06	100.57	
	10	9.88	98.81	
150%	15	15.11	100.73	
	15	14.92	99.50	
	15	14.97	99.77	

LOD: Detection limit of the Camylofin in this method was found to be 0.03µg/ml of Camylofin and 0.08µg/ml of Mefenamic acid.
LOQ: Quantification limit of the Camylofin in this method was found to be 0.08µg/ml of Camylofin and 0.23 µg/ml of Mefenamic acid

Table 8 LOD and LOQ Values for the Camylofin and Mefenamic acid

S. No	Name of the Drug	LOD (µg/ml)	LOQ (µg/ml)
1	Camylofin	0.03	0.08
2	Mefenamic acid	0.08	0.23

Robustness: Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions is calculated.

Table 9 Robustness Data

Parameter	%RSD of Mefenamic Acid	%RSD of Camylofin
Flow Minus	1.0	1.6
Flow Plus	0.5	1.4
Mobile phase Minus	1.5	1.2
Mobile phase Plus	0.5	1.1
Temperature minus	1.2	1.5
Temperature plus	0.7	1.9

ASSAY OF MARKETED FORMULATION
The marketed sample of the Anaforton MF (manufactured by Abbott pharma) with composition of Camylofin 50 mg and Mefenamic acid 250 mg. Standard solution and sample solution were injected separately into the system and chromatograms were recorded. Drug amount in sample was calculated using below mentioned formula.
Calculation Formula: Assay (%w/w)

$$\frac{\textbf{Sample Area} \times \textbf{test dilition} \times \textbf{Sample Purity} \times \textbf{1}}{\textbf{Standard Area} \times \textbf{Standardd Dilution} \times \textbf{Standard Purity} \times \textbf{Label Claim}} \times \textbf{100}$$

Dilution factor for Camylofin: 50
Dilution factor for Mefenamic Acid: 250

Table 10 Assay of Formulation

Sample No	Area of the Standard Mefenamic acid Sample	Area of the Mefenamic acid Sample	%Assay of Mefenamic Acid	Area of the Standard camylofin	Area of the Camylofin Sample	%Assay of Camylofin
1	1554803	1555298	**98.49**	383094	380797	**100.33**
2	1569031	1567364	**99.26**	380533	378626	**99.76**
3.	1566154	1567952	**99.30**	373827	378771	**99.79**
4.	1561828	1567630	**99.28**	377080	377907	**99.57**
5.	1582377	1587748	**100.55**	378613	378084	**99.61**
6.	1592286	1564501	**99.08**	381910	382365	**100.74**
AVG	1574335	1568416	**99.33**	379176	379425	**99.97**
STDEV	13829.8	10612.0	**0.672**	3405.6	1771.7	**0.47**
%RSD	0.9	0.7	**0.7**	0.9	0.5	**0.5**

The assay values for the Camylofin and Mefenamic acid were found to be 99.97 % 99.33.

4. Conclusion:

A simple analytical and rugged UPLC method was developed for the quantification of Camylofin and Mefenamic acid. The sophisticated method successfully met the system suitability criteria. A validated stability-indicating UPLC method was established for Camylofin and Mefenamic acid, enabling the effective separation of the drug substance from its degradation products. Various stress conditions were applied in degradation studies on the drug. No degradation products were detected during peroxide hydrolysis, neutral hydrolysis, thermal degradation, and UV degradation. However, a notable degradation product was observed

in 2N HCL and 2N Base hydrolysis. The results were obtained by validating the analytical method aligned with the limits outlined in ICH guidelines.

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