Introduction and Literate Review

1.1 - Definition

Validation of analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications (USP,37).

Method validation is defined as the process of proving that an analytical technique is acceptable for the intended use and this is an important requirement for analytical purpose. Validation is done according to the guidelines of ICH and FDA (Aulast, 2014).

The word validation originated from the Latin word validus meaning strong, and suggests that something has been proved to be true, useful, and of an acceptable standard(Araujo,2009).

Method validation can be defined: as the process of proving that a particular developed analytical method is acceptable for its intended use. Validation is an important requirement in the practice of an analytical process. Method validation is a continuous process, and the final goal of validation of an analytical method is to ensure that every future measurement in routine analysis will be close enough to the unknown true value for the content of the analyte in the sample(Gonzalez *et.al*, 2007).

The objective of validation of an analytical procedure is: to demonstrate that it is suitable for its intended purpose.

1.2 -Type of Analytical Procedure to be Validated

- Identification tests: The identification tests are intended to ensure the identity of an analyte in a sample this is achieved by comparison of property of the sample to that of a reference standard.
- Quantitative tests for impurities
- Limit tests for the control of impurities:Testing for impurities can be either a

quantitative test or a limit test for the impurity in a sample.

- Quantitative tests of the active moiety in sample of drug substance or other selected component in drug product:Assay procedure is intended to measure the analyte present in a given sample(ICH, 1995).

Typical validation Characteristic which should be considered are Listed **below:**

- Accuracy: It is the closeness to the true value, measured by % recovery of sample spikes or % error in the analysis of a reference sample.
- Precision: The degree of agreement between replicate analyses of a homogenous sample, usually measured as the relative standard deviation (RSD) of a set of replicates.

  The measured standard deviation can be subdivided into three categories: repeatability, intermediate precision, and reproducibility. (ICH, 1995).

  Repeatability is obtained when one operator using one piece of equipment over a relatively short time-span carries out the analysis in one laboratory. At least five or six determinations at two or three different concentrations should be done and the RSD calculated (Putheti *et al*, 2008).

  Repeatability expresses the precision under the same operating conditions over a short interval of time (ICH, 1995).

  Intermediate precision obtained when one operator using the same method and equipment through different days. Reproducibility: Reproducibility expresses the precision between laboratories. From the reproducibility standard deviation it is useful to calculate the 'reproducibility limit R', which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under reproducibility conditions, is **significant.**
- Specificity:Is the ability to assess unequivocally the analyte in the presence

of components which may be expected to be present (include impurities, degradants and matrix)

- Detection limit (LOD): The limit of detection (LOD) is the lowest concentration of the analyte in a sample that can be detected but not necessarily quantified(Putheti *etal* , 2008).
- Quantitation limit (LOQ): It is the concentration level above which the concentration can be determined with acceptable precision and accuracy (Swartz, *et.al* ,1997).
- Linearity: The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytesin samples within a given range (Hubert *et al*., 2007).

  Linearity may be demonstrated directly on the test substance by preparing a series of dilution of a standard stock solution or by using separate weighing of synthetic mixtures of the test product components, using the proposed procedure (Putheti *et al*., 2008).
- Range: Performed according to international conference harmonization guide lines (ICHG). Interval between the upper and lower levels of analyte (including theses level) that have been demonstrated to be determined with the suitable level of precision, accuracy and linearity (USP, 37).
- Robustness: The robustness of an analytical procedure is a measure of its capacity to remain unaffected by changes occur, but deliberate variations in method parameters and provides an indication of its reliability during normal usage (Heyden *et al*., 2001).

Table (1.1): Data elements required for validation

Analytical Procedure	Identification	Determinatio	Quantitation	Assay

Characteristics			n of impurities	limit	Dissolution Potency /content
Accuracy		_	_	+	+
Precision	Repeatability	_	_	+	+
	Interm. precision	_	_	+(1)	+(1)
Specificity (2)		+	+	+	+
Detection limit		_	+	_(3)	_
Quantitation limit		_	_	+	_
Linearity		_	_	+	+
Range		_	_	+	+

(-) signifies that this characteristic is not normally evaluated.
(+) signifies that this characteristic is normally evaluated.

1) Reproducibility has been performed, intermediate precision is not needed
2) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure.

3) May Be needed in Some Case (ICH, 1995).

1.3 - Strategy For Analytical Method Validation

The validation of a specific method must be demonstrated through laboratory experiments by routinely analyzing samples. The preparation and execution have to follow a validation protocol, meets criteria such as ease of use, ability to be automated and controlled by computer systems, costs per analysis, sample throughput, turnaround time, environmental, health, and safety requirements. Successful acceptance of the validation parameters and performance criteria by all involved parties requires a cooperative effort of several departments, including analytical development, quality control, regulatory affairs, and

individuals requiring the analytical data. The operating procedure or Validation Master Plan (VMP) must clearly define the rules and responsibilities of each involved department in the validation of analytical methods The validation experiments should be carried out by an expert analyst to avoid errors due to lack of experience. The analyst should also be very familiar with the technique and operation of the instrument. Before an instrument starting validating a method, its performance specifications must be verified by using generic chemical standards. Satisfactory results for a method can be only obtained with equipment that is performing well. A special attention must be paid to equipment characteristics which are critical for the method. For instance, if the detection limit is critical for a specific method, the specification of the instrument for baseline noise and some detectors must be verified

- Develop a validation protocol, an operating g procedure, or a validation master plan for the validation.
- For a specific validation project, define owners and responsibilities.
- Develop a validation project plan.
- Define the application, purpose, and scope of the method.
- Define the performance parameters and acceptance criteria.
- Define validation experiments.
- Verify relevant performance characteristics of equipment
- Qualify materials, e.g. standards and re agents for purity, ac curate amounts, and sufficient stability.
- Perform pre-validation experiments
- Adjust method parameters and/or acceptance criteria if necessary
- Perform full internal (and external) validation experiments
- Develop standard operation procedures for executing the method in the routine

- Define criteria for revalidation
- Define type and frequency of system suitability tests and/or analytical quality control checks for the routine
- Document validation experiments and results in the validation report. (ICH, 1995).

1.4 - High Performance Liquid Chromatography

High performance liquid chromatography is a powerful tool in analysis.

Drip through a column under gravity, it is forced through under high pressure of up to 400 atmospheres. It uses a very much smaller particle size (interactions between the stationary and mobile phase).

Mixed compounds can be separated to individual compound by absorption, distribution, ion exchange and size exclusion between sample, mobile phase and stationary phase.

HPLC can be used in separation and analysis of non-volatile or thermally-unstable compounds. It can also be used in identification and quantification of different chemical components.

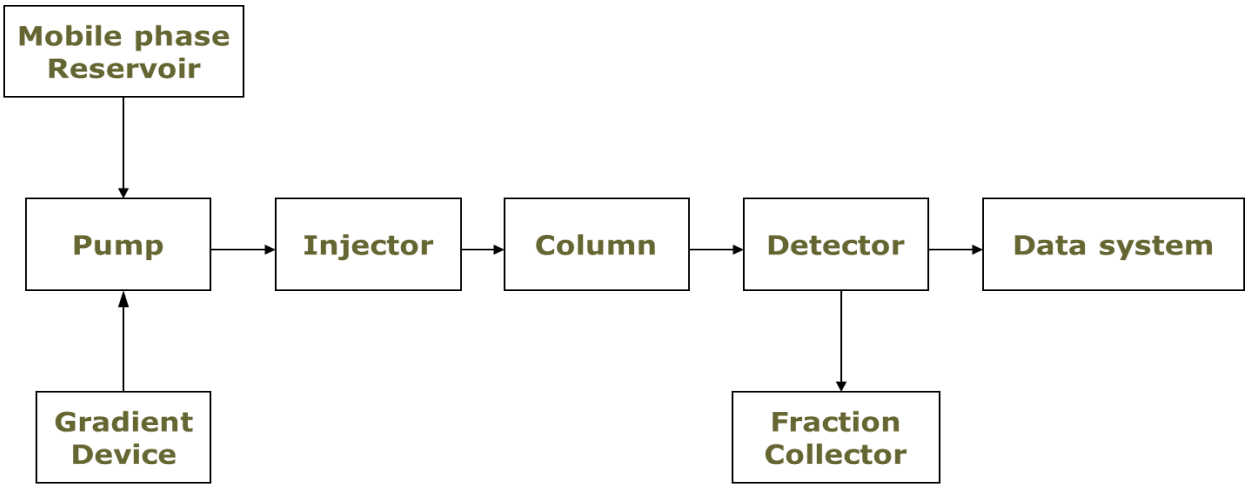


Figure 1.1: HPLC System configuration

HPLC basic components

- Solvent Delivery System (Pump):

Deliver the solvent (mobile phase) from the solvent reservoir to the injector

Advantage of pump

-Stability of Flow rate and Pressure

- Easy to use, Use of variable solvents
- Pulse elimination system
- Isocratic and Gradient mode can be used
- Endurance

Mode of pump operation

- Isocratic mode

  Maintain the solvent composition during analysis.

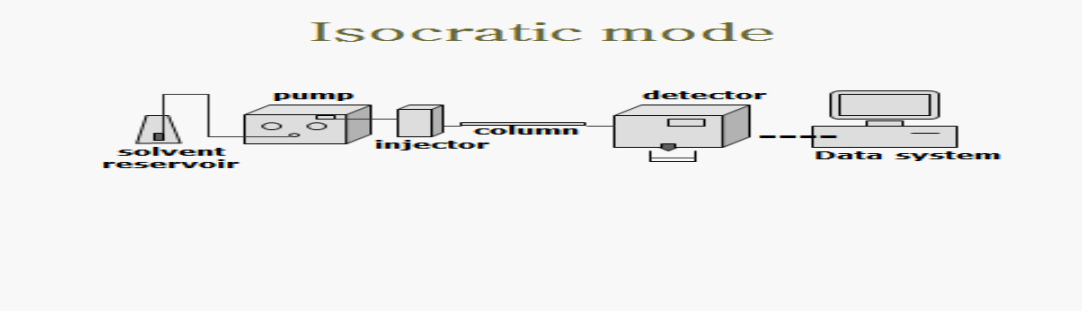


Figure 1.2: Isocratic mode

- **Gradient mode**

  Change the solvent composition according to time during analysis

Single pump: Low pressure

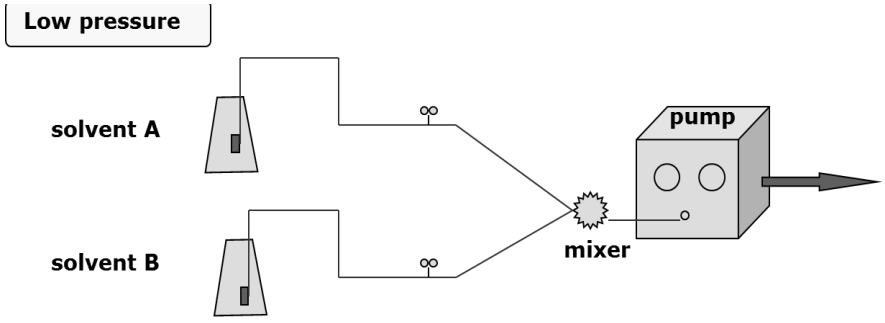


Figure 1.3: Gradient mode single pump

- **Injector:**

Loading sample through the mobile phase, so the sample move to the column.
Type of Injector
- Rheodyne injector (Valve injector)
- Automatic injector

- **Column:**

Column separates mixed compounds to individual compound.
Selection of column depend of
- Packing Material
- Particle Size
- Shape
- Pore Size

Separation Method
Normal phase: stationary phase is polar and mobile phase is non-polar.
Reverse phase: stationary phase is non-polar and mobile phase is polar (most commonly used)
Ion exchange: differences in ionization
ColumnStorage:
Column store with solvent that does not interact with packing material.

- **Detector**

Function: Output electrical signal proportional to sample amount.

Types of detector:

Optical detector
1. UV/Visible Detector
2. Fluorescence Detector
3. Refractive index Detector
4. Evaporative Light Scattering Detector

Electrochemical Detector
1. Conductivity Detector
2. Electrochemical Detector

UV/Visible Detector

Light of specific wavelength pass through the cell, some parts are absorbed, the others are transmitted
Specific sample has high absorbance to specific wavelength
Amount of absorbed light (A) is proportional to the concentration of sample

A =εbc (Lambert- Beer's Law)
A: Absorbance
ε : Mol absorbance factor
b: Cell path length
C: Concentration of sample

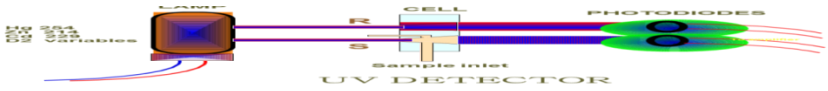


Figure 1.4: UV/Visible Detector

- **Mobile phase**

- HPLC grade (Water: 18 mega ohm)
- Low Viscosity
- Miscibility of solvents
- Do not change the stationary phase
- Solubility
- UV cut off, refractive index: Low

Advantages of HPLC

It is quick, automated and highly accurate.
Speed: the process can be completed in roughly 10 to 30 minutes.
Efficiency: it delivers high resolution.
Accuracy: it is accurate and highly reproducible.
Disadvantages of HPLC

Cost requiring large quantities of expensive organics and solvents.
Complexity is relatively easy to use; it can be complex to troubleshoot problems or to develop new methods.
Sensitivity and Resolution:

Versatile and extremely precise when it comes to identifying and quantifying chemical components
Does have low sensitivity for certain compounds, and some cannot be detected as they are irreversibly adsorbed. Volatile substances are better separated by GC (Satinder *et al*., 2005).

1.4 -Mefenamic Acid

Mefenamic acid is a non-steroidal anti-inflammatory drug (NSAID), its 2-

[(2,3-dimethylphenyl) amino] benzoic It is metabolized to 3-

Hydroxyl methyl mefenamic acid and further oxidation to a 3-carboxy mefenamic acid may. Used to treat from mild to moderate pain including menstrual pain (not more than 7 days), rheumatoid arthritis, osteoarthritis, inflammation and fever. It may cause an increased risk of serious and sometimes fatal heart and blood vessel problems (e.g., heart attack, stroke), high risk at patient with heart problems or long term use so it doesn't before or after bypass heart surgery. Also may increase risk of serious and sometimes fatal stomach ulcer and bleeding (high risk in elderly).

Some characteristics of mefenamic acid:

Mefenamic acid is a problematic drug in granulation, tableting, and dissolution due to its poor solubility, hydrophobicity, and tendency to stick to surfaces.

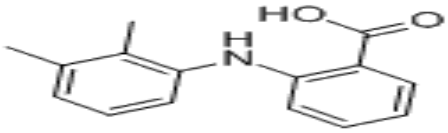


Figure 1.5: The Chemical structure of mefenamic acid.

Molecular formula: $C_{15}H_{15}NO_2$.

Molecular weight: 241.29.

Melting point: 230 °C.

Color: Light Yellow Solid.

Storage temperature: 15-30 °C. (Senthilkumar *et al*., 2010)

1.5 - Justification of The Study:

Literature survey revealed few analytical methods that have been reported concerning the development and validation of mefenamic acid. To the best of our knowledge there is no an HPLC method reported using methanol and

acidified water as mobile phase for the method development and validation of mefenamicacid.Hence an attempt has been made to develop a simple, precise, reliable, and sensitive indicating RP-HPLC method for development of mefenamic acid. The proposed method is to be validated according to ICH guidelines.And the Ophelia method of analysis of mefenamic acid (B.P and USP method) is titration method and the titration method in some case not accurate for analysis

1.6- Previous Study

Several studies were conducted to develop and validate mefenamic acid, either its present as the only active ingredient or combined with different active ingredient using reverse phase HPLC using different mobile phases and different detector.

- A simple assay method by HPLC was developed and validated for mefenamic acid tablet (Ponstan) ,were performed using HPLC- Uv-Visble at 275 nm on a reverse phase column.

  A binary mobile phase; A: 0.1% formic acid in deionised water, B: 100% acetonitrile. The validation aspects were selectivity, linearity, precision, accuracy and quantification limit. Linearity, 5-250 mgL-1, provided determination coefficients (R2) of 0.9995, and proved precise since the RSD% was less than 5% for three replications analysis. The recoveries obtained ranged from 99% to 108%. The retention time and drug content of mefenamic acid was 3.9 min and 97%, respectively.

  The mobile phase chosen for analytical method validation was 100% ACN.

This method is precise, accurate and very simple.

There is no significant difference between the results obtained with the mobile phase (100% ACN and ACN/DIW, 90: 10). However, 100% CAN provide better separation and shorter time, 100% methanol produced too late peak with area lower than last two mobile phases.

Flow rate was optimised with (0.8, 1.0, 1.5 and 2 mL/min). At 0.8 mL/min, there is no peak appeared with 3 replications.

In case of wavelength, there is no significant difference among the three wavelengths. While, volume injection appeared significant difference between 5 and 20 µL, this is related to the amount of analyte.

The results of recovery achieved 80-110 %.

The results show that the HPLC method presented here can be considered suitable for the analytical determination of mefenamic acid in tablets, being linear in the concentration range used, high selectivity and specificity, high precision and adequate accuracy at the concentrations studied. Statistical analysis give significant differences at particularly optimize aspect especially flow rate.(Fouad Fadhil,*et.al* 2014).

- The solvents methanol and water were used as a mobile phase in the ratio of 70:30 (v/v). The retention time of mefenamic acid was 5.80 min at the flow rate 1.25 ml/min. The maximum peak area was optimized at 370 nm. Statistically the limit of detection and limit of quantification were calculated 0.03 and 0.09 ppm by reverse phase high performance liquid chromatography (RP-HPLC) and 0.3 and 0.9 ppm by UV-Spectrophotometer, respectively. Good results were obtained with respect to linearity R2=0.993 by RP-HPLC and R2=0.996 by UV-Spectrophotometer. The inter-day and intra-day mean recoveries by RP-HPLC statistically were calculated 97.33 % and 97.66 % and for UV-Spectrophotometer 98.56 % and 97.13 %.

There were different ratios of the solvent of methanol and water (10:90, 20:80, 30:70, 40:60) tested at the ambient temperature 25 0C. The mobile phase methanol/water in the ratio of 70:30 (v/v) was given suitable retention time and better resolution.

The range of linearity of mefenamic acid was 5-100 ppm for both methods (RP-HPLC and UV-VIS Spectrophotometer). By Both methods (RP-HPLC and UV-VIS Spectrophotometer) the different parameters regression coefficient (R2), Limit of Detection (LOD) and Limit of Quantification (LOQ) were statistically calculated 0.993, 0.996, 0.03, 0.3, 0.09 and 0.9(Raju *et.al* ,2014).

- Mobile phase with Isocratic elution at a flow rate of 1.0ml/min was employed, the mobile phase consisted of: acetonitrile: Monobasic $[NH_4]_3PO_4$ buffer (0.05 M): tetra hydrofuran with the ratios 46:40:14 (V/V/V). The UV detection wavelength was 254 nm and 20 µl. The sample was injected. The retention time was 10.591 min.

  For the intermediate precision a study carried out on two consecutive days indicated a RSD of 0.0754. This indicates good method precision.

  The stability of MFNC is determined by storing the solutions at ambient temperature (27±100C). The data were compared with freshly prepared samples. They were stable for 48 hrs, as during this time the results did not decrease below 98%. This denotes that MFNC is stable and standard and sample solutions for at least 2 days at ambient temperature.

  The proposed method is simple, rapid, accurate, precise and specific. Its chromatographic run time of 15 min allows the analysis of a large number of samples in short period of time. Therefore, it is suitable for the routine analysis of MFNC in pharmaceutical dosage form. (Padmalatha,*et.al* , 2014)

- A simple, RP-HPLC stability indicating method was developed for determination of mefenamic acid in pharmaceutical formulations and its degradation products using mobile phase containing mixture of Buffer :

Acetonitrile + THF in the ratio of 55:45 v/v at a flow rate of 1.0 ml/min, retention time was 18.253 min with wavelength of 285 nm.

Linearity and Range was observed to be linear over 25-125%.

The proposed method was validated by testing its linearity, accuracy, and precision, limits of detection, and quantitation, and specificity. The method proved able to separate the peaks of active pharmaceutical ingredients (APIs) from the degradation products (produced during forced degradation studies). It is also clear from the chromatograms that both the active ingredient peaks under all the stress conditions were free from any sort of degradation impurities. These results allow us to conclude that the method can be successfully used for all stability and validation studies. (Dhumal,*et.al*., 2016).

1.7 - Objectives

To develop and validate a new analytical method according to ICH guidelinesfor mefenamic acid in tablet dosage form using reverse phase high performance liquid chromatography and methanol and acidified water as mobile phase .

Materials and Methods

2.1- Chemicals and Reagents

All chemical and reagents used were of analytical grade or HPLC grade and were used without any further purification.

- Standard Mefenamic acid used and mefenamic acid tablets were from Wafrapharma industry co. ltd pharmaceutical factory.
- Methanol HPLC grade, (Spain) and
- Phosphoric acid (India).

2.2 –Methods and chromatographic conditions

The analysis was carried out using high performance liquid chromatography (HPLC) system, UV/V is spectrophotometer detector. The analytical column was phenomenx® C 18, 4.6 mm, 2.5 mm . Mobile phase: methanol: acidified distilled water (90: 10) was used, the flow rate was 1.0 ml/minute, UV vis detection was set at 275 nm, 20 µl of the sample was injected into the HPLC and the data was processed.

Preparation of Mobile Phase

Preparation of acidified distilled water

Few drops of phosphoric acid was added to distilled water until pH reach 3.3, pH meter (Jenway) device was used to measured acidity of distilled water.

Exactly 900 ml was taken from methanol and put in 1000ml volumetric flask, then 100 ml of acidified distilled water which was added to volumetric flask, then mobile phase was placed in magnetic stirrer (BADDELIN electronic) for 5 minutes, then it was transferred to filter through filtration system.

Preparation of stock solution:

100mg of standard mefenamic acid was weighed in Petri dish and placed in a beaker, then 20ml of methanol was added to dissolved it, the solution was

placed in a sonictor for 10 minutes, then it transferred to 100ml volumetric flask and completed to the mark by mobile phase with concentration of 1000μg/ml.

Preparation of serial dilution:

0.25ml of stock solution was taken by micropipette and transferred to 50 volumetric flask and the solution was completed to the mark to give concentration of 5μg/ml.

0.5 ml of stock solution was taken by pipette and transferred to 50ml volumetric flask and the solution was completed to mark to give concentration of 10μg/ml.

0.75 ml of stock solution was taken by pipette and transferred to 50ml volumetric flask and the solution was completed to mark to give concentration of 15μg/ml.

1.0ml of stock solution was taken by pipette and transferred to 50ml volumetric flask and the solution was completed to mark to give concentration of 20 µg/ml.

1.25ml of stock solution was taken by pipette and transferred to 50ml volumetric flask and the solution was completed to mark to give concentration of 25µg/ml.

Calibration curve was plotted in the y axis the peak area and in the x axis the concentration and linear line was developed.

The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it stated in ICH guidelines. The method was validated for linearity, precision, recovery (accuracy), robustness, and specificity.

Standard calibration curve was plotted with 5 concentrations in the range of 5μg/ml to 25μg/ml prepared in triplicates to test linearity. The peak area of mefenamic acid was plotted against the concentration to obtain the calibration

graph.

Linearity is the ability of mefenamic acid to give a propositional relationship between the concentration and area of mefenamic acid and it give linear relationship form 5 µg /ml to 25µg/ml.

Serial dilution form 5µg/ml to 25µg/ml was injected and plotted against area which every concentration gave it and a linear relationship was appearing.

Precision was studied with respect to both repeatability and intermediate. Repeatability was calculated from five replicate injections of freshly prepared mefenamic acid. The experiment was repeated by assaying freshly prepared solution with different concentration at different days to determine intermediate precession.

Accuracy was tested by analyzing sample of mefenamic acid at four different levels using different concentrations. The results were expressed as percentage of mefenamic acid recovered in the samples.

The different excipients include, lactose, Mg stearate, MCC and talcum powder in the tablets was examined to know if this method is specific and accurate.

Few tablets were crushed and transferred in a flask, 10 ml of mobile phase was added and the flask was placed in asonicater for 20 minutes.

The solution was injected to HPLC device until each excipient appear with it specific peak.

Results and Discussion

As mentioned previously, the main aim of this study is to develop method for mefenamic acid using reverse phase column HPLC and validated to ensure it's reliable, simple, validated and accurate.

Linearity: the ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range.

Five points graph was constructed covering concentrations from 5 µg /ml to 25 µg/ml. linear relationship was found between the peak area signal of mefenamic acid and mefenamic acid concentration.

Acceptance Criteria: linearity regression coefficient must be more than 0.999.

Serial dilution from 5/ µg ml to 25 µg/ml were conducted and shown in figures 3.1, 3.5.

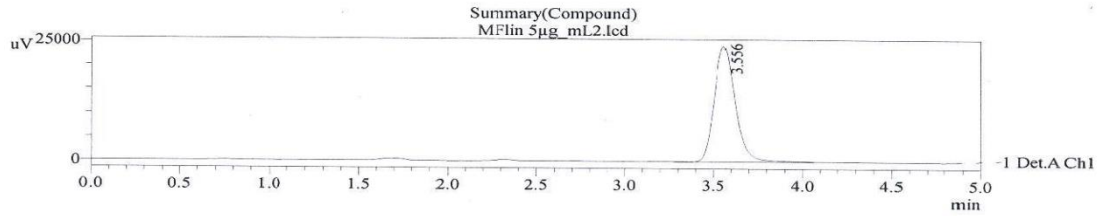
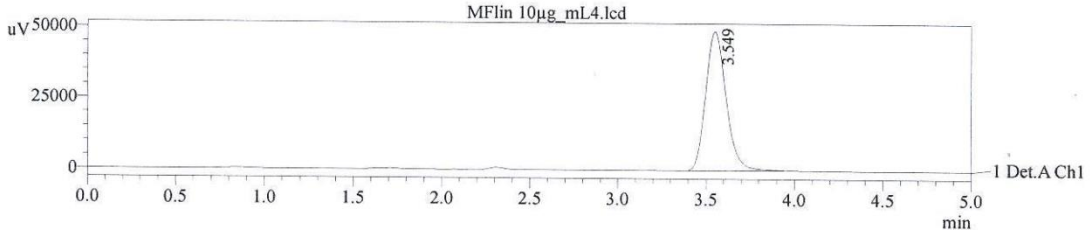


Figure 3.1 showing chromatogram of 5μg/ml



Min

Figure 3.2 showing chromatogram of 10μg/ml

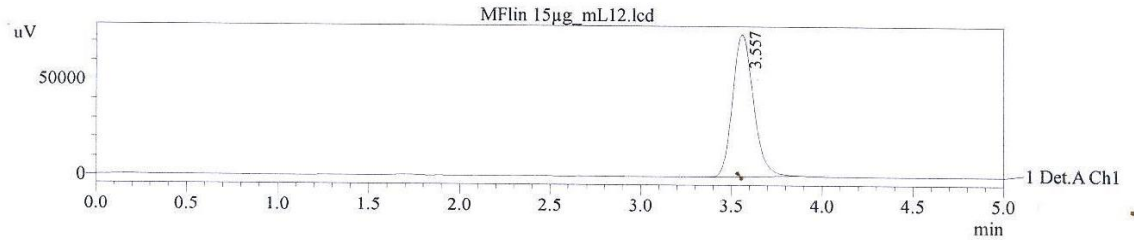


Figure 3.3showing chromatogram of 15μg/ml

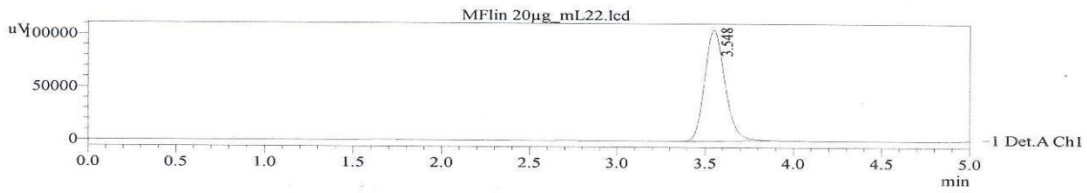


Figure 3.4 : showing chromatogram of 20µg/ml

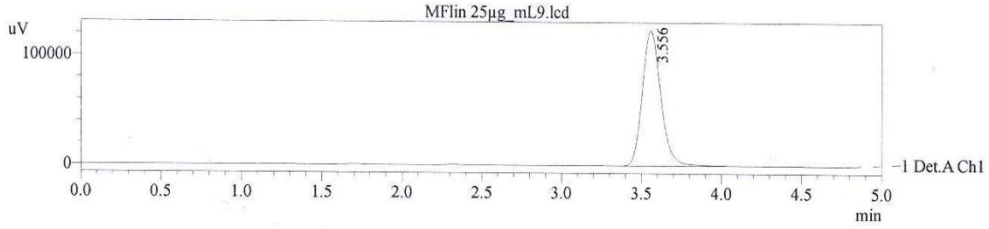


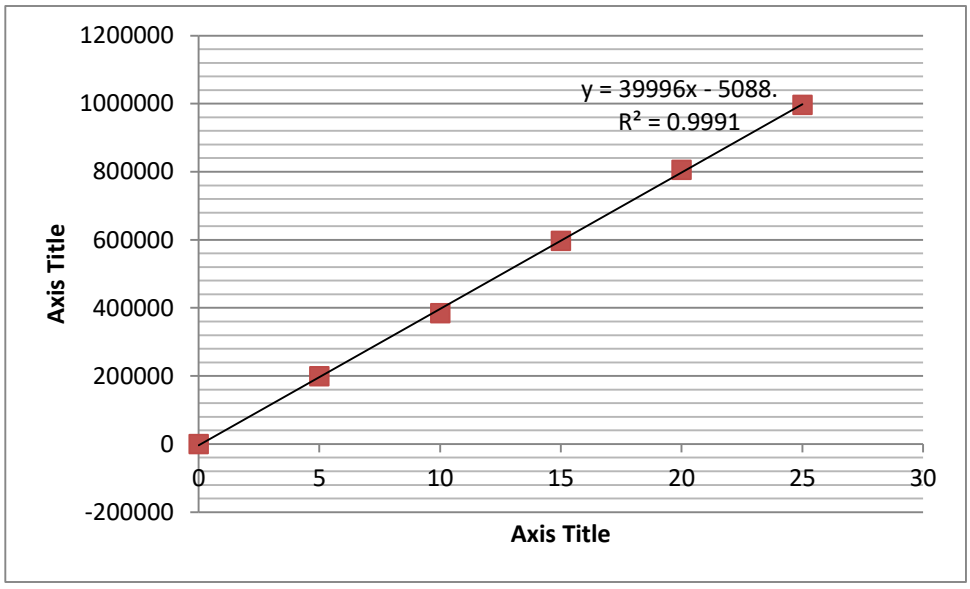
Figure 3.5 :showing chromatogram of25 µg /ml

Serial dilution was prepared and the diluted concentration was plotted against peak area and results was shown in table 3.1

Table 3.1: it Showing Concentration of Mefenamic acid from 5 to 25µg/mg

Title	Area	Height	Concentration
Mefenamic acid 5µg/ml	199141	24203	5.000
Mefenamic acid 10µg/ml	394472	48971	9.981
Mefenamic acid 15µg/ml	597036	74702	15.038
Mefenamic acid 20µg/ml	836618	104788	20.486
Mefenamic acid 25µg/ml	996196	123616	24.666
Average	604693	75256	15.034
%RSD	53.318	53.601	52.450
Maximum	996196	123616	24.666
Minimum	199141	24203	5.000
Standard Deviation	322408	40338	7.885

Area



µg/ml

Figure 3.6: The Linear Relationship between Concentration and Area of mefenamic acid ($R^2 = 0.9991$)

Table 3.2: Showing the Results of Calibration Curve of Mefenamic Acid

Number	Concentration	Area
1	5.000µg/ml	199141
2	10.000 µg/m	394472
3	15.000 µg/ml	597035
4	20.000 µg/ml	836617
5	25.000 µg/ml	996196

Slope:442145

Mefenamic acid from 5 to 25μg/ml gave a linear relationship.

We chose 15 μg/ml to be the 100% concentration and through calculation we ensure it's the 100% concentration.and the correlation coefficient was found

$R^2 = 0.9991$

Limit of detection

It is the lowest concentration of the analyte in a sample that can be detected but not necessarily quantified.

DL= 3.3 α/S

α=Standard deviation from the high concentration (120 %)=5624

S= the slope of calibration curve

LOD was found to be 0.041975

Limit of Quantification: \

It is the concentration level above which the concentration can be determined with acceptable precision and accuracy.

QL=10 α/S

LOQ was found to be 0.12719

Precision

The degree of agreement between replicate analyses of a homogenous sample, usually measured as the relative standard deviation (RSD) of a set of replicates. The sample was analyzed three consecutive days with different concentration to measure repeatability and intermediate precision.

Acceptance Criteria: % of relative standard deviation > 2 %

Figures 3.7 -3.15Shows Precision Chromatograms at the First Day

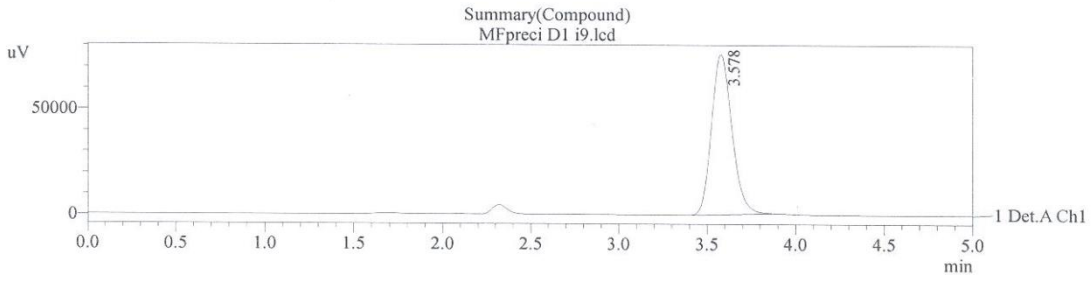


Figure 3.7showing the precision chromatogram at first day(injection 1)

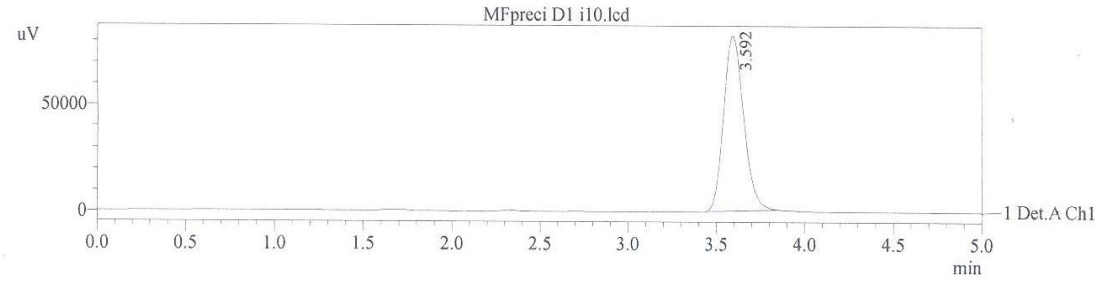


Figure 3.8 showing the precision chromatogram at first day (injection 2)

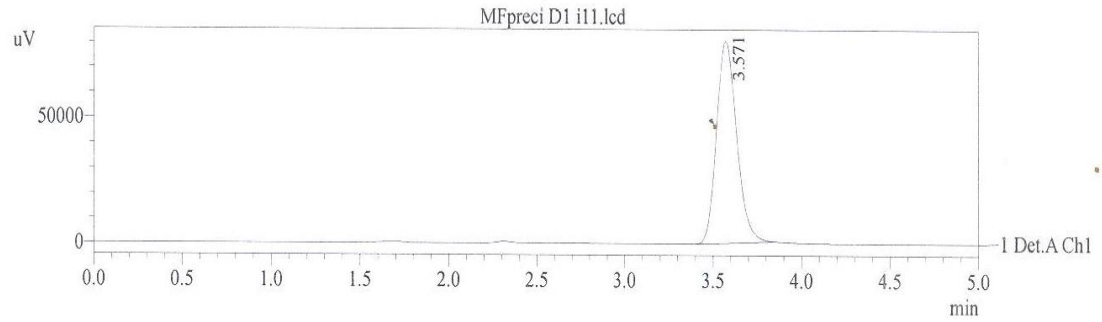


Figure 3.9 :showing the precision chromatogram at first day(injection 3)

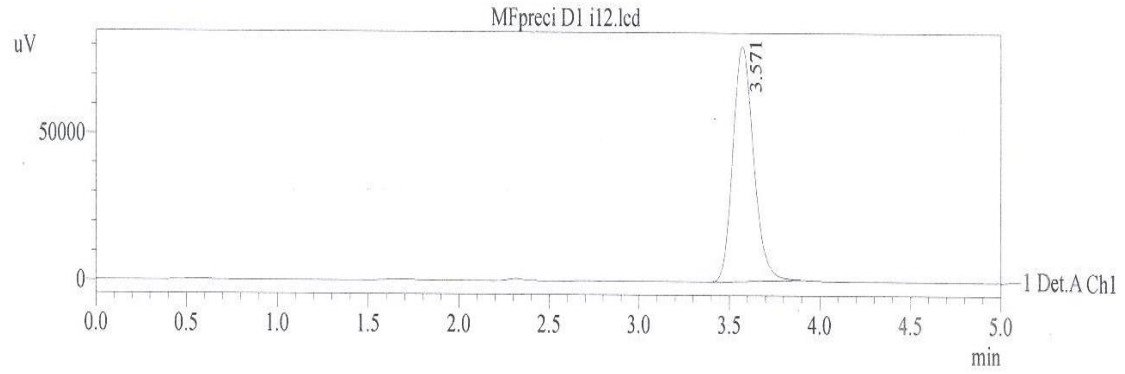


Figure 3.10 showing the precision chromatogram at first day(injection 4)

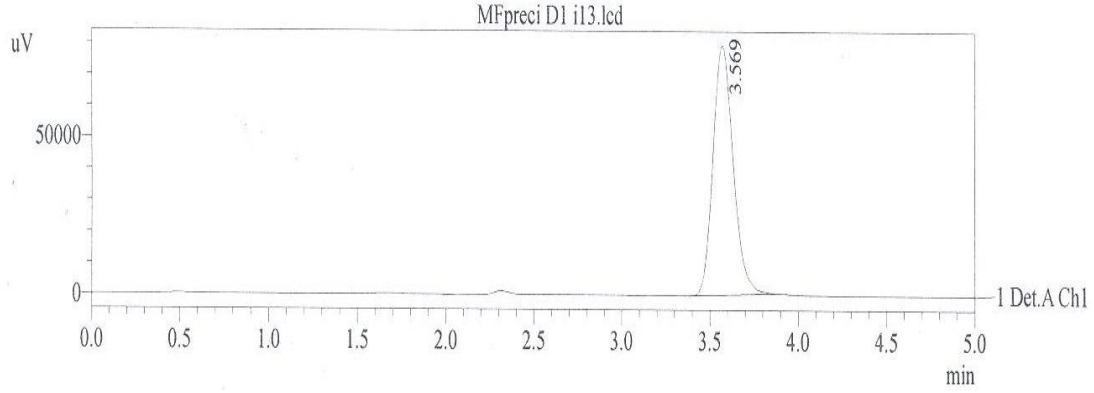


Figure 3.11:showing the precision chromatogram at first day(injection 5)

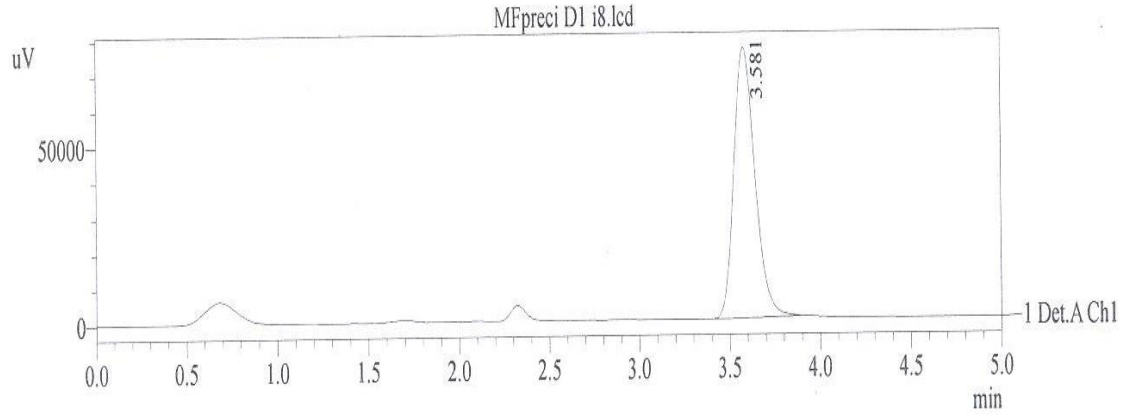


Figure 3.12: showing the precision chromatogram at first day(injection 6)

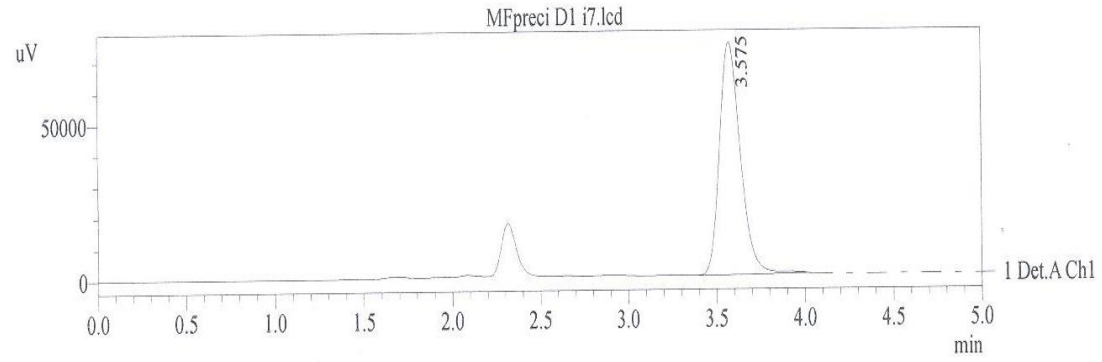


Figure 3.13: showing the precision chromatogram at first day(injection 7)

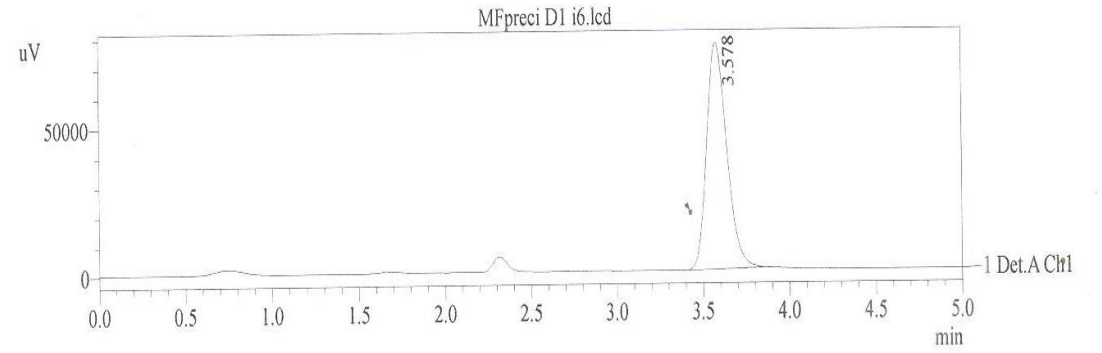


Figure 3.14 :showing the precision chromatogram at first day(injection 8)

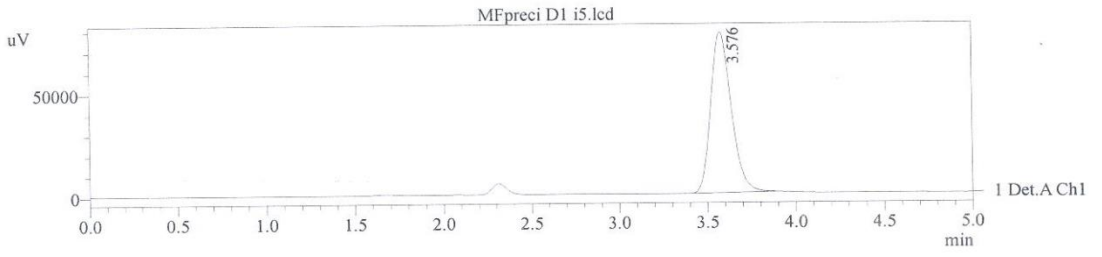


Figure 3.15: showing the precision chromatogram at first day (injection 9)

Table 3.3: Showing Precision of Mefenamic Acid at First Day

Title	Retention time	Area	Area of mefenamic acid 15 µg/ml

Mefeamic acid (injection 1)	3.578	611144	597035
Mefeamic acid (injection 2)	3.592	646275	597035
Mefeamic acid (injection 3	3.571	638440	597035
Mefeamic acid (injection 4)	3.571	637564	597035
Mefeamic acid (injection 5)	3.569	629685	597035
Mefeamic acid (injection 6)	3.581	616735	597035
Mefeamic acid (injection 7)	3.575	609563	597035
Mefeamic acid (injection 8)	3.578	618229	597035
Mefeamic acid (injection 9)	3.567	629689	597035
Average	3.577	626369	
%RSD	0.198	2.084	
Maximum	3.592	646275	
Minimum	3.569	609563	

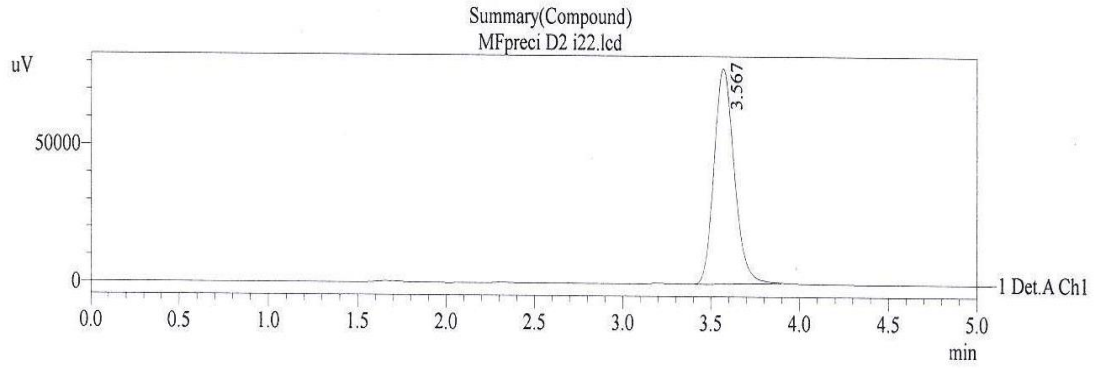


Figure 3.16:showing the precision chromatogram at second day(injection 10)

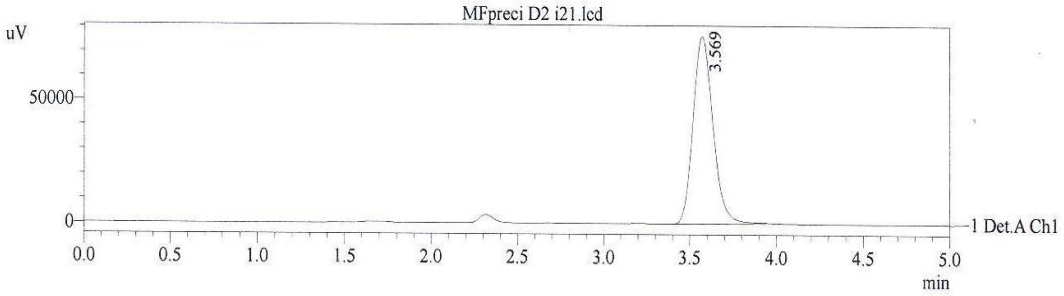


Figure 3.17 :showing the precision chromatogram at second day(injection 11)

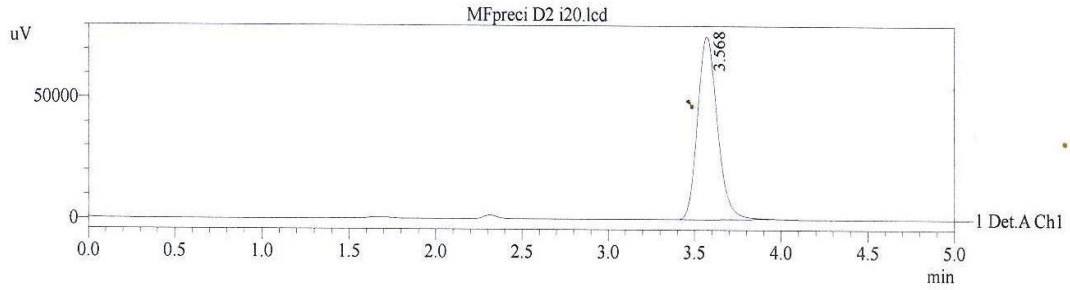


Figure3.18:showing the precision chromatogram at second day(injection 12

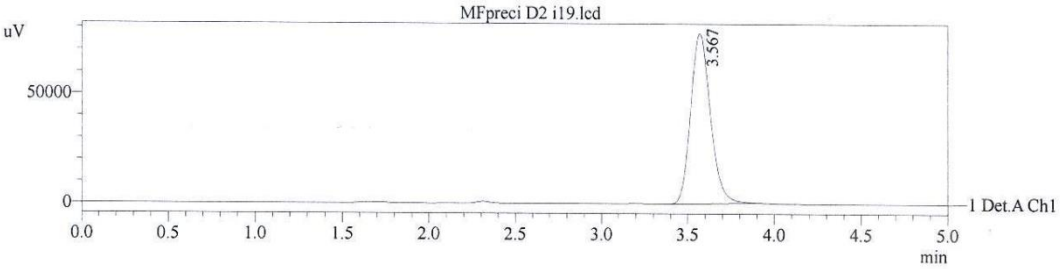


Figure 3.19 : showing the precision chromatogram at second day(injection 13)

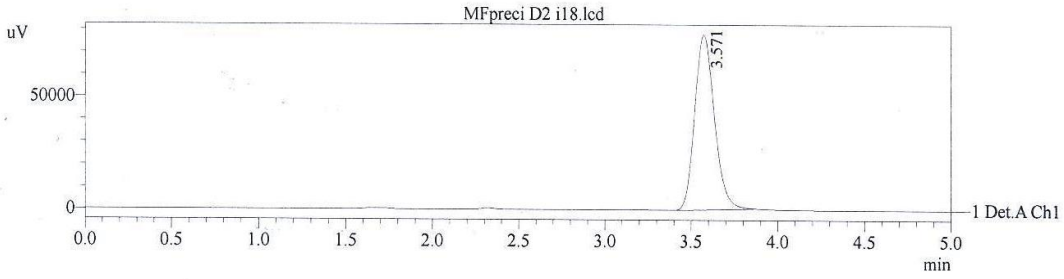


Figure 3.20: showing the precision chromatogram at second day(injection 14)

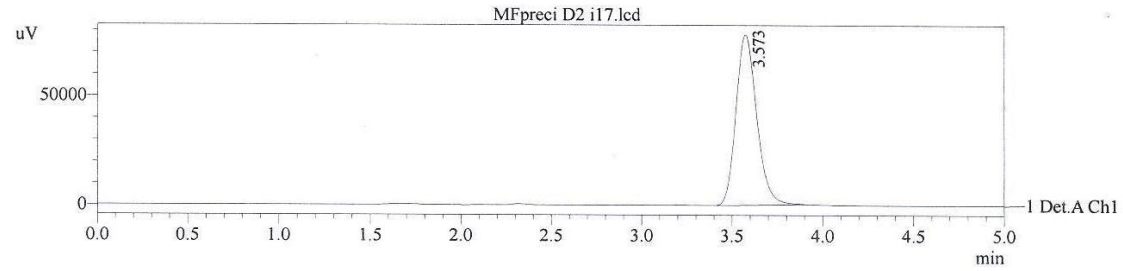


Figure 3.21 : showing the precision chromatogram at second day(injection 15)

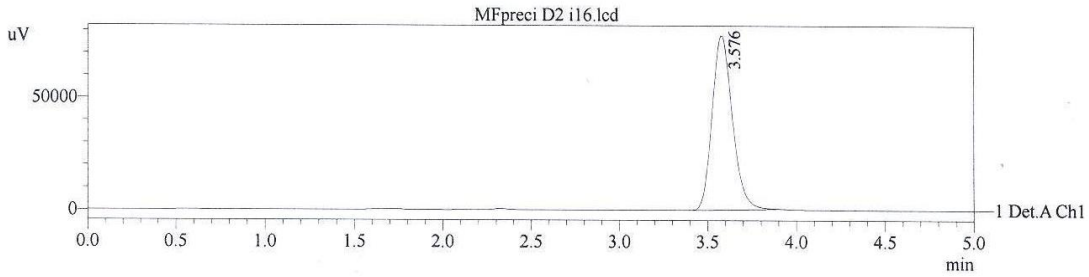


Figure 3.22: showing the precision chromatogram at second day(injection 16)

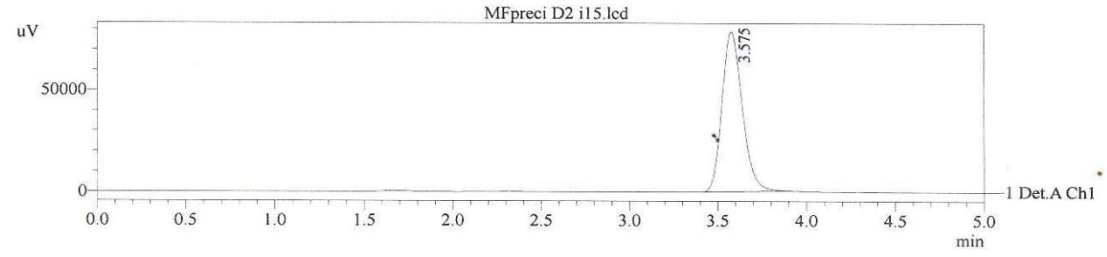


Figure 3.23 : showing the precision chromatogram at second day(injection 17)

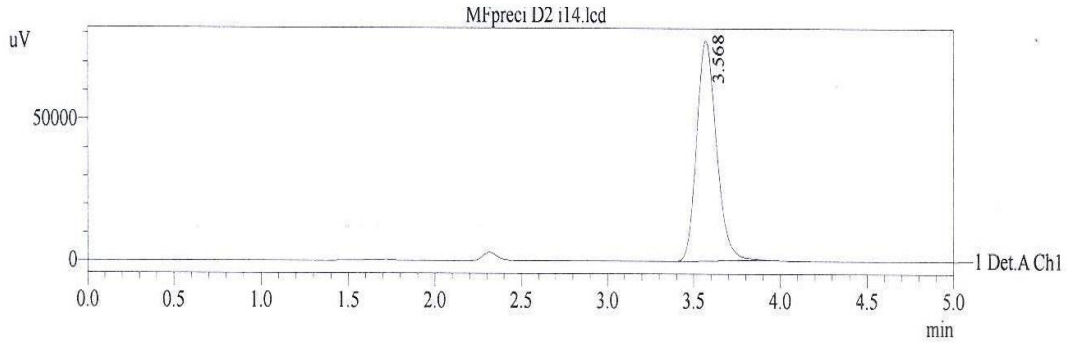


Figure 3.24 :showing the precision chromatogram at second day(injection 18)

Table 3.4 : Showing the Precision of Mefenamic Acid at the Second Day

Title	Retention time	Area	Area of mefenamic acid 15 µg/ml
Mefeamic acid(injection 10)	3.567	623072	597035
Mefeamic acid(injection 11)	3.569	608430	597035
Mefeamic acid(injection 12)	3.568	602032	597035
Mefeamic acid(injection 13)	3.567	614369	597035
Mefeamic acid(injection 14)	3.571	615610	597035
Mefeamic acid (injection 15)	3.573	620879	597035
Mefeamic acid (injection 16)	3.576	616580	597035
Mefeamic acid(injection 17)	3.575	624189	597035
Mefeamic acid(injection 18)	3.568	619462	597035
Average	3.570	616096	
%RSD	0.091	1.160	

Maximum	3.576	624189	
Minimum	3.567	602032	
Standard deviation	0.003	7145	

Figures 3.25– 3.33: Show the Chromatograms Precision at the Third Day.

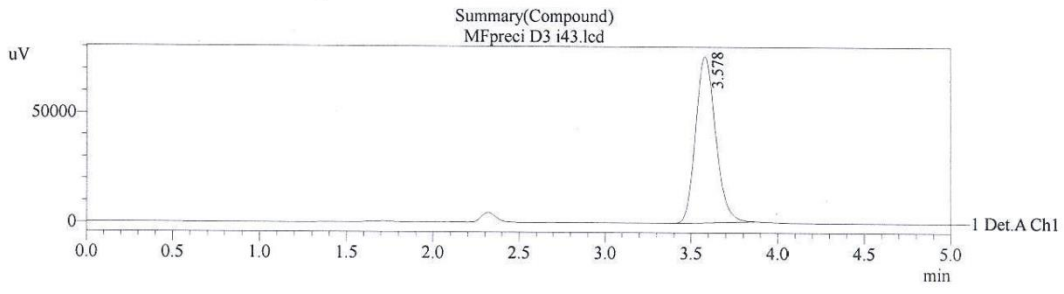


Figure 3.25 : showing the precision chromatogram at the third day(injection 19)

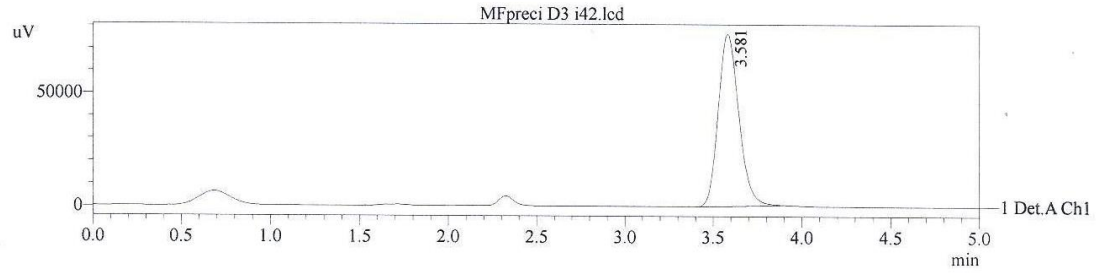


Figure 3.26 :showing the precision chromatogram at the third day(injection 20)

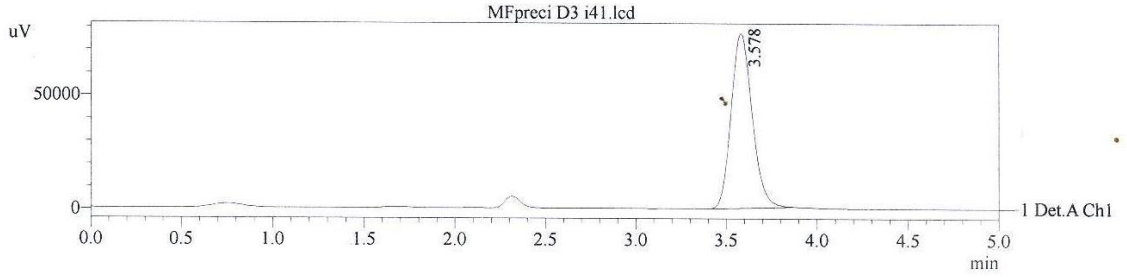


Figure3.27: showing the precision chromatogram at the third day(injection 21)

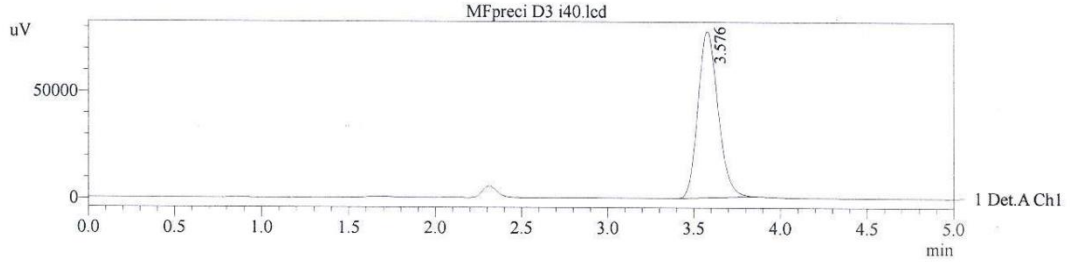


Figure 3.28 :showing the precision chromatogram at the third day(injection 22)

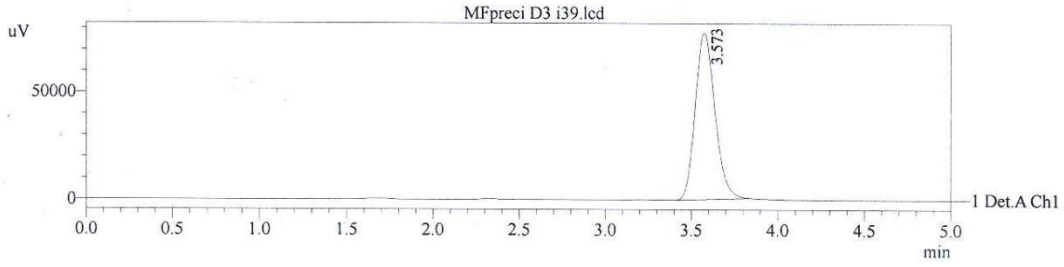


Figure 3.29 : showing the precision chromatogram at the third day(injection 23)

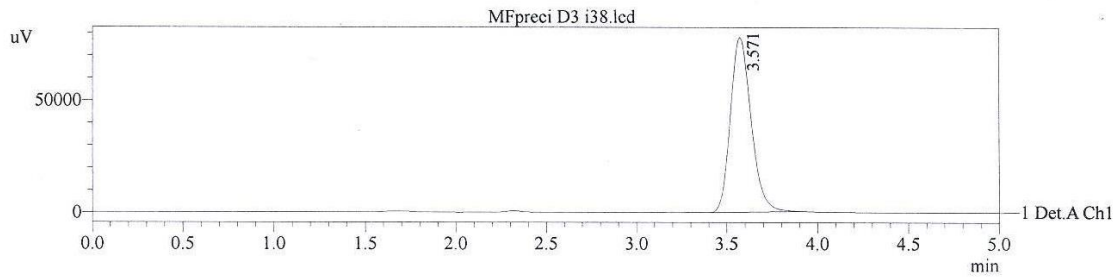


Figure 3.30 :showing the precision chromatogram at the third day (injection 24)

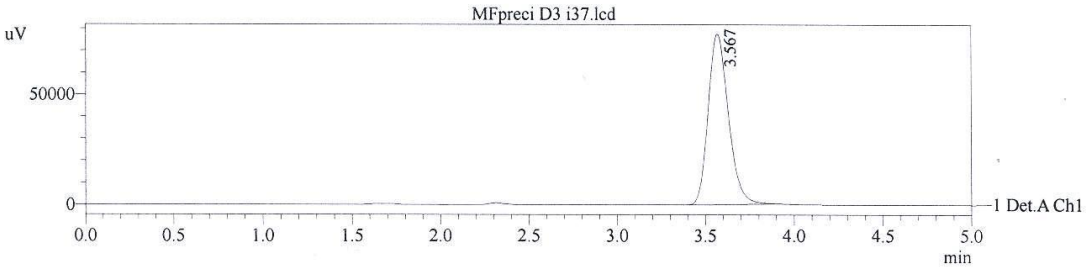


Figure 3.31 : showing the precision chromatogram at the third day(injection 25)

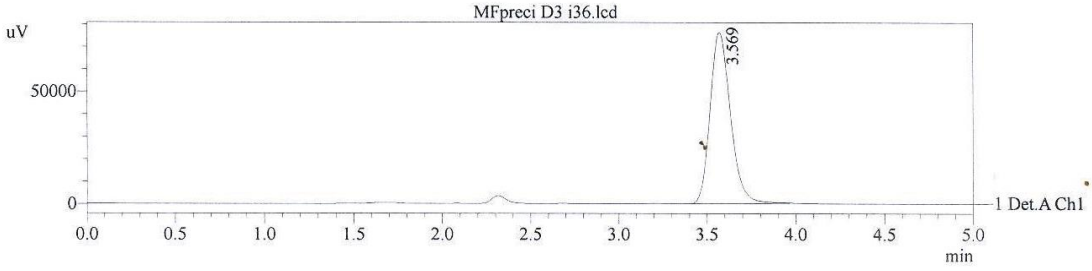


Figure3.32 : showing the precision chromatogram at the third day (injection 26)

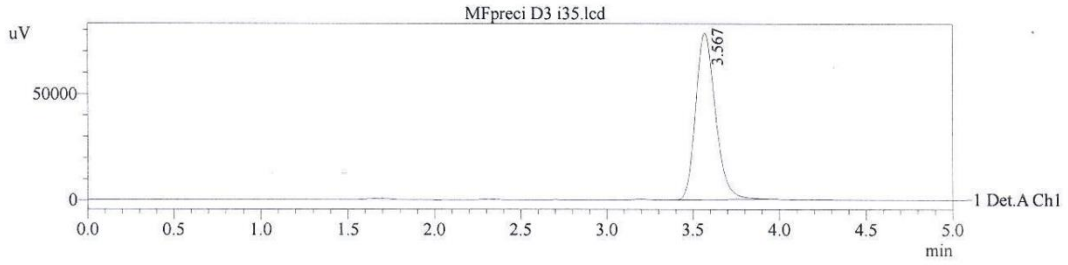


Figure3.33: showing the precision chromatogram at the third day(injection 27)

Table 3.5: Showing Precision of Mefenamic acid at Third day

Title	Retention time	Area	Area of mefenamic acid 15 µg/ml
Mefeamicacid (injection19)	3.578	610869	597035
Mefeamic acid(injection 20)	3.581	615306	597035
Mefeamic acid(injection 21)	3.578	619119	597035
Mefeamic acid(injection 22)	3.576	625987	597035
Mefeamic acid (injection 23)	3.573	614065	597035
Mefeamic acid(injection 24)	3.571	618891	597035
Mefeamic acid(injection 25)	3.567	614316	597035
Mefeamic acid(injection 26)	3.569	610189	597035
Mefeamic acid(injection 27)	3.567	624837	597035
Average	3.573	617064	
%RSD	0.142	0.911	
Maximum	3.581	625987	
Minimum	3.567	610189	
Standard deviation	0.005	5622	

Table3.6: Precision Result for Tablets assay on Three Consecutive Day

DAY	Mean	% RSD
Day 1	104.0 %	0.198 %
Day 2	103.0 %	0.091 %
Day 3	102.96 %	0.142 %

The precision of the method was assessed by repeatability was determination by analyzing nine sample for three consecutive day, day one RSD=0.198 % less than 2, day two RSD=0.091% less than 2 and day three RSD=0.142 % less than 2

Robustness: of an analytical procedure is a measure of its capacity to remain unaffected by changes occurs but remains and produced similar results.

Parameters were optimized; mobile phase concentration, wave length, flow rate and pH.

Mobile phase (methanol concentration) with different composition 91%and 89% .

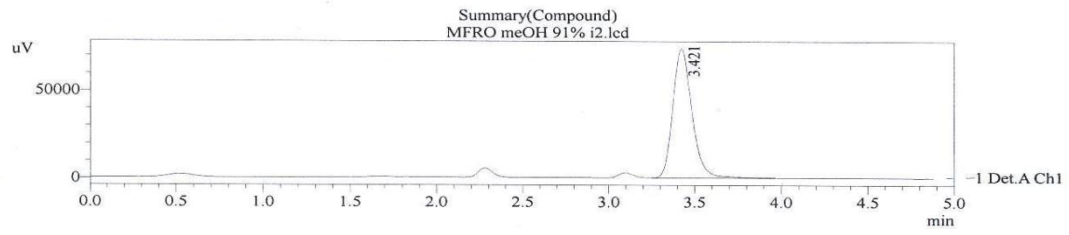


Figure 3.34: showing the chromatogram of methanol 91 %(injection 1)

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid at methanol 91% (injection 1)	576931	597035
Mefenamic acid at methanol 91% (injection 2)	575964	597035
Average	576447	

Table 3.7 : Showing the Result of Robustness in Methanol 91%

%RSD	0.119	
Maximum	576931	
Minimum	575964	
Standard deviation	684	

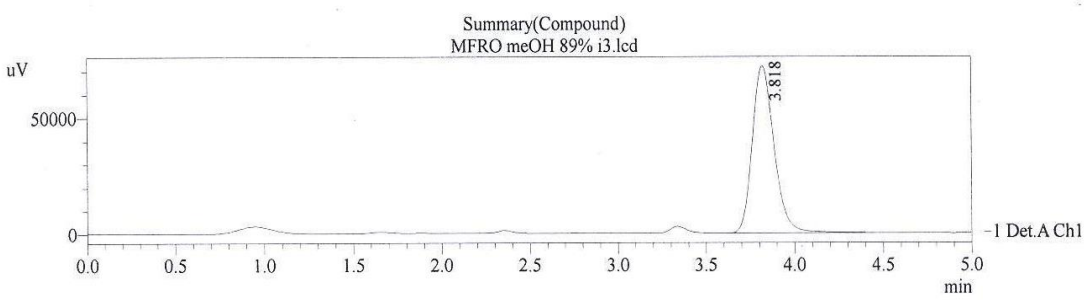


Figure3.36 :showing the chromatogram of methanol 89 %(injection 1)

Table 3.8 : Showing the Result of Robustness in Methanol 89%

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid at methanol 89%(injection1)	619219	597035
Mefenamic acid at methanol 89% (injection2)	597447	597035
Average	608333	

%RSD	2.531	
Maximum	619219	
Minimum	597447	
Standard deviation	15395	

The mefenamic acid was examined under wave length 274nm and 276nm, which shown bellow

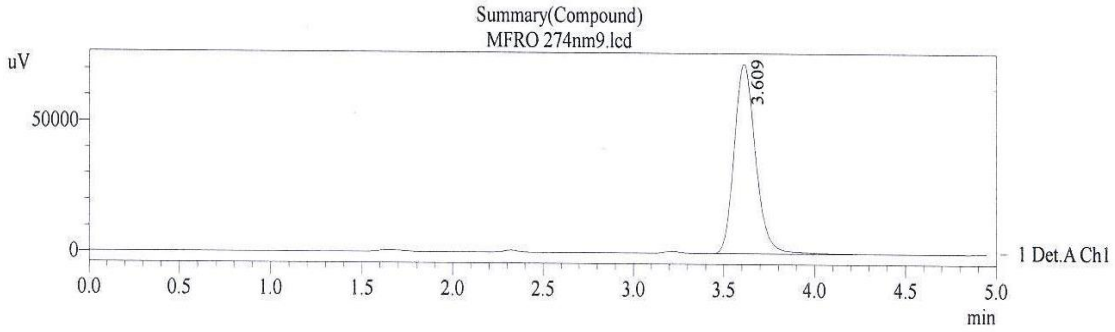


Figure 3.37 : showing the chromatogram at wave length 274nm (injection 1)

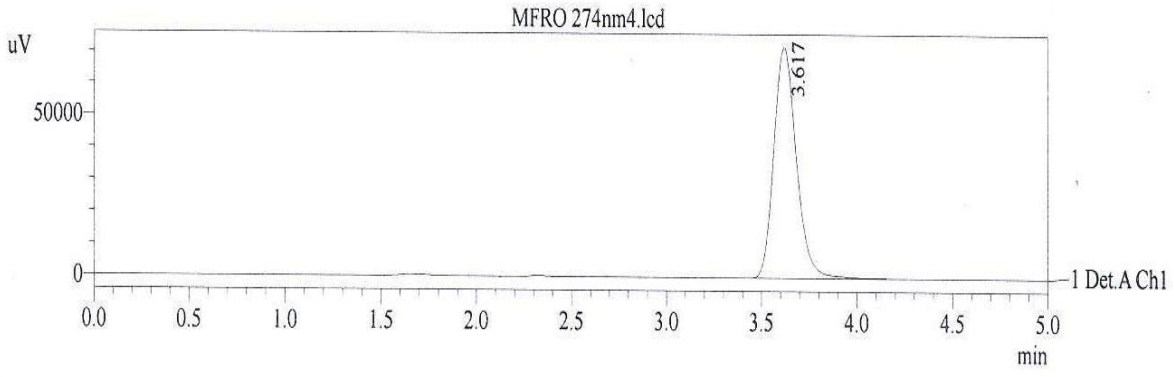


Figure 3.38: Showing the chromatogram at wavelength 274nm (injection 2)

Table 3.9 : Showing the Result of Robustness at Wavelength 274 nm

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid at wavelength 274nm (injection 1)	600304	597035
Mefenamic acid at wavelength 274nm(injection 2)	594121	597035
Average	597213	
%RSD	0.732	
Maximum	600304	
Minimum	594121	
Standard deviation	4373	

Figures 3.39 – 3.: Shows the chromatograms of Mefenamic acid at Wavelength 276nm:

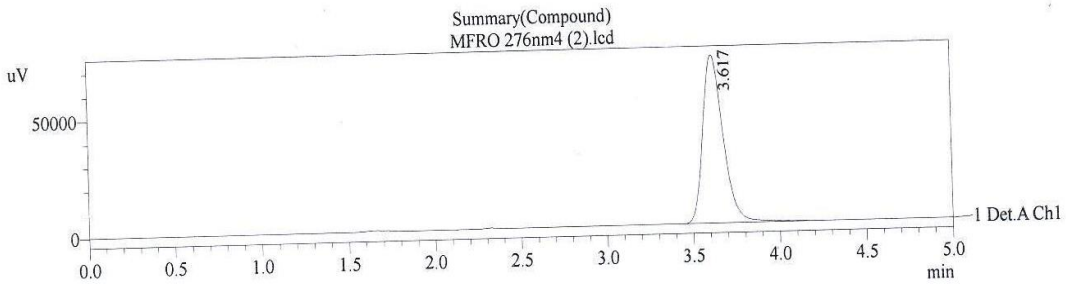


Figure 3.39 : showing the chromatogram at wave length 276nm (injection 1)

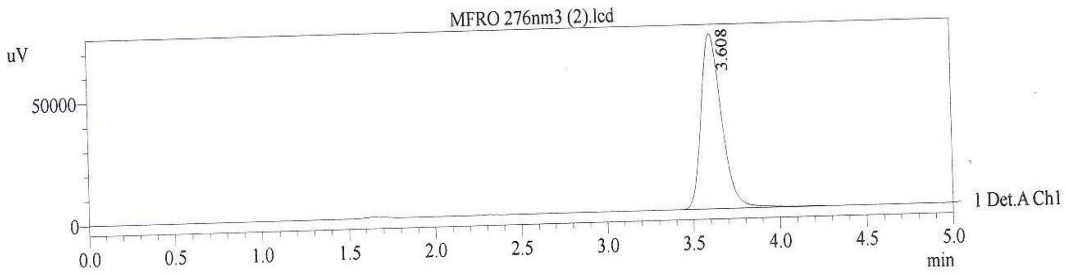


Figure 3.40: showing the chromatogram at wave length 276nm (injection 2)

Table 3.10: Showing the Result of Robustness in Wavelength to 276nm

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid at wavelength 276nm (injection 1)	594121	597035
Mefenamic acid at wavelength 276nm (injection 2)	599164	597035
Average	596643	
%RSD	0.598	
Maximum	599164	
Minimum	594121	
Standard deviation	3566	

The flow rate was change to 1.1ml/min and 0.9ml/min, the results were shown bellow

Figures 3.41– 3.43 :shows the chromatograms of mefenamic acid at flow rate 1.1ml/min

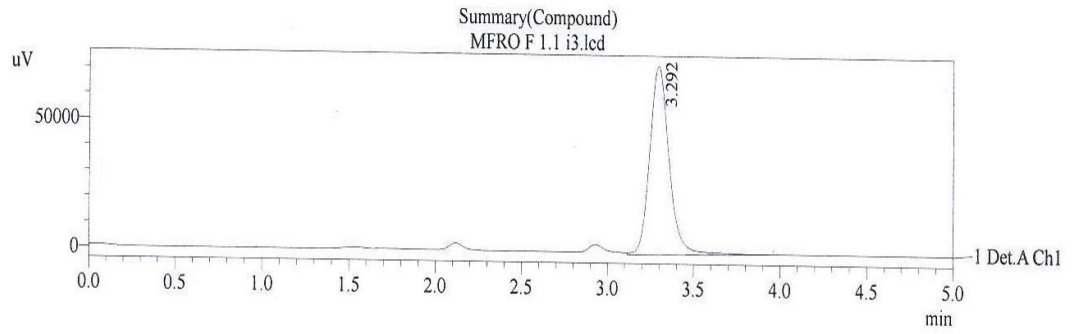


Figure 3.41 showing the chromatogram at flow rate 1.1(injection 1)

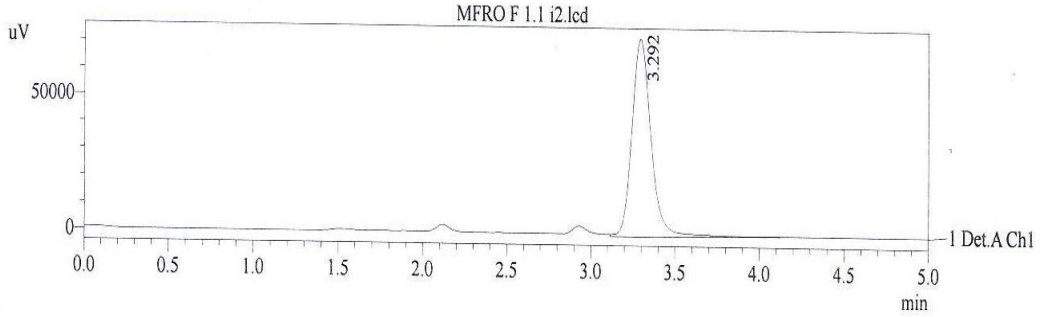


Figure 3.42 : showing the chromatogram at flow rate 1.1(injection 2)

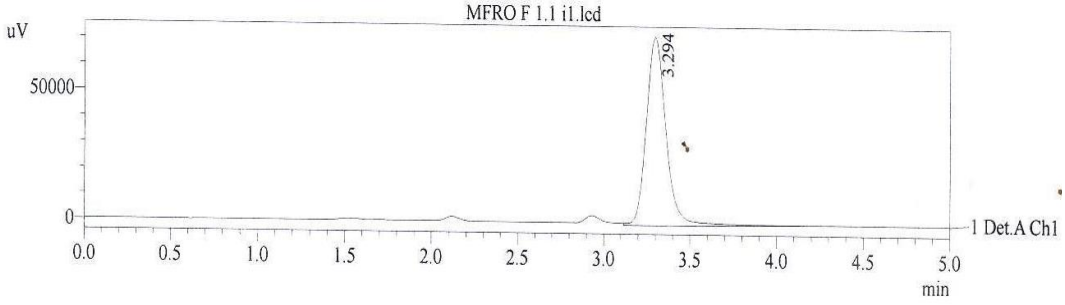


Figure 3.43 : showing the chromatogram at flow rate 1.1(injection 3)

Table 3.11: showing the result of robustness in flow rate to 1.1ml/min

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid at flow rate 1.1ml/min(injection1)	57504	597035

Mefenamic acid at flow rate 1.1ml/min(injection2)	581085	597035
Mefenamic acid at flow rate 1.1ml/min(injection3)	577200	597035
Average	577777	
%RSD	0.530	
Maximum	581085	
Minimum	575047	
Standard deviation	3036	

Figures 3.44-3.45 show the Chromatograms of Mefenamic acid at Flow Rate 0.9 ml/min

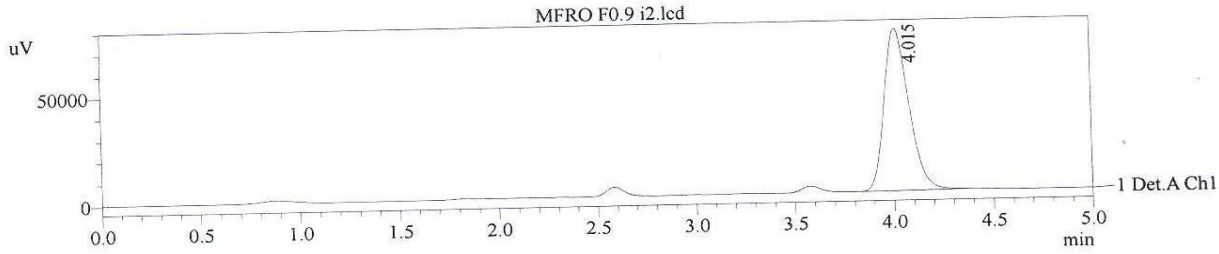


Figure 3.44 :showing the chromatogram at flow rate 0.9ml/min (injection1)

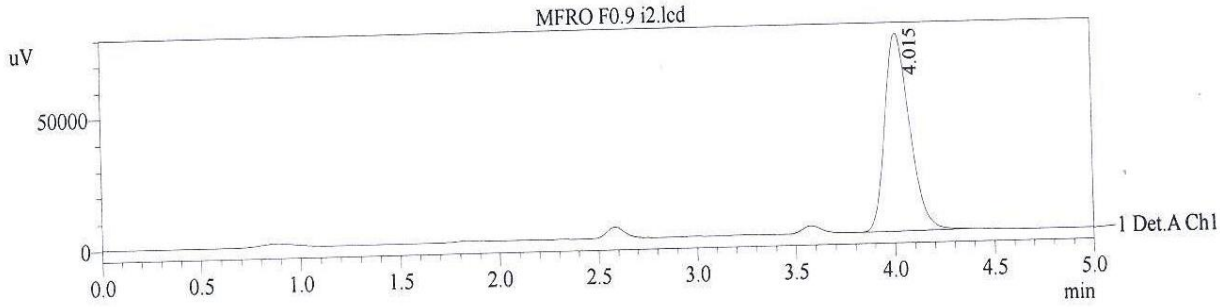


Figure 3.45: showingthe chromatogram at flow rate 0.9ml/min (injection 2)

Table 3.12: Showing the Result of Robustness in Flow Rate to 0.9ml/min

Title	Area	Area of mefenamic acid 15 µg/ml
Mefnamic acid with flow rate 0.9ml/min (injection1)	649090	597035
Mefenamic acid with flow rate0.9ml/min(injection 2)	651532	597035
Average	650311	597035
%RSD	0.266	
Maximum	651532	
Minimum	649090	
Standard deviation	1727	

Also pH was altered to 2.8 and 3.5 to ensure best results which were shown bellow

Figures 3.46- 3.48 shows the chromatograms of mfenemic acid at pH 2.8

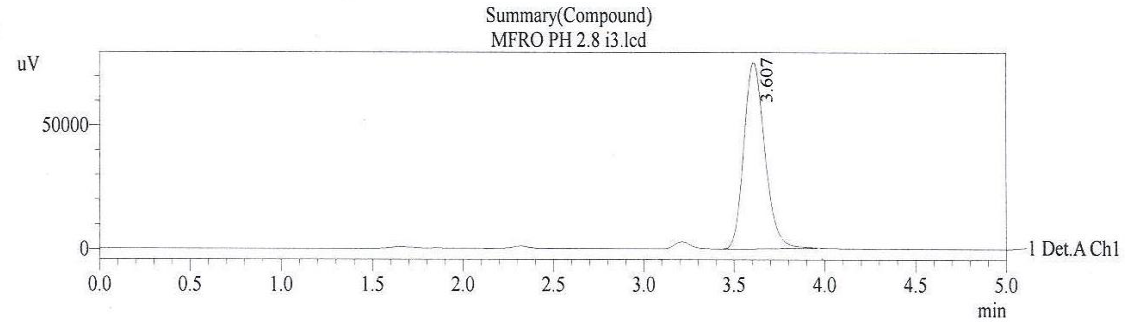


Figure 3.46 : showing chromatogram at pH 2.8(injection 1)

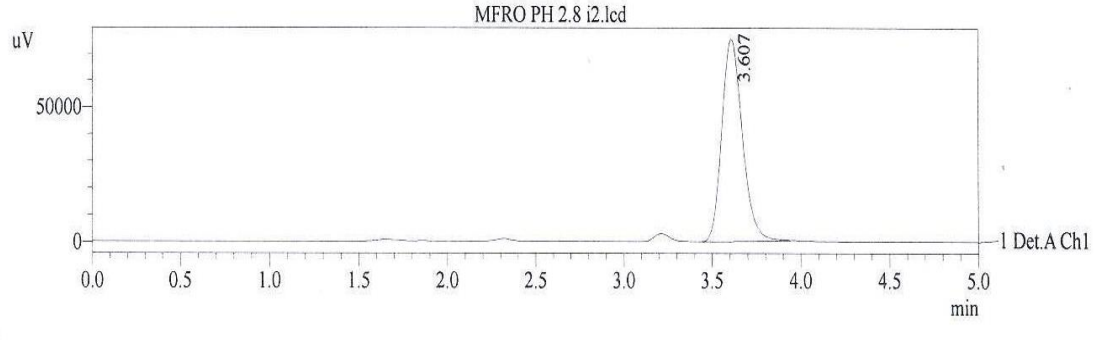


Figure 3.47 showing chromatogram at pH 2.8(injection 2)

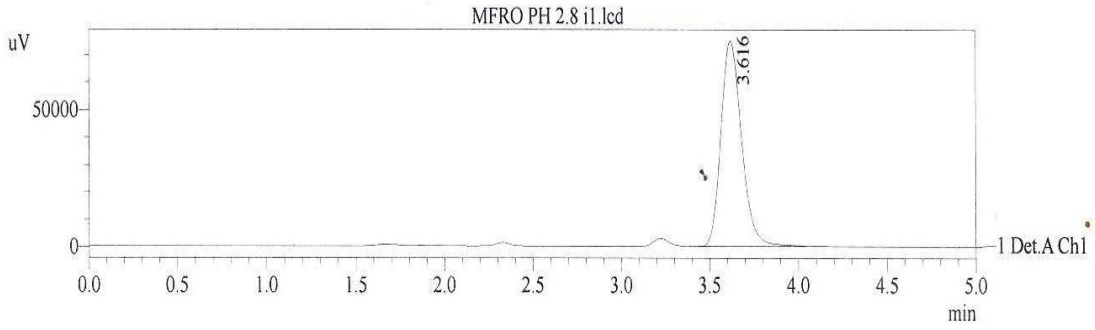


Figure 3.48: showing chromatogram at pH 2.8 (injection 3)

Table 3.13: Showing the Result of Mefenamic Acid at pH 2.8

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid with pH 2.8 injection 3	605796	597035
Mefenamic acid with pH 2.8 injection 2	604509	597035
Mefenamic acid with pH 2.8 injection1	606177	597035

Average	605494	
%RSD	0.144	
Maximum	606177	
Minimum	604509	
Standard deviation	874	

Figures 3.49 – 3.51: Shows the Chromatograms of Mefenamic acid at pH 3.5

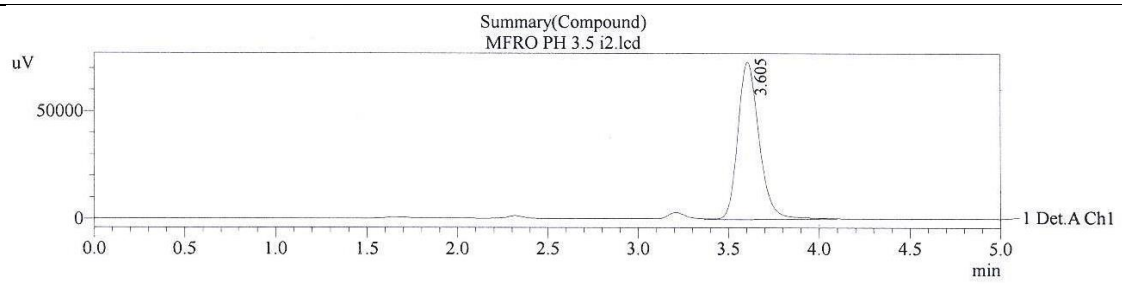


Figure 3.49: showing the chromatogram at pH 3.5(injection 1)

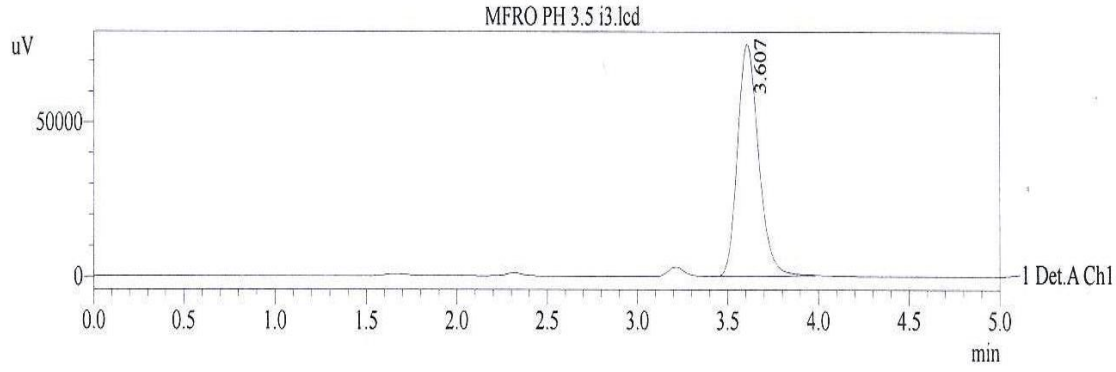


Figure 3.50 :showing the chromatogram at pH 3.5(injection 2)

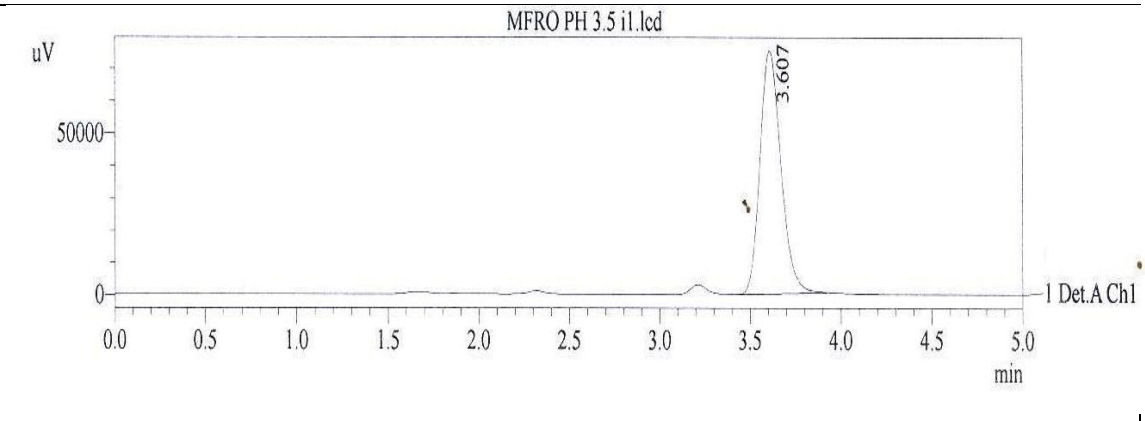


Figure 3.51 :showing the chromatogram at pH 3.5(injection 3)

Table 3.14: Showing the Result of Robustness in changing pH to 3.5

Title	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid at pH 3.5(injection 1)	599373	597035
Mefenamic acid at pH 3.5(injection 2)	605488	597035
Mefenamic acid at pH (3.5 injection 3)	603377	597035
Average	602746	
%RSD	0.515	
Maximum	605488	
Minimum	599373	
Standard deviation	3106	

Area of mefenamic acid with methanol 90% was found to be 597036, while with methanol 91% was found to be 576447, there is no significant difference between results.

Height of mefenamic acid with methanol 90% was found to be 74702, while with methanol 91% was found to be 74197, which show no big difference.

Relative stander deviation of area and height of Mefenamic acid with methanol 91% were found to be 0.119and 0.558 respectively, and were less than 2.

So the replacement of methanol 90 %(90ml) with methanol 91% (91) doesn't show difference, so we can use both 90%, and 91%.

Area of mefenamic acid with methanol 90% was found to be 597036, while with methanol 89% was found to be 608333, which show a slight difference between the results

Height of mefenamic acid with methanol 90% was found to be 74702, while with methanol 89% was found to be 70769, which show a slight difference between the results.

Relative standard deviation of area and height of Mefenamic acid with methanol 89% were found to be 2.531and 2.511 respectively, and more than 2.

So the replacement of methanol 91% with methanol 89% gives different results and it's not applicable.

Retention time of mefenamic acid with flow rate 1 ml/min was found to be 3.557 minutes, while retention time of mefenamic acid with flow rate 1.1 ml/min was found to be 3.292minutes.

Mefenamic acid with flow rate 1.1 ml/min show earlier peak than the peak produced with flow rate 1 ml/min. Area of mefenamic acid with flow rate 1 ml/min was found to be 597036, while area of mefenamic acid with flow rate 1.1 ml/min show earlier peak than the peak produced with flow rate 1 ml/min.

Area of mefenamic acid with flow rate 1 ml/min was found to be 597036, while area of mefenamic acid with flow rate 1.1 ml/min was found to be 577777, which show no significant difference.

Height of mefenamic acid with flow rate 1 ml/min was found to be 74702, while height of mefenamic acid with flow rate 1.1 ml/min was found to be 72802, which show no significant difference.

Relative standard deviation of area, and height were found to be 0.530, and

0.530 respectively, and were less than 2.

So results of mefenamic acid with flow rate 1.1ml/min doesn't differ from results of mefenamic acid with flow rate 1ml/min, so this flow rate can be used to give the similar results.

Retention time of mefenamic acid with flow rate 1 ml/min was found to be 3.557 minutes, while retention time of mefenamic acid with flow rate 0.9 ml/min was found to be 4.015 minutes.

Mefenamic acid with flow rate 0.9 ml/min produce later peak than the peak produced with flow rate 1 ml/min.

Area of mefenamic acid with flow rate 1 ml/min was found to be 597036, while area of mefenamic acid with flow rate 0.9 ml/min was found to be 650311,which show a slight difference between the results.

Height of mefenamic acid with flow rate 1 ml/min was found to be 74702, while height of mefenamic acid with flow rate 0.9ml/min was found to be 75412, which show no significant difference.

Relative standard deviation of area, and height were found to be 0.266, and 0.097 respectively, and were less than 2.

The use of flow rate 0.9ml/min produced almost similar results with flow rate 1.1 ml/min.

The difference in retention time in flow rate 1.1ml/min show earlier peak while in flow rate 0.9ml/min show later peak, and gave larger peak area, so both flow rates could be use. Retention time of mefenamic acid at274nm was found to be 3.613 which is later peak than mefenamic acid at 275nm which was found to be 3.557min.

Area of mefenamic acid at 274nm was found to be 597213, while area of mefenamic acid in 275nm was found to be 597036, and show no difference.

Height of mefenamic acid at 274nm was found to be 72146, while height of mefenamic acid in 275nm was found to be 74702, and show no significant

difference.

Relative standard deviation for area and height were found to be 0.732 and 0.614, all were less than 2.

Results of mefenamic acid at 274nm is near to results of mefenamic acid at 275nm,the only difference is the retention time of peak, where it later at 274nm than 275nm.

Retention time of mefenamic acid at 276nm was found to be 3.612 which is appeared after the peak of mefenamic acid at 275nm which was found to be 3.557 min.

Area of mefenamic acid at 276nm was found to be 596643, while area of mefenamic acid in 275nm was found to be 597036, and show no difference.

Height of mefenamic acid at 276nm was found to be 71897, while height of mefenamic acid in 275nm was found to be 74702, and show no significant difference.

Relative standard deviation for area and height were found to be 0.598 and 0.125, all were less than 2.

Results of mefenamic acid at 276nm is near to results of mefenamic acid at 275nm,the only difference is the retention time of peak, where it later at 276nm than 275nm.

Both wave lengths 274 and 276 could be used.

Retention time of mefenamic acid at PH2.8 was found to 3.61min, while retention time of mefenamic acid at PH3.3 was found to be 3.557min.

Area of mefenamic acid at PH 2.8 was found to be 605494, while area of mefenamic acid at PH 3.3 was found to be 597036, and show no significant difference.

Height of mefenamic acid at pH 2.8 was found to be 75024, while height of mefenamic acid at pH 3.3 was found to be 74702, and show no significant difference.

Relative standard deviation of area and height were found to be 0.144, and 0.323 respectively, and were less than 2.

So the use of pH 2.8 instead of 3.3 doesn't changes the result significantly.

Retention time of mefenamic acid at pH 3.5 was found to 3.606min, while retention time of mefenamic acid at pH3.3 was found to be 3.557min.

Area of mefenamic acid at pH 3.5was found to be 602746, while area of mefenamic acid at pH 3.3 was found to be 597036, and show no significant difference.

Height of mefenamic acid at pH 3.5 was found to be 74442, while height of mefenamic acid at pH 3.3 was found to be 74702, and show no difference.

Relative stander deviation of area and height were found to be 0.515, and 1.591 respectively.

Relative stander deviation of area and relative standard deviation of the height were less than 2. So the use of pH 3.5 instead of 3.3 may alter the retention time and height a little bit.

So the pH of 2.8 is closer result to pH of 3.3 than the pH of 3.5.

Accuracy

Figures 3.52 –3.54: shows the chromatograms of mefenamic acid at 50% of mefenmaic acid:

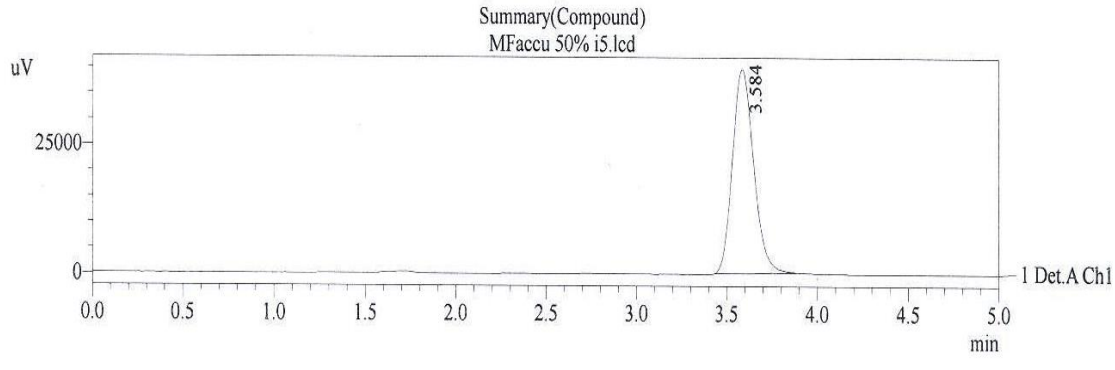


Figure 3.52: showing the chromatogram of 50% mefenamic acid(injection 1)

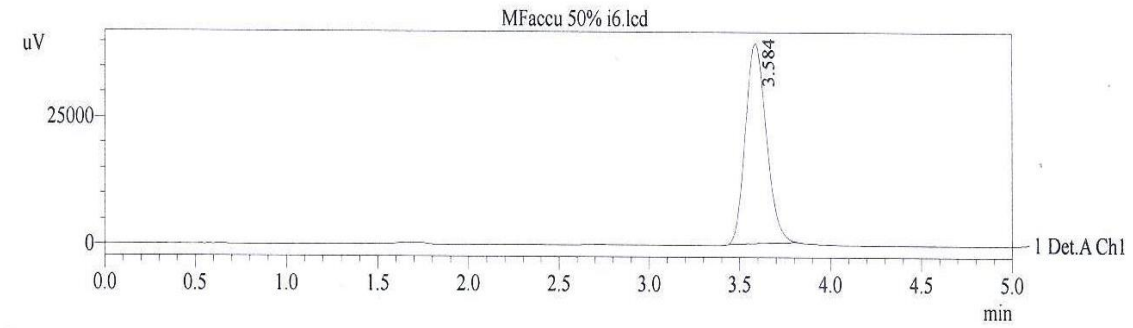


Figure 3.53 : showing the chromatogram of 50% mefenamic acid (injection 2)

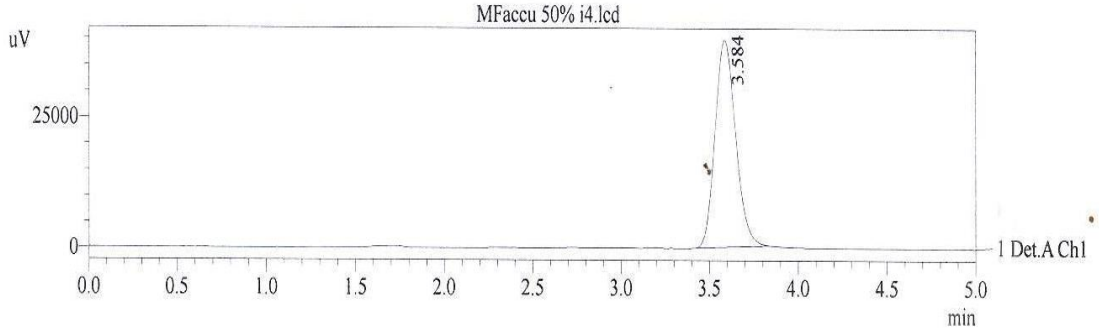


Figure 3.54 : showing the chromatogram of 50% mefenamic acid (injection 3)

Table 3.15: ShowingMefenamic acid as 50%:

Title	Retention time	Area	Area of mefenamic acid 15 µg/ml
Mefenamicacidas50% (injection1)	3.584	322453	597035
Mefenamic acid as 50% (injection2)	3.584	316317	597035
Mefenamic acid as 50% (injection3)	3.584	319550	597035

Average	3.584	319440	
RSD%	0.000	0.961	
Maximum	3.584	322453	
Minimum	3.584	316317	
Stander deviation	0.000	3070	

Figures 3.55-3.57shows the chromatograms of mefenamic acid at 80% of mefenamic acid

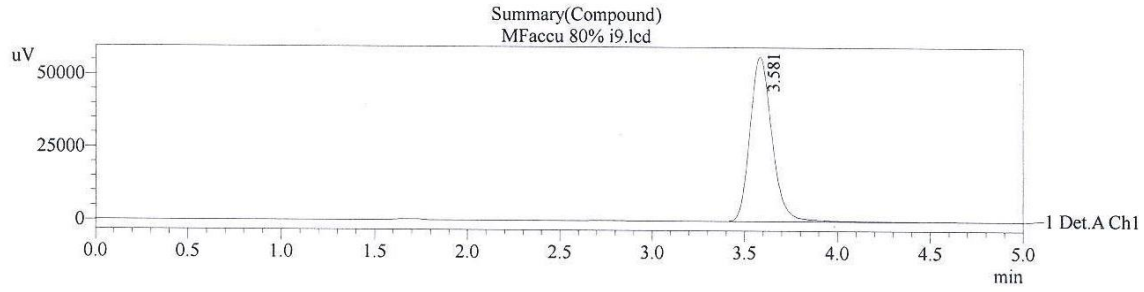


Figure 3.55 : showing the chromatogram of 80% mefenamic acid (injection 1)

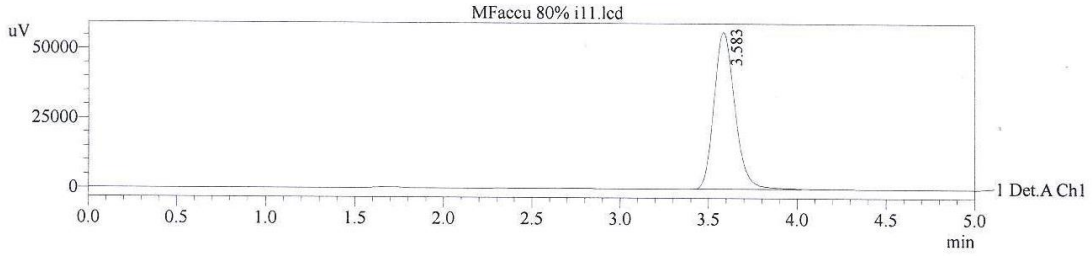


Figure 3.56: showing the chromatogram of 80% mefenamic acid (injection 2)

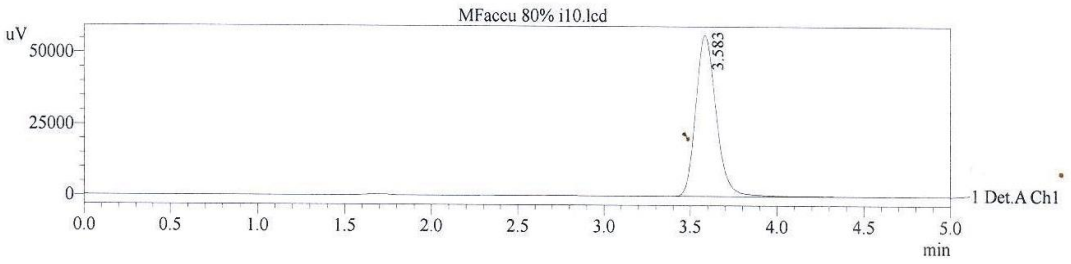


Figure 3.57 :showing the chromatogram of 80% mefenamic acid (injection 3)

Table 3.16:Showing the Mefenamic acid as 80%

Title	Retention time	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid as 80% (injection number 1)	3.581	469189	597035
Mefenamic acid as 80% (injection number 2)	3.583	461340	597035
Mefenamic acid as 80% (injection number 3)	3.583	466754	597035
Average	3.582	465761	
%RSD	0.040	0.863	
Maximum	3.583	469189	
Minimum	3.581	461340	
Stander deviation	0.001	4017	

Figures 3.58- 3.60 shows chromatograms of mefenamic acid at 100% of mefenamic acid

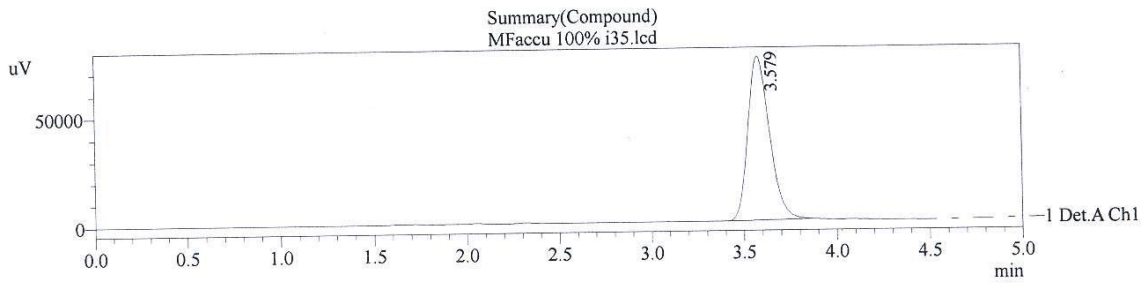


Figure 3.58 : showing the chromatogram of 100% mefenamic acid (injection 1)

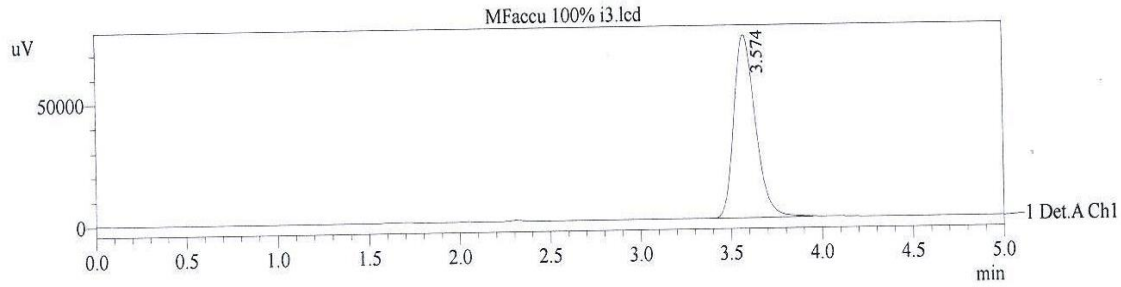


Figure 3.59 : showing the chromatogram of 100% mefenamic acid (injection 2)

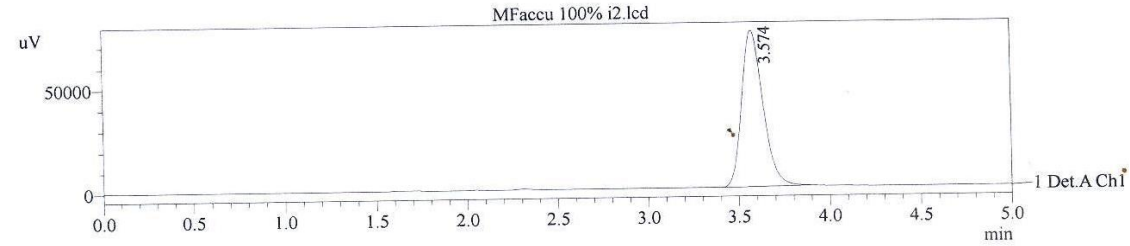


Figure 3.60 : showing the chromatogram of 100% mefenamic acid(injection 3)

Table 3.17: Showing the Results of Mefenamic acid as 100%

Title	Retention time	Area	Area of mefenamic acid 15 µg/ml
Mefenamic acid as100(injection1)	3.579	607957	597035
Mefenamicacidas100%(injection2)	3.574	616025	597035
Mefenamicacidas100%(injection3)	3.574	611368	597035
Average	3.576	61178	

		3	
%RSD	0.088	0.662	
Maximum	3.579	616025	
Minimum	3.574	617957	
Stander deviation	0.003	4050	

Figures 3.61–67: Shows the Chromatograms of Mefenamic acid at 120%

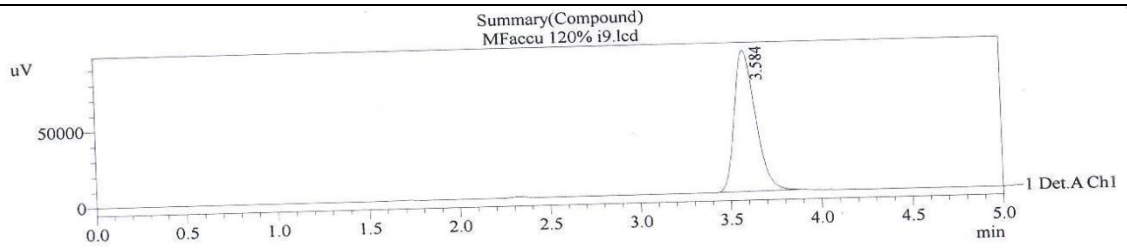


Figure 3.61 :showing the chromatogram of 120% mefenamic acid(injection 1)

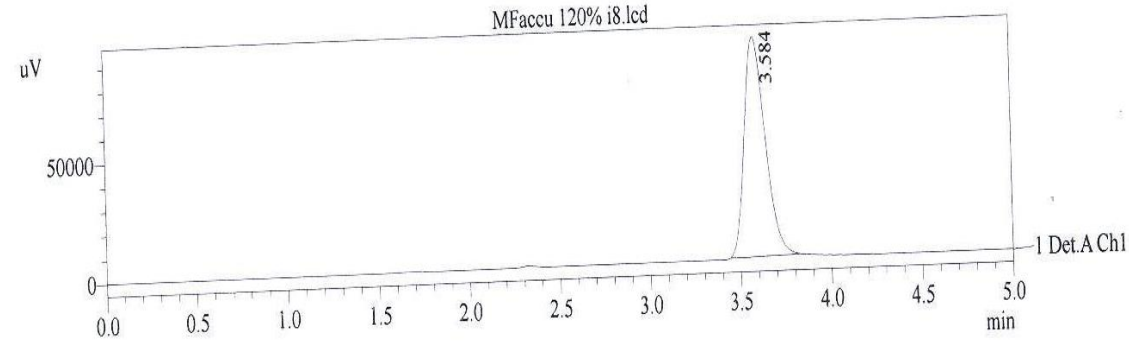


Figure 3.62 : showing the chromatogram of 120% mefenamic acid (injection 2)

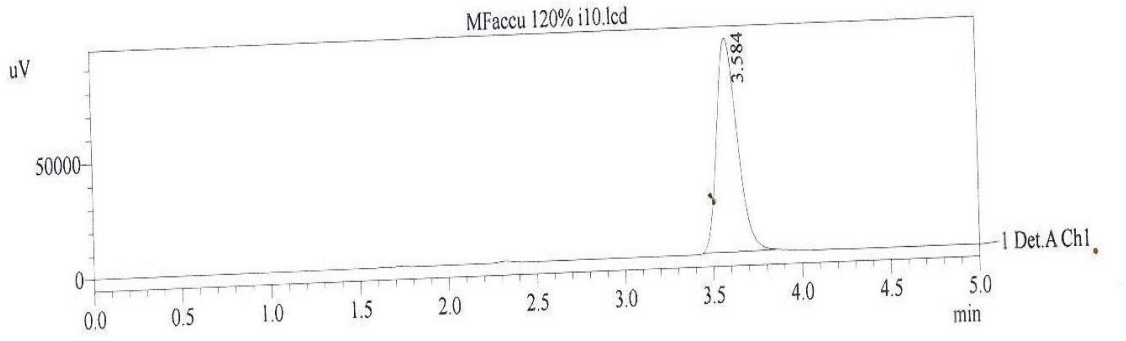


Figure 3.63 : showing the chromatogram of 120% mefenamic acid (injection 3)

Table 3.18: Showing the Results of Mefenamic acid as120%

Title	Area	Area of mefenamic acid 15 µg/ml
Mfenamic acid as 120% (injection number 1)	748359	597035
Mfenamic acid as 120% (injection number 2)	737249	597035
Mfenamic acid as 120% (injection number 3)	741277	597035
Average	742295	
%RSD	0.758	
Maximum	748359	
Minimum	737249	
Stander deviation	5624	

It is the closeness to the true value, measured by % recovery of sample.

We assume that the concentration of is the 15 µg/ml 100% concentration.

For 50 % concentration (7.9 15μg/ml)

Percent recovery = peak area of drug in sample/ peak area of drug in stander *100

PR= $\frac{319440}{611783}$ ×100= 52.2%

PR = 52.2%

For 80 % (11µg/ml)

PR = $\frac{465761}{611783}$ ×100% = 76.1%

For 100 % with concentration (15 µg/ml)

PR= $\frac{611783}{611783}$ ×100% = 100%

PR= 100%

For 120 % with concentration (18 µg/ml)

PR = $\frac{742295}{611783}$ ×100 % = 120 %

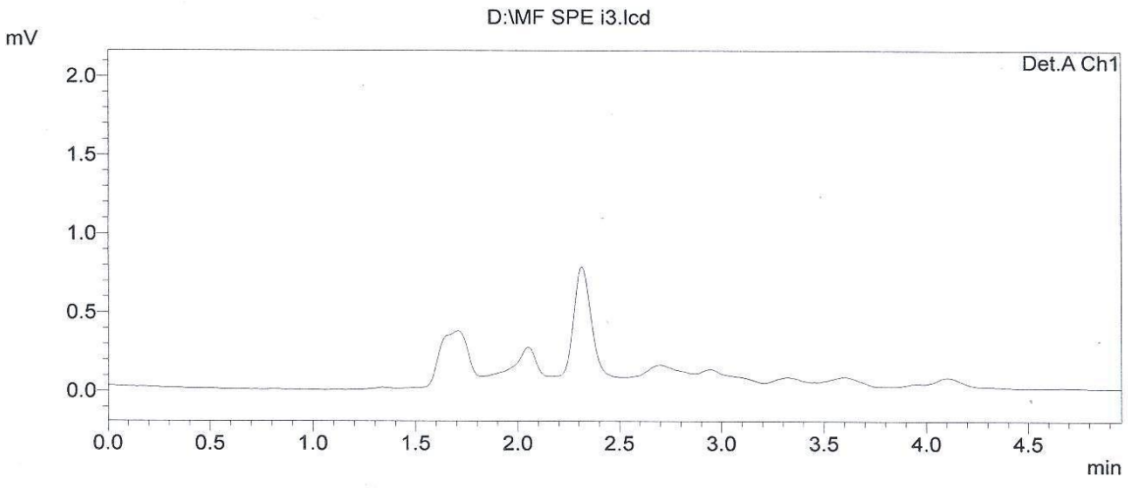
For mefenamic acid 80% the relative stander deviation for retention time, area, height and concentration were found to be 0.040, 0.863, 0.283, and 0,863, all were less than 2.

For mefenamic acid 100% the relative stander deviation for retention time, area, height, and concentration were found to be 0.088, 0.662, 0.096, 0.662 respectively, and all were less than 2.

For mefenamic acid 120% the concentration was greater a little bit which was found to be 18 µg/ml ml.

For mefenamic acid 120% the relative stander deviation for area, height, and concentration were found to be 0.758, 0.231, 0.758 respectively, and were less than 2.

Specificity:



Figure

3.64: Showing the Chromatogram of Excipients

From this chromatogram showed that no interference between the sample (Active pharmaceutical ingredient –mefnamicacid) and other ingredient)

Conclusion

A precise reverse phase HPLC method was developed for the development and validation in pharmaceutical dosage form and this method was found to be valid according to ICH guideline in terms of specificity, linearity, accuracy, precision and robustness and therefore it could be used to meet requirements for a global regulatory filing.

From the results obtained in this study the following conclusions could be

drawn:

The run time around 3.55 minutes enables its application for routine analysis of mefenamic acid in pharmaceutical formulations.

Mobile phase consist of methanol 91% give better results than methanol 89%.

The pH (2.8 and 3.5), wavelengths (274nm and 276nm), flow rate (1.1 ml/min and 0.9 ml/min)

The obtained results are valuable not only from the scientific viewpoint but can also have practical value.

It was concluded that the newly method meets the development requirements for pharmaceutical analysis purposes pertaining mefenamic acid tablets.

Recommendation

Other mobile phase should be investigated to expand the range of solvents which can be used with mefenamic acid.

Develop and validate method for mefenamic acid with combination with other drug.

References

Araujo,P. (2009): Key aspects of analytical method validation and linearity evaluation. *J Chromatogr B AnalytTechnol Biomed Life Sci*; Aug, 2224–2234

Senthilkumar, B., Saravanakumaand, M. And Thirmurthy, R. (2010): Estimatiom of mefenamic acid and Drotaverine HCL combined dosage. *Dar pharma chemica*, 2(5): 170-177.

Breaux, J., Jones K., and Boulas , P. (2003): Understanding and implementing efficient analytical methods development and validation. *Pharm Technol Anal ChemTest*, 5: 6-13.

Dhumal B.R., Bhusari. K.P., Tajne M.R., Ghante M.H. and Jain NS.(2016): Stability Indicating Method for the Determination of Mefenamic Acid in Pharmaceutical Formulations by HPLC. *Journal* of *Applied Pharmaceutical Science*(4), *60-64.*

Fouad Fadhil Al-qaima,b, MdpauziAbdullahb,c, MohamedRozaliOthmanb,c and Wan mohdafiq wan mohdkhalik(2014) : Development and validation of hplc analytical assay method for mefenamic acid tablet (ponstan) *Int. J. Chem. Sci*.12(1), 62-72.

Gonzalez, G. and Herrador, M.A. (2007): A practical guide to analytical method validation, including measurement uncertainty and accuracy profiles. *Trends Anal Chem*; 26(3), 227-380.

Hubert, P., Nguyenhuu, J.J., Boulanger, B., Chapuzet, E. and Compagnon P. A, (2007): Harmonization of strategies for the validation of quantitative analytical procedures A SFSTP proposal-Part III. *J Pharm Biomed Anal,* 45(1),82-96.

Padmalatha.H.,.Dr.Vidyasagar,G., Teena Oswaletal.(2014):Development of Validated Analytical Method of Mefenamic Acid in an Emulgel. *International Journal of Pharma Sciences and Research (IJPSR),* 5: 232-237, India.

Heyden, Y., Nijhuis, A., Smeyers-Verbeke, J., Vandeginste, B.G.,and Massart, D. L., (2001):Guidance for robustness: ruggedness tests in method validation. *J Pharm Biomed Anal,* 24 (5),723-753.

Satinder Ahuja and Michael Dong (2005): Hand book of Pharmaceutical Analysis by HPLC, 600.

ICH Topic Q 2 (R1) (1995): Text and Methodology, note for guidance on validation of analytical procedures (1995), London.

ICH of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1), Complementary Guideline on Methodology, (1996), November, London.

Kumar Aulast, (2014): Method development and validation, *Drug development andtherapeutics*. (5): 109-114.

Natesan S., Thanasekaran D., Krishnaswami V., and Ponnusamy C. (2011): Improved Rp- Hplc Method for the Simultaneous Estimation of Tranexamic Acid and Mefenamic Acid in Tablet Dosage Form. *Pharm Anal Acta*,(2),115.

PuthetiR.R.,Okigbo R.N., Patil S.C., Advanapu M.S. and Leburu, R., (2008): Method development and validation s Characterization of critical elements in the development of pharmaceuticals. *Int J Health Res;* 11-20.

Padmalatha,H.,.Dr.G.Vidyasagar. Teena Oswaland *et.al*,(2014):Method devolpment and validation of mefnamic acid ,*international Journal of Pharma Sciences and Research*(5),232.

Raju Chandra, AshwaniSanghi, Deepak Kumar and Vinod Singh. (2014): A method validation for the estimation of mefenamic acid from marketed tablets by reversed phase high performance liquid chromatography (RP-HPLC) and UV-VIS simultaneous spectrophotometer,Der Pharmacia Lettre, (4):138-14

Swartz, M. E.andKrull, IS.,(1997): Analytical method development and validation by Michael. Journal of American chemical society(14) ,3540–3540New York .