International Journal of Pharmacy and Industrial Research (IJPIR)

ISSN: 2231-3656
Print: 2231-3648

IJPIR |Vol.13 | Issue 1 | Jan - Mar -2024
www.ijpir.com
DOI : https://doi.org/10.61096/ijpir.v13.iss1.2024.51-62

Research

Rp- Hplc Method Development And Validation Of Drotaverin And Mefanamic Acid In Bulk And Pharmaceutical Dosage Forms

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Published on:15 Feb 2024

Published by:
DrSriram Publications

Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Thiocolchicoside and Lornoxicam, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Altima C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol and water (5:95% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 285nm. The retention time of the Thiocolchicoside and Lornoxicam was 2.088, 6.068 ±0.02min respectively. The method produce linear responses in the concentration range of 10-50mg/ml of Thiocolchicoside and 20-100mg/ml of Lornoxicam. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Thiocolchicoside, Lornoxicam, RP-HPLC, Validation.

INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry,

physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.[1]

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance. [2]

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable. [1, 2]

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample. E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture. E.g. Potentiometry, Conductometry, Amperometry.[2]

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics. E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC). [2]

Miscellaneous Techniques

Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques

GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

INTRODUCTION TO HPLC

HPLC is also called as high pressure liquid chromatography since high pressure is used to increase the flow rate and efficient separation by forcing the mobile phase through at much higher rate. The pressure is applied using a pumping system. The development of HPLC from classical column chromatography can be attributed to the development of smaller particle sizes. Smaller particle size is important since they offer more surface area over the conventional large particle sizes. The HPLC is the method of choice in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages.

1. Improved resolution of separated substances
2. column packing with very small (3,5 and 10 µm) particles
3. Faster separation times (minutes)
4. Sensitivity
5. Reproducibility
6. continuous flow detectors capable of handling small flow rates
7. Easy sample recovery, handling and maintenance. [6]

Types of HPLC Techniques

Based on Modes of Chromatography

These distinctions are based on relative polarities of stationary and mobile phases

Reverse phase chromatography: In this the stationary phase is non-polar and mobile phase is polar. In this technique the polar compounds are eluted first and non polar compounds are retained in the column and eluted slowly. Therefore it is widely used technique.

Normal phase chromatography: In this the stationary phase is polar and mobile phase is non-polar. In this technique least polar compounds travel faster and are eluted first where as the polar compounds are retained in the column for longer time and eluted.[4]

Based on Principle of Separation

Liquid/solid chromatography (Adsorption): LSC, also called adsorption chromatography, the principle involved in this technique is adsorption of the components onto stationary phase when the sample solution is dissolved in mobile phase and passed through a column of stationary phase. The basis for separation is the selective adsorption of polar compounds; analytes that are more polar will be attracted more strongly to the active silica gel sites. The solvent strength of the mobile phase determines the rate at which adsorbed analytes are desorbed and elute. It is widely used for separation of isomers and classes of compounds differing in polarity and number of functional groups. It works best with compounds that have relatively low or intermediate polarity.[3]

Liquid/Liquid chromatography (Partition Chromatography): LLC, also called partition chromatography, involves a solid support, usually silica gel or kieselguhr, mechanically coated with a film of an organic liquid. A typical system for NP LLC column is coated with ß, ß'-oxy dipropionitrile and a non-polar solvent like hexane as the mobile phase. Analytes are separated by partitioning between the two phases as in solvent extraction. Components more soluble in the stationary liquid move more slowly and elute later.[1,2]

Ion exchange: In this the components are separated by exchange of ions between an ion exchange resin stationary phase and a mobile electrolyte phase. A cation exchange resin is used for the separation of cations and anion exchange resin is used to separate a mixture of anions. [3.16,17]

Size exclusion: In this type, the components of sample are separated according to their molecular sizes by using different gels (polyvinyl acetate gel, agarose gel). ex: separation of proteins, polysaccharides, enzymes and synthetic polymers. [3,15]

Chiral chromatography: In this type of chromatography optical isomers are separated by using chiral stationary phase.

Affinity chromatography: In this type, the components are separated by an equilibrium between a macromolecular and a small molecule for which it has a high biological specificity and hence affinity. [3]

Based on elution technique

Isocratic separation: In this technique, the same mobile phase combination is used throughout the process of separation. The same polarity or elution strength is maintained throughout the process.

Gradient separation: In this technique, a mobile phase combination of lower polarity or elution strength is followed by gradually increasing polarity or elution strength. [3]

Based on the scale of operation

Analytical HPLC: Where only analysis of samples are done. Recovery of samples for reusing is normally not done, since the sample used is very low. Ex: µg quantities.

Preparative HPLC: Where the individual fractions of pure compounds can be collected using fraction collector. The collected samples are reused. Ex: separation of few grams of mixtures by HPLC.[4]

Based on type of analysis

Qualitative analysis: Which is used to identify the compound, detect the presence of impurities to find out the number of components. This is done by using retention time values.

Quantitative analysis: This is done to determine the quantity of individual or several components of mixture. This is done by comparing the peak area of the standard and sample.

MATERIALS AND METHODS

Drotaverine-Sura labs, Mefenamic acid-Sura labs,Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck, Orthophosphoric acid-Sura labs, Trimethyl amine-Sura labs.

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Drotaverine and Mefenamic acid working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.8ml of Drotaverine and 2.5ml of Mefenamic acid from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: TEA Buffer in proportion 30:70 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry ODS C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	40ºC
Column	:	Symmetry C18 (4.6mm×150mm, 5.0 µm) particle size
pH	:	4.2
Mobile phase	:	Acetonitrile: TEA buffer pH 4.2 (40:60v/v)
Flow rate	:	1ml/min
Wavelength	:	275nm
Injection volume	:	10 µl
Run time	:	6 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine (TEA) buffer (pH-3.8)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.2. Fliter and sonicate the solution by vaccum filtration and ultra sonication.

Preparation of mobile phase

Accurately measured 400 ml (40%) of Acetonitrile and 600 ml of buffer (60%) a were mixed and degassed in digital ultra sonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Acetonitrile: TEA pH 4.2 (40:60)
Column : Symmetry C18 (4.6mm×150mm, 5.0 µm) particle size
Flow rate : 1 ml/min
Wavelength : 275 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 6 minutes

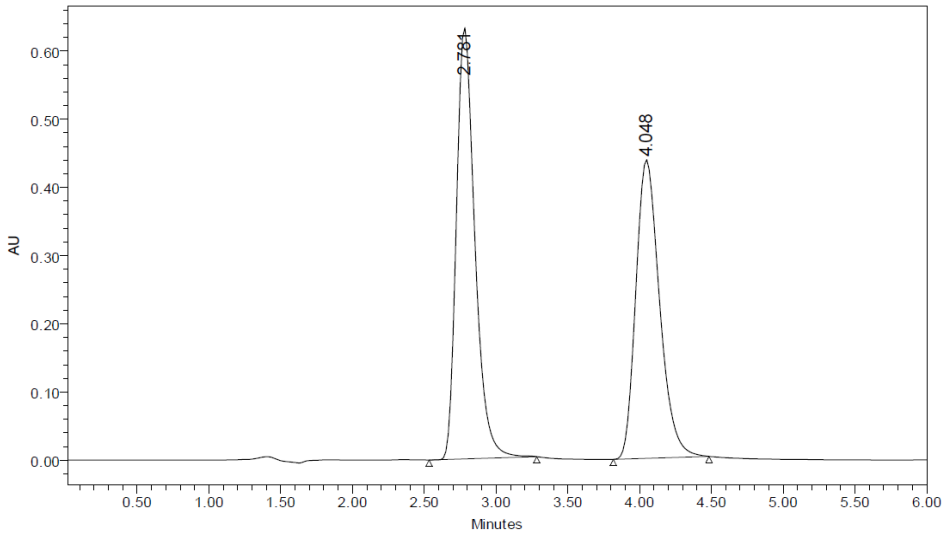

Fig 1: Optimized Chromatogram

Table 1: Peak Results for Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Drotaverine	2.781	2774027	299752		1.2	6314
2	Mefenamic Acid	4.048	2533532	210321	4.6	1.3	5521

From the above chromatogram it was observed that the Drotaverine and Mefenamic Acid peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

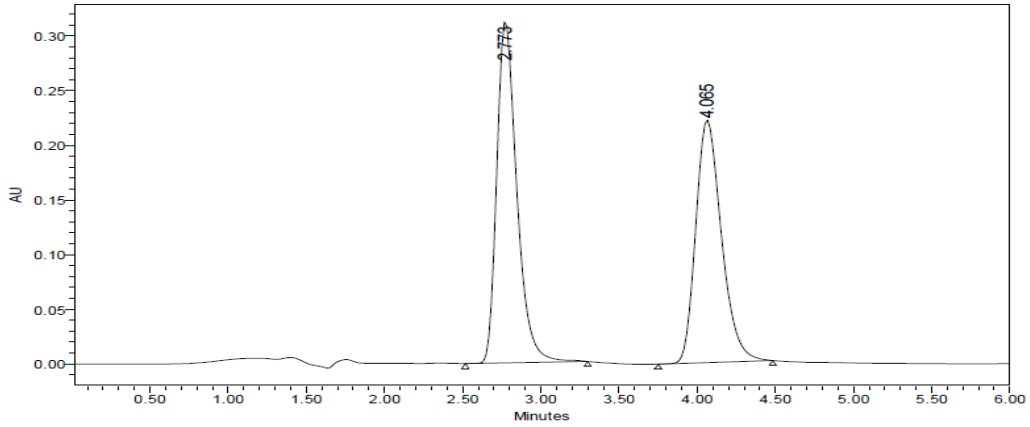

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Drotaverine	2.773	2770123	285417		1.6	5057
2	Mefenamic Acid	4.065	2522041	252546	3.3	1.5	5952

- Resolution between two drugs must be not less than 2
- Theoretical plates must be not less than 2000
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Assay (Standard)

Table 3: Peak results for assay standard of Drotaverine

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate
1	Drotaverine	2.782	2762937	356859	1.3	6344.7
2	Drotaverine dinitrate	2.766	2774613	387847	1.3	6368.2
3	Drotaverine	2.767	2762937	399481	1.3	6354.1
4	Drotaverine dinitrate	2.795	2774613	386985	1.3	6341.7
5	Drotaverine	2.768	2776429	365478	1.3	6347.2
Mean			2770306			
Std. Dev.			6767.495			
% RSD			0.2			

Table 4: Peak results for assay standard of Mefenamic Acid

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Mefenamic Acid	4.049	2540214	237854	4.6	1.3	5948.7
2	Mefenamic Acid	4.025	2541284	225688	4.7	1.3	5254.8
3	Mefenamic Acid	4.029	2534375	215324	4.6	1.3	5948.7
4	Mefenamic Acid	4.067	2526189	224859	4.7	1.3	5265.8

5	Mefenamic Acid	4.030	2546248	232547	4.7	1.3	5994.7
Mean			2537662				
Std. Dev.			7677.647				
% RSD			0.3				

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample)

Table 5: Peak Results for Assay sample

S.No	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Drotaverine	2.764	2732203	296854		1.3	6353	1
2	Mefenamic Acid	4.012	2507543	217548	4.6	1.3	5984	1
3	Drotaverine	2.767	2751843	286524		1.3	6398	2
4	Mefenamic Acid	4.016		216685	4.6	1.3	5965	2
5	Drotaverine	2.764	2744776	318546		1.3	6355	3
6	Mefenamic Acid	4.013	2515628	204584	4.6	1.3	5998	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

27429401/2770306×10/28.125×28.125/0.035×99.7/100×0.1342/37.5×100
100. 9%.
The % purity of Drotaverine in pharmaceutical dosage form was found to be 100. 9%.

LINEARITY: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:
Drotaverine

Concentration µg/ml	Average Peak Area
60	1992464
70	2316364
80	2677423
90	3019213
100	3361317

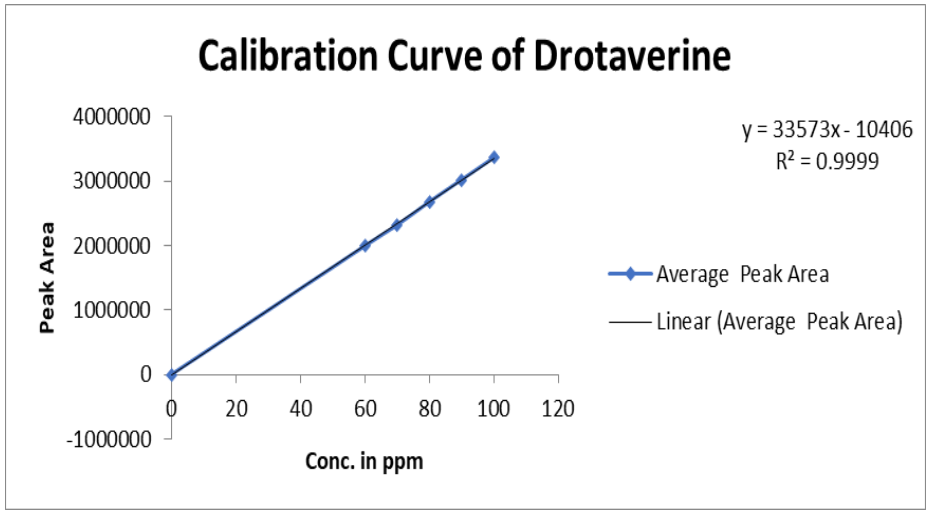

Fig 3: Calibration Graph for Drotaverine

Mefenamic Acid

Concentration µg/ml	Average Peak Area
187.5	2080032
218.75	2452782
250	2821426
281.25	3226009
312.5	3587393

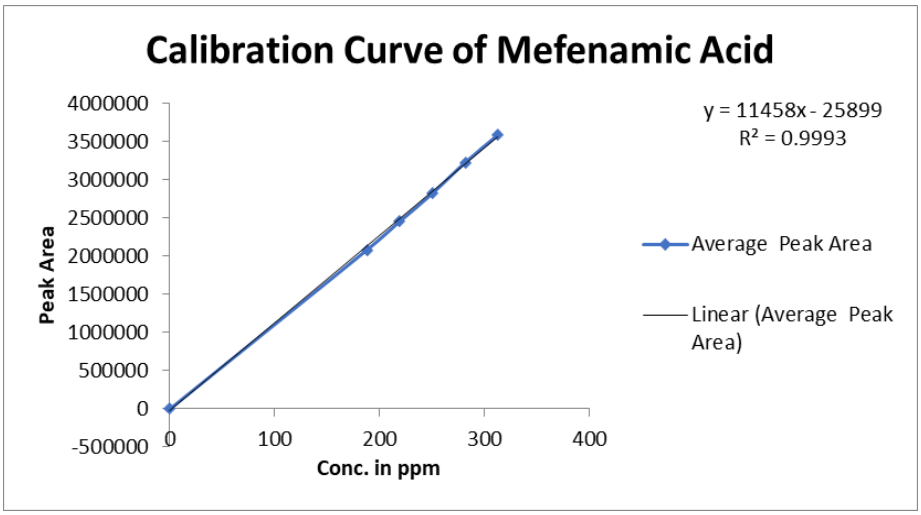

Fig 4: Calibration Graph for Mefenamic Acid

REPEATABILITY

Table 6: Results of repeatability for Drotaverine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Drotaverine	2.766	2766870	294578	6684	1.3
2	Drotaverine	2.774	2771971	286541	6347	1.3
3	Drotaverine	2.770	2771958	302657	6674	1.3

4	Drotaverine	2.772	2780299	293412	6451	1.3
5	Drotaverine	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.896			
% RSD			0.3			

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 7: Results of method precession for Mefenamic Acid

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Mefenamic Acid	4.025	2534539	193240	5761	1.3	4.7
2	Mefenamic Acid	4.040	2539247	201647	5489	1.3	4.6
3	Mefenamic Acid	4.032	2544661	193472	5367	1.3	4.6
4	Mefenamic Acid	4.041	2548839	196475	5845	1.3	4.6
5	Mefenamic Acid	4.036	2558822	201394	5347	1.3	4.7
Mean			2545222				
Std.Dev			9329.852				
% RSD			0.3				

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision

Table 8: Results of Intermediate precision for Drotaverine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Drotaverine	2.781	2715421	296585	6785	1.3
2	Drotaverine	2.780	2778540	284584	6856	1.3
3	Drotaverine	2.782	2754247	275698	6934	1.3
4	Drotaverine	2.780	2780545	282451	6484	1.3
5	Drotaverine	2.782	2777021	283654	6669	1.3
6	Drotaverine	2.774	2780254	296587	6584	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

- %RSD of five different sample solutions should not more than 2.

Table 9: Results of Intermediate precision for Mefenamic Acid

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Mefenamic Acid	4.048	2506927	212541	5486	1.4	4.6
2	Mefenamic Acid	4.050	2504522	203658	5659	1.4	4.6
3	Mefenamic Acid	4.049	2541270	198458	5857	1.4	4.7
4	Mefenamic Acid	4.050	2507885	207554	5968	1.4	4.6
5	Mefenamic Acid	4.049	2504587	206455	5784	1.4	4.6
6	Mefenamic Acid	4.040	2504780	214521	5969	1.4	4.6
Mean			2511662				
Std. Dev			14572.01				
% RSD			0.5				

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table 10: Results of Intermediate precision Day 2 for Drotaverine

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Drotaverine	2.764	2781856	295682	6698	1.3
2	Drotaverine	2.759	2761510	284857	6764	1.3
3	Drotaverine	3.015	2748811	276532	6942	1.3
4	Drotaverine	2.773	2790831	282354	6461	1.3
5	Drotaverine	2.765	2785112	285698	6659	1.3
6	Drotaverine	2.764	2781932	295663	6685	1.3
Mean			2775009			
Std. Dev			16222.05			
% RSD			0.5			

- %RSD of five different sample solutions should not more than 2

Table 11: Results of Intermediate precision for Mefenamic Acid

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Mefenamic Acid	4.015	2536301	212532	5569	1.4	4.6
2	Mefenamic Acid	4.007	2541972	205682	5596	1.4	4.6
3	Mefenamic Acid	4.323	2521259	199686	5754	1.4	4.7
4	Mefenamic Acid	4.065	2537081	202548	5996	1.4	4.6
5	Mefenamic Acid	4.020	2549869	208989	5785	1.4	4.6
6	Mefenamic Acid	4.015	2536301	201245	5964	1.4	4.6
Mean			2537131				
Std. Dev			9370.087				
% RSD			0.3				

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

ACCURACY

The accuracy results for Drotaverine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1361022	40	40.228	100.57	
100%	2698948	80	80.079	100.098	**100.387%**
150%	4059065	120	120.592	100.493	

The accuracy results for Mefenamic Acid

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1459598	125	125.126	100.100	
100%	2894368	250	250.346	100.138	100.098**%**
150%	4325099	375	375.213	100.056	

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 12: Results for Robustness

Drotaverine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Mefenamic Acid

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5
Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Drotaverine and Mefenamic Acid in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Drotaverine was found to be Soluble in DMSO, Methanol and Water and Mefenamic Acid was found to be high solubility in dipolar aprotic solvents (N,N-dimethylacetamide, N,N-dimethylformamide, ethyl acetate, and propanone), moderate solubility in polar protic solvents (ethanol and propan-2-ol), and poor solubility in apolar aprotic solvents (hexane, heptane, and cyclohexane). Acetonitrile: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Drotaverine and Mefenamic Acid in bulk drug and in Pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The Authors are thankful to the Management and Principal, Department of Pharmacy, Samskruti college of pharmacy in Ghatkesar, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

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