International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 7 | Issue 2 | APR-JUN| 2017 | 211-222
Original Research Article – Pharmaceutical Sciences| Open Access| UGC Approved | MCI Approved Journal

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR DETERMINATION OF DROTAVERINE HYDROCHLORIDE AND MEFENAMIC ACID IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING RP–HPLC

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ABSTRACT

A simple and accurate, precise method was developed for the simultaneous estimation of the Drotaverine Hydrochloride and Mefenamic Acid in bulk and pharmaceutical dosage form. The chromatography was performed by running through X-Bridge C18 Column which is having 4.6X150mm I.D with 5µm particle size. Column temperature was maintained at ambient, with a mobile phase phosphate buffer: Methanol at the ratio of 30:70v/v. The flow rate was 1ml/min and the UV detections was carried out at 290nm. Retention time of Drotaverine Hydrochloride and Mefenamic Acid were found to be 2.089 mins and 5.372 mins. The percentage purity of the Drotaverine Hydrochloride and Mefenamic Acid were found to be 99.30% and 101% respectively. The average percentage recovery of Drotaverine Hydrochloride and Mefenamic Acid was found to be 99.50% and 100.1%. The LOD values of Drotaverine Hydrochloride and Mefenamic Acid was 0.38 µg/ml, 0.11 µg/ml and LOQ values were 1.16 µg/ml and 0.34 µg/ml. The %RSD was found to be less than 2%, the method was validated as per ICH guidelines. The percentage recovery was in good agreement and the method is simple, specific, precise, and accurate for the determination of Drotaverine Hydrochloride and Mefenamic Acid which can be applied for the routine quality control analysis. The statistical parameters and recovery studies were carried out and reported.

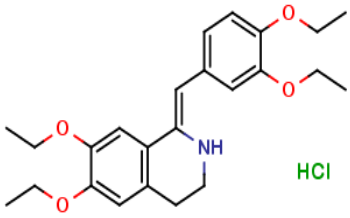
KEY WORDS

Drotaverine HCl, Bulk & Dosage Form, Development, Validation, RP-HPLC

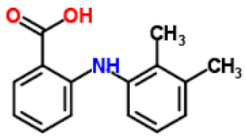
INTRODUCTION

Drotaverine hydrochloride is chemically known as 1-[(3, 4-[diethoxyphenyl) methylene]-6, 7 diethoxy-1, 2, 3, 4 – tetra hydro isoquinolene hydrochloride [1]. Drotaverine hydrochloride is highly potent spasmolytic agent [2]. It acts as an antispasmodic agent by inhibiting phosphodiesterase IV enzyme, specific for smooth muscle spasm and pain, used to reduce excessive labor pain [3]. Drotaverine hydrochloride is official in Polish Pharmacopoeia [4]. A few UV spectrophotometric and HPLC [methods have been reported for estimation of drotaverine hydrochloride. HPLC methods [5-9].

Mefenamic acid, 2- [(2, 3-dimethyl phenyl) amino] benzoic acid, is an orally active analgesic and anti-inflammatory drug, used to relieve pain [10]. Mefenamic acid is official in IP [11], BP [12] and USP [13]. Several UV spectrophotometric [14,15], HPLC [16-17] and HPTLC [18] methods for the estimation of mefenamic acid have been reported.



Drotaverine hydrochloride



Mefenamic acid

Literature survey revealed a need for a method capable of simultaneous estimation of drotaverine hydrochloride and mefenamic acid [19]. The objective of this study was to develop and validate a specific, accurate, precise and reproducible quality control method for drotaverine hydrochloride and mefenamic acid in their combination. To our knowledge there is no HPLC method reported for the combination, availability of HPLC method with high sensitivity and selectivity will be very useful for the estimation of Mefenamic acid and Drotaverine HCl in combined pharmaceutical dosage forms [20-21]. Therefore, the aim of the study was to develop a sensitive, precise, accurate and specific HPLC method for the determination of Mefenamic acid and Drotaverine HCl simultaneously in formulation. The present work describes a simple RP-HPLC PDA method for the determination of Mefenamic acid and Drotaverine HCl in tablets. The method was validated according to ICH guidelines and was found to be reproducible with good resolution between Mefenamic acid and Drotaverine HCl.

MATERIALS AND METHODS

Reagents & Chemicals

Mefenamic acid and Drotaverine HCl were obtained as a gift sample from Sura Laboratories (Hyderabad, India), Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Phosphate buffer, Ortho phosphoric acid, Milli Q water and Methanol (HPLC grade, MERCK). Mobile phase was filter through a 0.45µ membrane filter were used for the preparation of sample Solutions. All chemicals were of an analytical grade and used as received.

Instrumentation

Chromatographic separation was achieved by using a Waters 2489 UV 2695 pump, Waters 2998 PDA 2695 pump Software Empower2 photodiode array detector was used.

Chromatographic conditions

A Symmetry C-18 (Make: Waters, 150 mmx4.6 mm I.D; particle size 5µm) Column was used for analysis at ambient column temperature. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 20µL. The photodiode array detector was set to a wavelength of 290nm for the detection and Chromatographic runtime was 30minutes.

Preparation of Standard

Weighted accurately 10mg of Drotaverine HCl and Mefenamic acid working standards and transferred into 10ml volumetric flask and added 7ml of diluents and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further, 0.9ml from the above stock solution was transferred into 10ml volumetric flask and diluted with the diluent.

Preparation of mobile phase

Buffer preparation

Buffer was prepared by dissolving 2.95gms of Potassium dihydrogen phosphate and 5.45gms of Dipotassium hydrogen phosphate into a 1000mL of purified water and mixed. Adjusted pH to 3.0 with dilute ortho phosphoric acid solution. The solution was sonicated and Filter the solution through 0.45µm membrane filter.

Mobile phase preparation

Mobile Phase - A:

Use filtered and degassed buffer as mobile phase A

Mobile Phase – B:

Prepare a filtered and degassed mixture of Buffer and methanol in the ratio of 300:700 v/v respectively.

Diluent preparation

Mobile phase-B is used as diluent

Sample Preparation

Accurately weighed 10 combination tablets crushed to fine powder with the help of mortar and pestle. Powder equivalent to 10mg of Drotaverine HCL and Mefenamic acid was weighed and transferred to 10ml dry volumetric flask and 7ml of diluent was added followed by sonication for 5mins. Solution was allowed to cool at room temperature. The solution was filtered through 0.45µm membrane filters. Then 0.9ml of the above stock solution was transferred into 10ml of volumetric flask and was diluted up to the mark with the diluent.

METHOD DEVELOPMENT

To develop a suitable and robust RP-HPLC method for the determination of Drotaverine HCL and Mefenamic acid, different mobile phases were employed to achieve the best separation and resolution. The method development was started with AGILENT 150mm Column with the mobile phase composition of Methanol and Phosphate buffer with the ratio of 80:20 v/v. The flow rate is maintained 0.5 ml/min. At the first trail, no peaks were identified. At the second trial, maintained flow rate at 1.0 ml/ min. Here we got more retention time for Drotaverine HCL and Mefenamic acid was 7.116 mins and 12.745 mins. For the next trail 3, analysis was run through C18 X-Bridge Column with 4.6X150mm I.D with mobile phase ratio 75:25 v/v with flow rate 0.5ml/ min. We got retention times at 4.890 mins and 6.019mins for Drotaverine HCL and Mefenamic acid but the baseline is not improper. Trail 4, flow rate was maintained 1.0ml/ min. mobile phase composition was kept in the ratio of 70:30 v/v. R_t for Drotaverine HCL and Mefenamic acid was 2.020 mins, 4.499 mins but we got improper baseline. For next trail 5, mobile phase composition was 75:25 v/v, flow rate was maintained 1.0ml/ min but R_t for Drotaverine HCL and Mefenamic acid was 2.420mins and 10.284 mins, more R_t value we got. Next trail 6, mobile phase composition was maintained as 70:30 v/v, flow rate is 0.5ml/min. We got retentions times as 2.018 mins, 4.483 mins Drotaverine HCL and Mefenamic acid. Here we got impurities peaks. Next trail 7, mobile phase composition is 75:25v/v, flow rate was 1.0ml/ min. We got retention time for Drotaverine HCL and Mefenamic acid was 3.528 mins and 5.414 mins. But Improper base line was attainted. At last trail 8, mobile phase composition was 70:30 v/v, flow rate 1.0ml/ min. Retention times for Drotaverine HCL and Mefenamic acid was 2.089 mins and 5.327 mins. At this trail the separation was completely done with good peak shapes.

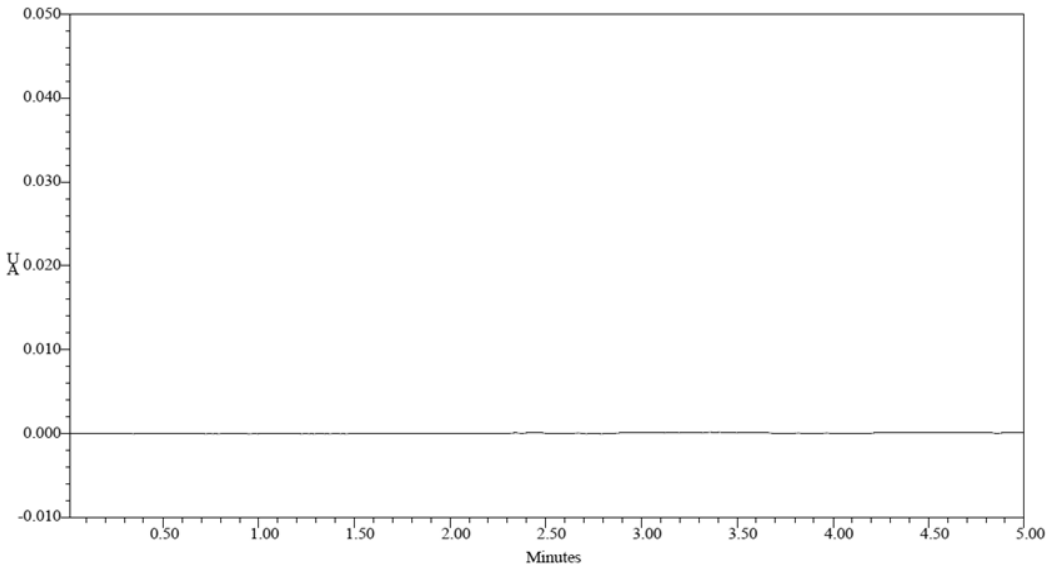


Figure 1: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 1

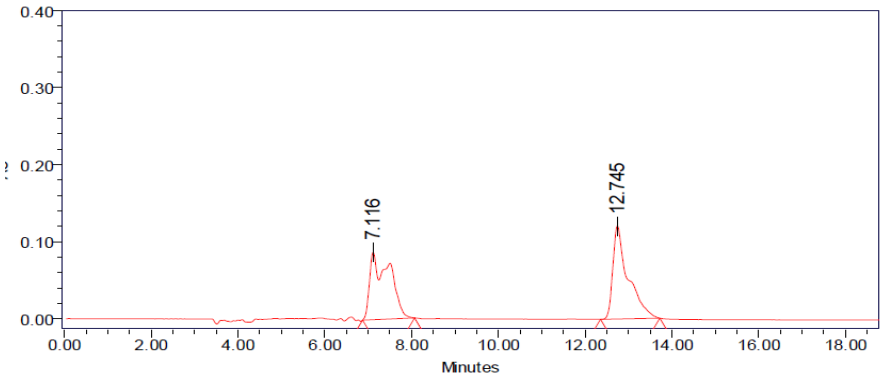


Figure 2: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 2

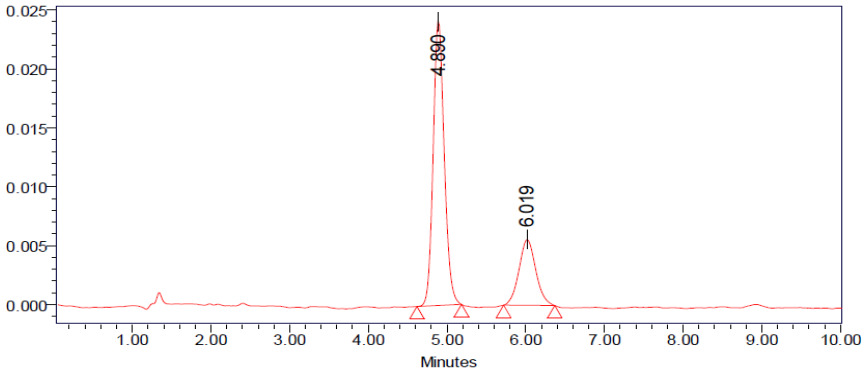


Figure 3: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 3

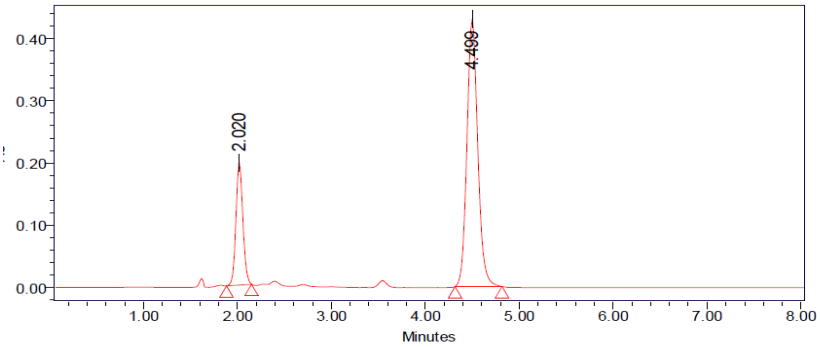


Figure 4: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 4

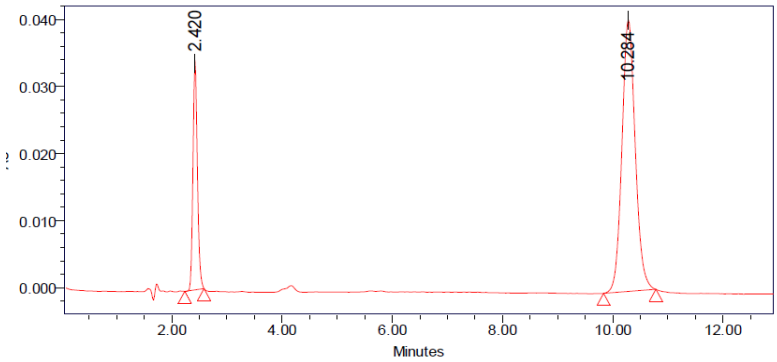


Figure 5: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 5

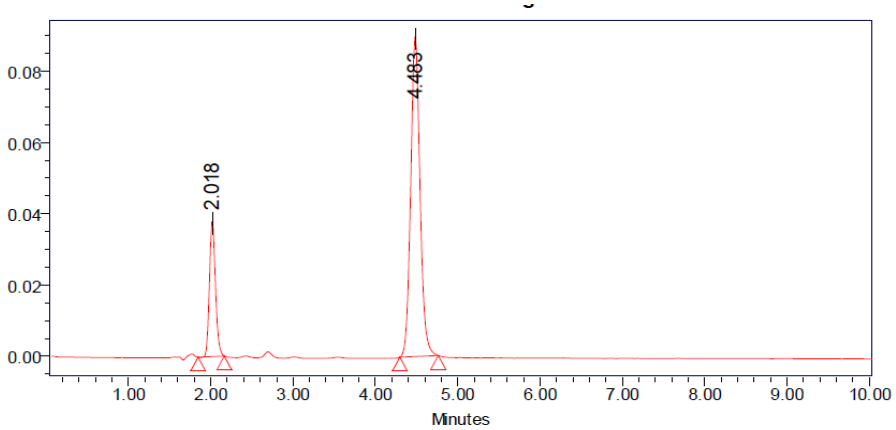


Figure 6: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 6

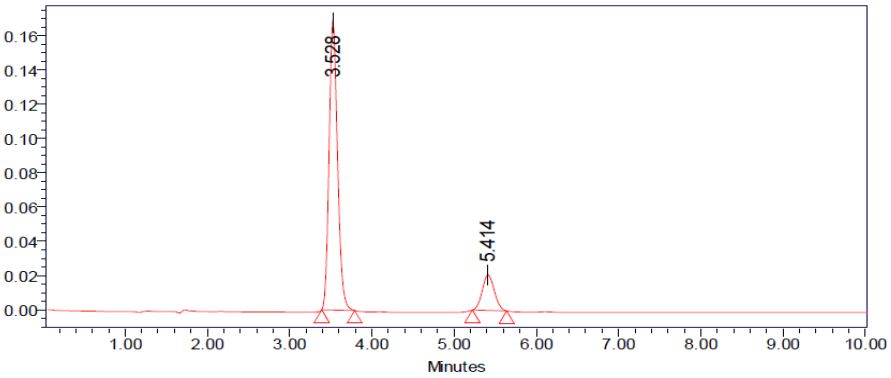


Figure 7: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 7

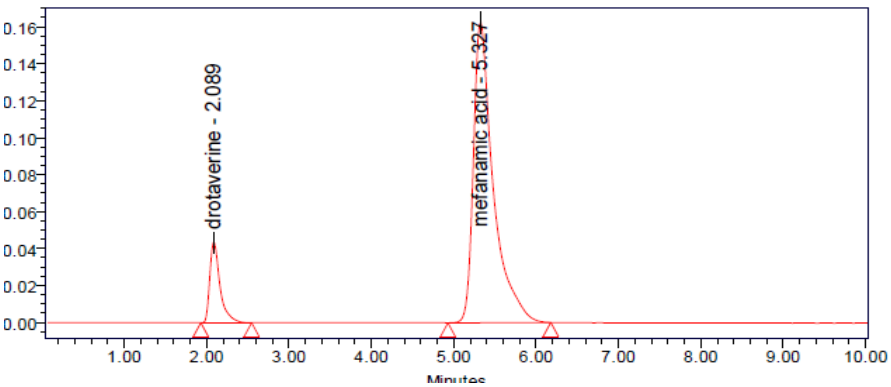


Figure 8: Chromatogram for Drotaverine HCL and Mefenamic acid at Trail 8

METHOD VALIDATION

System Suitability

To demonstrate system suitability, the standard solution prepared as per method. Weighed accurately 10 mg of Drotaverine HCL and Mefenamic acid working standard was weighed and transferred to 10 ml clean and dry volumetric flask. Add 7ml of diluent and sonicate to dissolve it completely and dilute it with diluent. From the above solution, transfer 0.9 ml of stock solution into 10 ml of flask and again dilute it with diluent. This solution is injected six replicate injections into the HPLC system as per methodology. The system suitability parameters were evaluated from the standard solution and found to be within the acceptance criteria. The % RSD for Drotaverine HCL and Mefenamic acid peak areas from six replicate injections of standard solution was found to be within the limits. The results are summarized in Table-1 and Figure 9.

Table 1: System Suitability for Drotaverine HCL and Mefenamic acid

S.No	Name of the Compound	R_t mins	Peak Area	Height	USP Theoretical Plate Count	USP Tailing Factor	%RSD
1.	Drotaverine HCl	2.089	376138	43857	2243	1.68	0.2
2.	Mefenamic acid	5.327	2729173	162591	2714	1.86	0.43

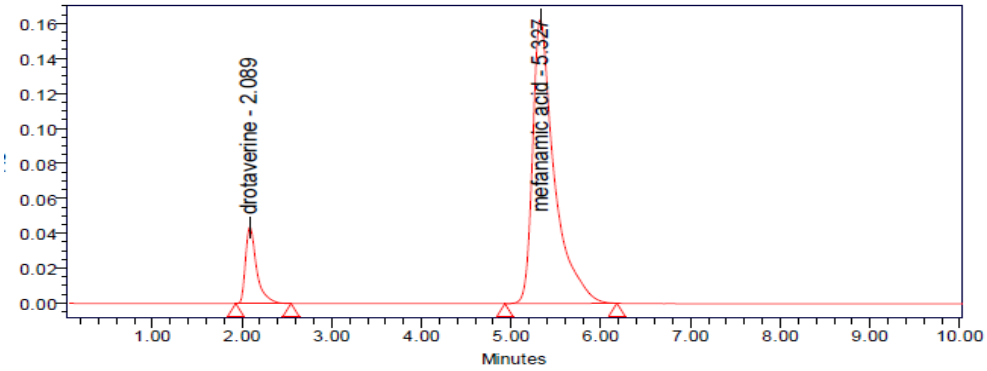


Figure 9: System Suitability Chromatogram for Drotaverine HCL and Mefenamic acid

Specificity

Blank interference

The specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The study was performed by injecting blank. No interferences were found in the Drotaverine HCL and Mefenamic acid retention times.

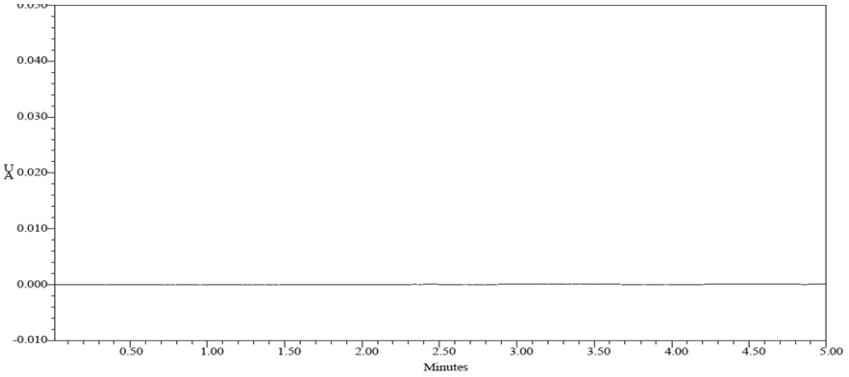


Figure 10: A typical HPLC Chromatogram showing the no interference of diluent

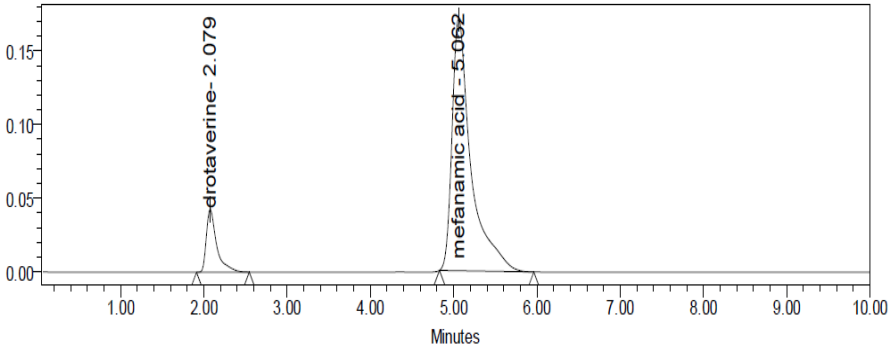


Figure 11: Representative Chromatogram of Standard

Table 2: Specificity Chromatogram for Drotaverine HCL and Mefenamic acid

S.No.	Name of the Compound	R_t (mins)	Peak Area	Height	USP Theoretical Plate Count	USP Tailing Factor	Resolution
1.	Drotaverine HCl	2.079	369866	42795	2589.0	1.9	8
2.	Mefenamic acid	5.062	2696009	171710	3164.1	2.1	10.0

Establishment of Limit of Detection and Limit of Quantification:

A study was conducted to establish the limit of detection (LOD) and limit of quantification (LOQ) of Drotaverine HCL and Mefenamic acid based on slope method. Prepared a series of solutions from 10% to 50% of standard concentration of Drotaverine HCL and 30% to 150% of Mefenamic acid These solutions were injected into the HPLC system as per methodology. Plotted a graph by taking concentration on X-axis and area on Y-axis, calculated the standard error and slope of the calibration curve. The predicted LOQ concentration and LOD concentration are calculated by using formula given below. The results are summarized in the Table 3-4.

$$LOQ = \frac{10 \times \sigma}{S} \qquad LOD = \frac{3.3 \times \sigma}{S}$$

σ = Standard Error of the calibration curve

S = Slope of the calibration curve

Table 3: Results of LOD and LOQ of Drotaverine HCL and Mefenamic acid

S.No.	Name of the Compound	LOD	LOQ
1.	Drotaverine HCl	0.38µg/ml	1.16µg/ml
2.	Mefenamic acid	0.11µg/ml	0.3µg/ml

Linearity:

Linearity is carried out under LOD-LOQ establishment experiment, the same linearity establishment data can be used to deduce the linearity from 10 ppm to 50 ppm for Drotaverine HCl and 30 ppm to 150 ppm for Mefenamic acid. A graph was plotted to concentration in ppm on X-axis versus response on Y-axis. Calculated % y-intercept and correlation coefficient. The calibration curve for both Drotaverine HCL and Mefenamic acid are shown as in the Figures 12, 13.

Table 4: The results and the linearity graph of Drotaverine HCL

Drotaverine HCL			
Name of the Level	**Concentration In ppm**	**Area Response**	**R_t mins**
Level - 1	0	0	0
Level - 2	10	132855	2.081
Level - 3	20	243880	2.080
Level - 4	30	369866	2.073
Level - 5	40	497340	2.073
Level - 6	50	610491	2.074
Correction Coefficient			0.999

Figure 12: Calibration Curve for Drotaverine HCL

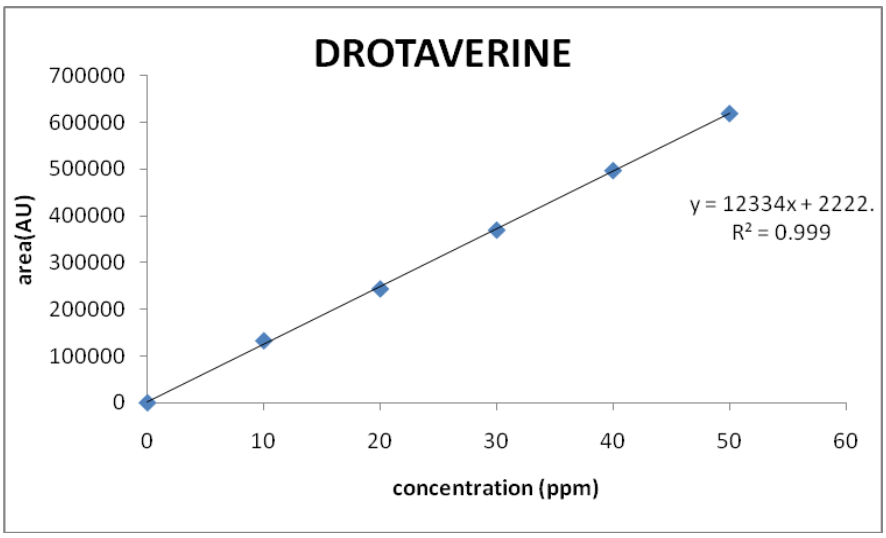
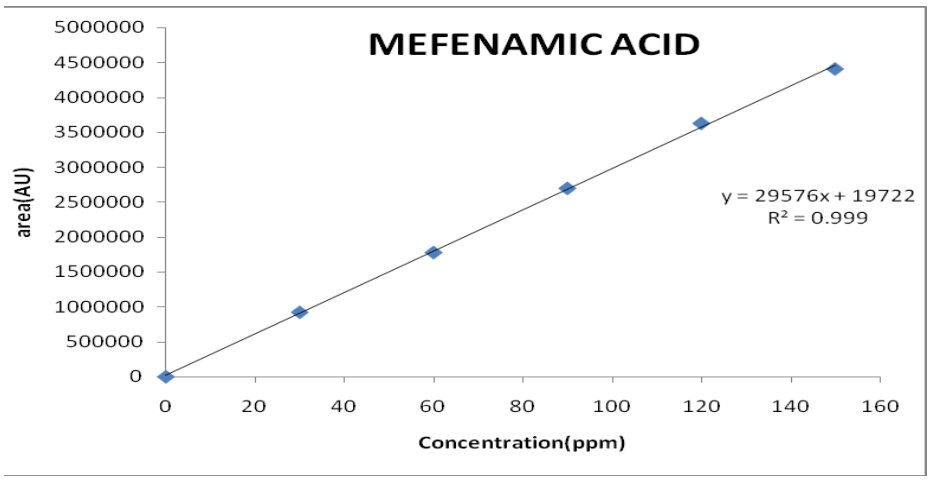


Table 5: The results and the linearity graph of Mefenamic acid

Mefenamic acid			
Name of the Level	**Concentration In ppm**	**Area Response**	**R_t mins**
Level - 1	0	0	0
Level - 2	30	922308	5.086
Level - 3	60	1677910	5.084
Level - 4	90	2696009	5.062
Level - 5	120	3626466	5.047
Level - 6	150	4404849	5.052
Correction Coefficient			**0.999**

Figure 13: Calibration Curve for Mefenamic acid



Precision:

Repeatability

The standard solution was injected for the five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injection was found to be within specified limits.

Table 6: Results showing Repeatability of Drotaverine HCL

Drotaverine HCL				
S.No.	**Injections**	**Area Response**	**R_t (mins)**	**Peak Height**
1.	I	369212	2.086	42515
2.	II	369988	2.083	42308
3.	III	370020	2.083	42776
4.	IV	370868	2.081	42944
5.	V	371366	2.081	42225
		Average		**370290.8**
		Standard Deviation		**839.3514**
		% RSD		**0.226**

Table 7: Results showing Repeatability of Mefenamic acid

Mefenamic acid				
S.No.	**Injections**	**Area Response**	**R_t (mins)**	**Peak Height**
1.	I	2731586	5.199	164371
2.	II	2732792	5.202	166468
3.	III	2732792	5.178	167541
4.	IV	2734689	5.235	166530
5.	V	2735120	5.206	167929
		Average		**2733396**
		Standard Deviation		**1470.523**
		% RSD		**0.053**

Intermediate precision / Ruggedness

The standard solution was injected for five times and measured the area for all five injections. The % RSD for the area of five replicate injections was found to be within the acceptable limits.

Table 8: Results showing for Intermediate precision of Drotaverine HCL

Drotaverine HCL				
S.No.	**Injections**	**Area Response**	**R_t (mins)**	**Peak Height**
1.	I	370351	2.083	42361
2.	II	370941	2.083	42205
3.	III	368833	2.089	42416
4.	IV	369642	2.082	42616
5.	V	370013	2.078	43015
		Average		**369956**
		Standard Deviation		**788.8637**
		% RSD		**0.02**

Table 9: Results showing for Intermediate precision of Mefenamic acid

Mefenamic acid				
S.No.	**Injections**	**Area Response**	**R_t (mins)**	**Peak Height**
1.	I	2731586	5.229	165429
2.	II	2732792	5.203	167393
3.	III	2732792	5.132	169637
4.	IV	2734689	5.112	171655
5.	V	2735120	5.151	169607
		Average		**2727488**
		Standard Deviation		**11738.96**
		% RSD		**0.43**

Accuracy

The study was performed for 50 %, 100 % and 150 % for Drotaverine HCL and Mefenamic acid. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. The results were summarized below.

Table 10: Results showing for Accuracy of Drotaverine HCL

S.No.	% Concentration (At Specification Level)	Average Peak Area	Amount Added (mg)	Amount found (mg)	% Recovery	Mean Recovery %
1.	50%	464023.3	5	4.99	99.80	99.50
2.	100%	601467	10	9.95	99.50	99.50
3.	150%	789470	15	14.88	99.20	99.50

Table 11: Results showing for Accuracy of Drotaverine HCL

S.No.	% Concentration (At Specification Level)	Average Peak Area	Amount Added (mg)	Amount found (mg)	% Recovery	Mean Recovery
1.	50%	4887.69	5	5.1	102	100.1
2.	100%	6522456	10	9.9	99	100.1
3.	150%	6905412	15	14.90	99.33	100.1

Robustness

Similarly, Robustness also evaluated and found that the method is robust enough for various robustness parameters such as flow variation, column temperature variation, mobile phase composition variation.

All the system suitability criteria are meeting in all the robust parameters, this indicates that the proposed analytical method is robust enough for the estimation of Drotaverine HCL and Mefenamic acid by using the analytical method.

Table 12: Results showing for Robustnessof Drotaverine HCL

S.No.	Flow Rate (ml/min)	USP Plate Count	USP Tailing Factor
1.	0.8	2558	1.01
2.	1.0	2243	1.68
3.	1.2	2391	1.03

Table 13: Results showing for Robustness of Drotaverine HCL

S.No.	Flow Rate (ml/min)	USP Plate Count	USP Tailing Factor
1.	0.8	3438	1.14
2.	1.0	2714	1.86
3.	1.2	2783	1.33

Assay

Standard preparations were made from the API and sample preparation are from formulation. Both the sample and standards are injected six homogenous samples. Drug in the formulation was estimated by taking the standard as the reference.

Table 14: Results showing Assay for Drotaverine HCL and Mefenamic acid

S.No.	Name of the Compound	Amount Present (ppm)	Amount Found (ppm)	% Purity
1.	Drotaverine HCL	30	29.8	99.3
2.	Mefenamic acid	90	91	101

CONCLUSION

A simple, economic, accurate and precise RP-HPLC method was successfully developed. In this method, it was carried out by using symmetry C18, (150× 4.6mm) with 5µm particle size. Injection volume of 20µl is injected and eluted with the mobile phase A as buffer of KH_2PO_4, pH 3.0 adjusted with dilute ortho phosphoric acid and buffer and methanol as mobile phase B over gradient program, which is pumped at a flow rate of 1.0 ml/min. Detection, was carried out at 290 nm. The two compounds are well resolved from each peak and there is no interference from blank. The results obtained were accurate and reproducible. The method developed was statistically validated in terms of Selectivity, accuracy, linearity, precision, robustness, stability of solution and mobile phase stability.

For Selectivity, the chromatograms were recorded for standard and sample solutions of Drotaverine HCL and Mefenamic acid. Selectivity studies reveal that the peak is well separated from each other. Therefore, the method is selective for the determination of Drotaverine HCL and Mefenamic acid.

The limit of detection (LOD) and limit of quantitation (LOQ) was found to be for 0.038µg/ml and 1.16µg/ml respectively for Drotaverine HCL, 0.11µg/ml and 0.34 µg/ml for Mefenamic acid. The linearity results for Drotaverine HCL and Mefenamic acid in the specified concentration range are found satisfactory, with a correlation coefficient 0.999% and 0.999%. Calibration curve was plotted and correlation co-efficient for Drotaverine HCL and Mefenamic acid found to be more than 0.95.

The accuracy studies were shown as % recovery for Drotaverine HCL and Mefenamic acid at 50%, 100% and 150%. The limit of % recovered shown is not less than 80% and the results obtained were found to be within the limits. Hence the method was found to be accurate. The accuracy studies showed % recovery of the Drotaverine HCL and Mefenamic acid was found to be 99.50 and 100.1% respectively.

For Precision studies six (6) replicate injections were performed. %RSD was determined from the peak areas of Drotaverine HCL and Mefenamic acid. The acceptance limit should be not more than 10 %RSD, and the results were found to be within the acceptance limits. For intermediate precision, the bias is not more than ± 1.0.

Hence, the chromatographic method developed for Drotaverine HCL and Mefenamic acid are rapid, simple, sensitive, precise, and accurate. Therefore, the proposed method can be successfully applied for the routine analysis of Drotaverine HCL and Mefenamic acid in API and Pharmaceutical dosage form.

REFERENCE

1. Rockville, MD (2005). United States Pharmacopoeia 28th ed, National Formulary 23, U.S Pharmacopeial convention. p. 1064.
2. L.R. Snyder, J.J. Kirkland, J.L (1997). Glajch, Practical HPLC Method Development, 2nd ed., John Wiley and Sons Inc., New York. p. 709.
3. L.R. Snyder, J.J. Kirkland, J.L (1997). Glajch, Practical HPLC Method Development, 2nd ed., John Wiley and Sons Inc., New York. p. 19.
4. ICH, (November 1996). Q2B Analytical Validation Methodology Answers. Chromatography Questions. What detectors are used in HPLC [Online].2012[cited2012]; Available From: URL: http://wiki.answers.om/Q/what detectors are used in HPLC.
5. ICH, (October 1994). Q2A, Text on validation of analytical procedure
6. ICH, (2005). Q2 (R1), Technical Requirements for registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology.
7. Skoog, Holler, Crouch **(**2011**)**. *Instrumental Analysis*. 8th Ed. New Delhi:Cengage Learning India Pvt. Ltd, p. 839-924.
8. Drug Profile, mefenamic acid, http://www.drugs.com/ppa/mefenamic acid.html, as accessed on.

9. Sharma A and Gangwal S (1996). Simultaneous spectrophotometric determination of mefenamic acid and paracetamol on combined pharmaceutical dosage forms. Indian J. Pharm. Sci, p. 216-18.
10. Parimoo P, Bhara A and Padma K (1996). Simultaneous estimation of mefenamic acid and paracetamol in drug preparations by UV-Absorption spectroscopy. Indian J. Pharm. Sci. p. 92-290.
11. Dhake AS, Sonaje DB, Nikam PT, Talekar RS and Kasture VS (2001). Simultaneous determination of mefenamic acid and paracetamol from combined dosage froms by spectroscopy. Indian J. Pharm. Sci. p. 50-60.
12. Rouini MR, Asadipour A, Ardakani YH and Aghdasi F (2004). Liquid chromatography method for determination of mefenamic acid in human serum. *J. Chromatogr*. p. 92-189.
13. http://www.wikipedia.com/drotaverine hydroxichloride
14. Martindale (2002). The complete Drug Reference. 32nd edition, Pharmaceutical Press. p. 1574-1582.
15. Prasad R.K., Sharma R (2010). Int. J. Pharm. Sci. and Drug Research, Vol. 2, p. 67-70.
16. Budavari S (2004). The Merck Index. Merck & Co., Inc., New Jersey. p. 609-1100.
17. Sweetman SC (2002). Martindale: The Complete Dug Reference. Pharmaceutical Press, London. P. 160-170.
18. Dahivelkar PP, Bari SB and Surana SJ (2007) Simultaneous derivative and multicomponent spectrophotometric determination of drotaverine hydrochloride and mefenamic acid in tablets. Indian J. Pahrm. Sci. p. 14-30.
19. Dahivelkar PP, Bari SB and Surana SJ (2006). Spectrophotometric method for simultaneous estimation of drotaverine hydrochloride and mefenamic acid in bulk and tablet formulation. Indian Drugs. p. 896-900.
20. British Pharmacopoeia, 2002. Vol II. A. Constable London. P. 1198-1202.
21. The Merck index, Encyclopedia of chemical drugs and biologicals, 13th edition, Merck Research Laboratory, Merck & Co., Inc. Whitehouse Station, NJ, pp. 1036, 609.

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