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Reverse Phase High Performance Liquid Chromatography for Simultaneous Validation of Aceclofenac and Drotaverine Hydrochloride in Bulk and Pharmaceutical Dosage form

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Abstract : Simultaneous validation of Aceclofenac and Drotaverine hydrochloride from combined dosage form i.e. tablets was described by high performance liquid chromatography method with separation of drugs on BDS HypersilC18 (150 x 4.6 mm i.d.) and 5 µ particle size. A mixture of buffer and acetonitrile (55:45 % (v/v)) was constituted as mobile phase. The chromatograms were studied at 230 nm as wavelength. The mobile phase was also used as a diluent. A validated of method was studied for linear regression, accuracy, method as well as system precision. The robustness study was done for change in wavelength, mobile phase composition and flow rate as per ICH guidelines. The method has been successfully used to analyze Aceclofenac and Drotaverine from combined dosage form i.e. tablets.
Keywords : Aceclofenac, drotaverine Acetonitrile, tri-ethyl amine, ortho phosphoric acid.

Introduction

Aceclofenac is, the non steroidalanti inflammatory, analgesic in nature ,{[[2-[(2,6-Dichlorophenyl) amino] phenyl]acetyl]oxy}Acetic acid.

Drotaverine hydrochloride is a highly potent spasmolytic drug with smooth muscle relaxant by increasing intracellular levels of cyclic adenosine mono-phosphate (cAMP) secondary to inhibition of phosphor-diesterase. It is [(1 - (3, 4 – diethoxybenzylidene) - 6, 7 – diethoxy - 1, 2, 3, 4tetrahydroisoquinoline) hydrochloride], a benzylisoquinoline derivative. UV spectrophotometric methods [1.2], HPLC[3-5]were reported for simultaneous determination of drotaverine and aceclofenac in combined dosage form and other miscellaneous[6-16] methods were reported for validation of drugs with other combinations in literature .This new work presents reproducible reverse phase high performance liquid chromatographic method for simultaneous assay of aceclofenac and drotaverine in tablet dosage form.

Chemical and reagents

Standard of Aceclofenac and Drotaverine were used which are validated as per pharmacopeia. All chemicals of analytical grade were used such as tri-ethyl amine, acetonitrile and ortho phosphoric acid.The HPLC grade water was used from Millipore. For preparation of standard and sample solutions,the diluent was mobile phase i.e. mixture of buffer of pH 4 and acetonitrile (53:47 % (v/v).

Instrumentation

The MERCK Hitachi HPLC system equipped with -

a) D 7200 separation module as auto sampler
b) D- 7400) as UV detector
c) PC based EZ Chrom Elite software.
d) A analytical balance (0.01 mg) made of SHIMADZU was used.

Preparation of Standard preparation

Standard solution

A 1000 µg /ml of aceclofenac and 800 µg /ml of drotaverine standard solution was prepared by using diluent [mixture of buffer of pH 4 and acetonitrile [53:47 % (v/v)] respectively.

Sample preparation

With the help of twenty tablets, average weight of each tablet was calculated. A tablet powder equivalent to 10mg of standard aceclofenac and 8 mg of drotaverine was used for preparation of sample solution to give concentration as 1000 µg /ml of aceclofenac and 800µg /ml. of respectively.

Chromatographic condition

Chromatographic study was performed on Hypersil BDS C18 (150 x 4.6 mm i.d.) with 5 µ particle size column. The mobile phase was buffer of pH 4 and acetonitrile [53:47 % (v/v)]. The buffer constituted as 0.01% (v/v) tri-ethyl amine.The pH4 of buffer solution was made with ortho-phosphoric acid. The flow rate of 1.2 ml /min was maintained in study at wavelength 230 nm. and injection volume as 10.0 µl.(Fig.1)

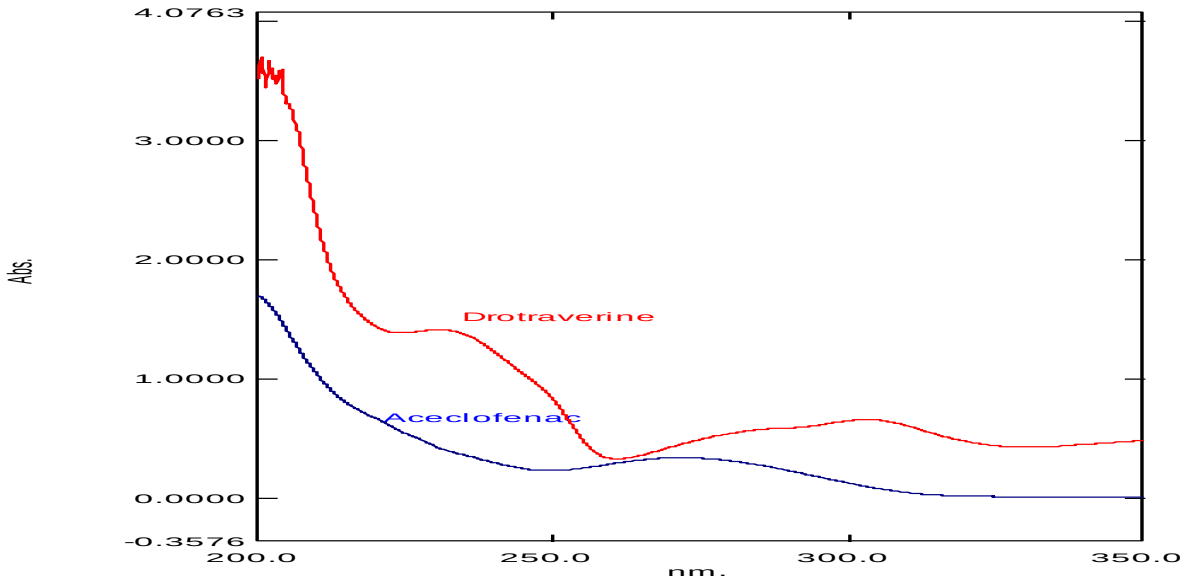

Figure 1: Overlay UV spectra of aceclofenac and drotaverine

Method validation

System suitability

For study of System and method precision six replicates were used. From such data,Parameter i.e.theoretical plates (N), asymmetry, resolution and area were calculated. The results are shown in table 1 which indicates system suitability of the system.

Table 1: parameters of system suitability

Parameter	Aceclofenac	Drotaverinehydrochloride
Retention time	4.40	6.45
Theoretical plates (N),	2752	3166
Area	6676042	4926950
resolution	-	3.68279

Specificity

Specificity study was performed by injecting blank, standards. The chromatogram of the standard and sample assayed are given in figure 2 and 3 respectively.

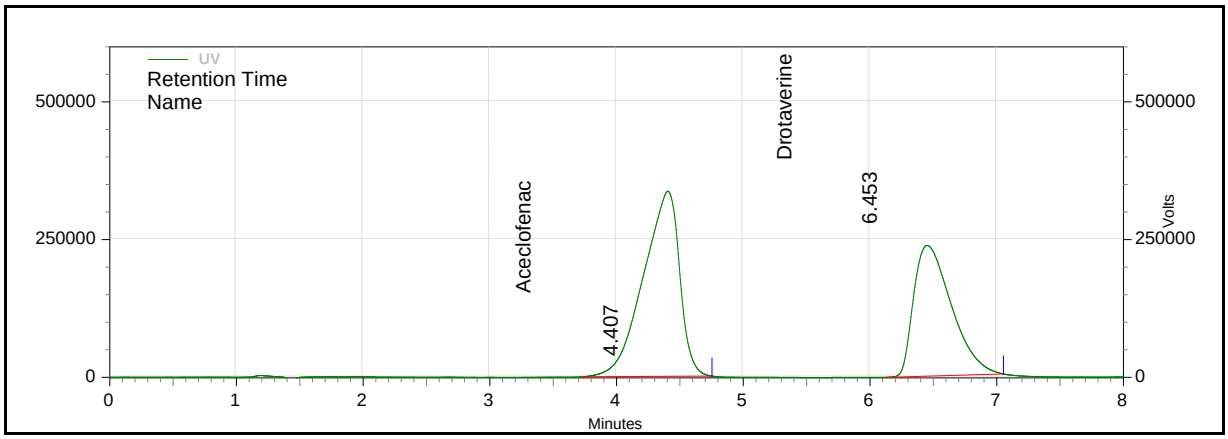

Fig.2: Chromatogram of aceclofenac and drotaverine(standard)

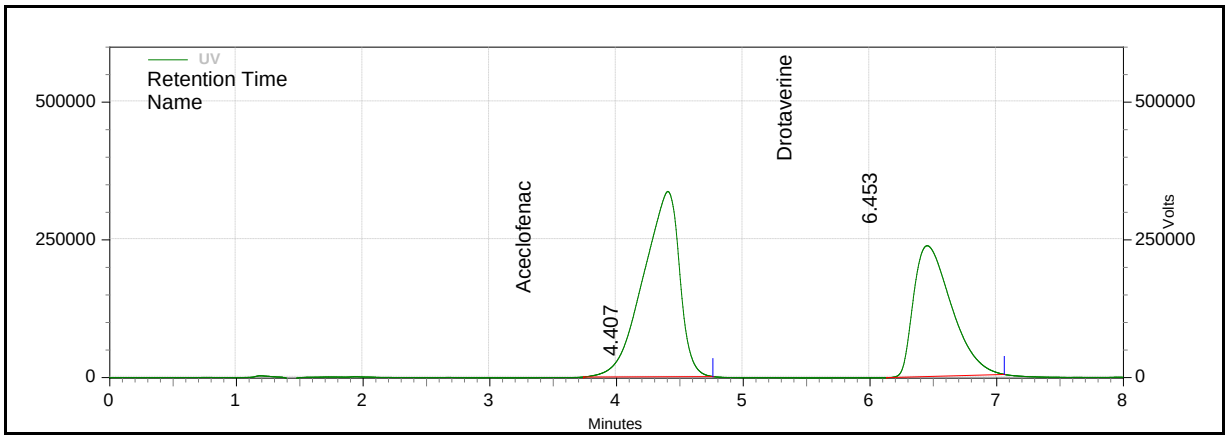

Fig.3: Chromatogram of Aceclofenac and Drotaverine(sample)

Linearity

From the given data of 50% 80%, 100% 120% and 150%, linear calibration was drawn as peak area (y) of different concentration v/s concentration (x). The data is tabulated in table no. 2. The linearity graph is given in fig no.4(a), (b)

Table 2: parameters of linear graphs

Parameter	Aceclofenac	Drotaverine hydrochloride
Range of linearity	50-150 µg /ml	40-120 µg /ml
Slope	65596	60084
Intercept	- 34417	50428
Coefficient of correlation	0.9999	0.9999

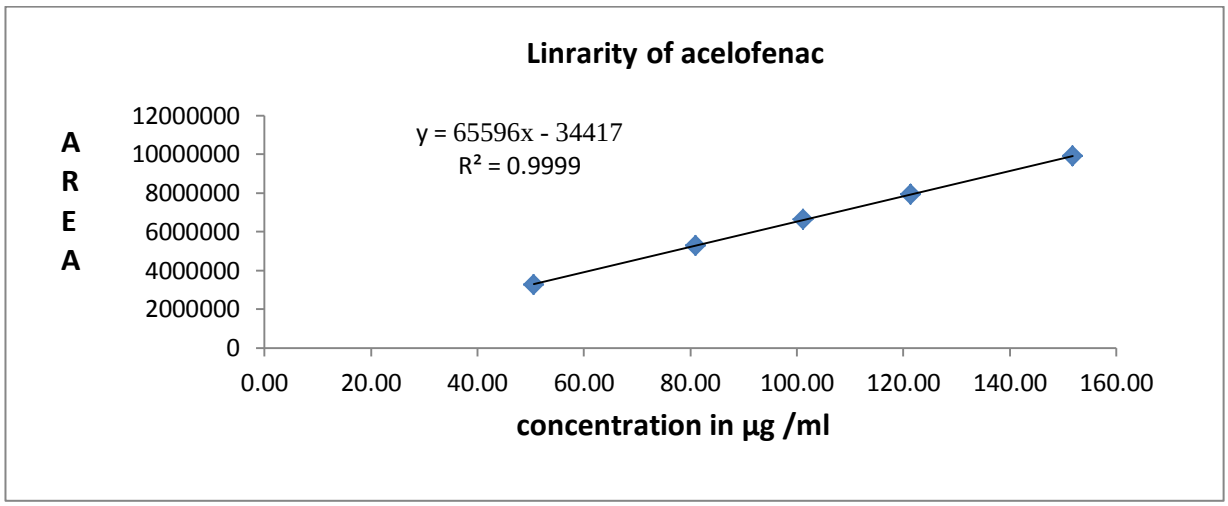

Fig. 4(a): linearity graph of Aceclofenac

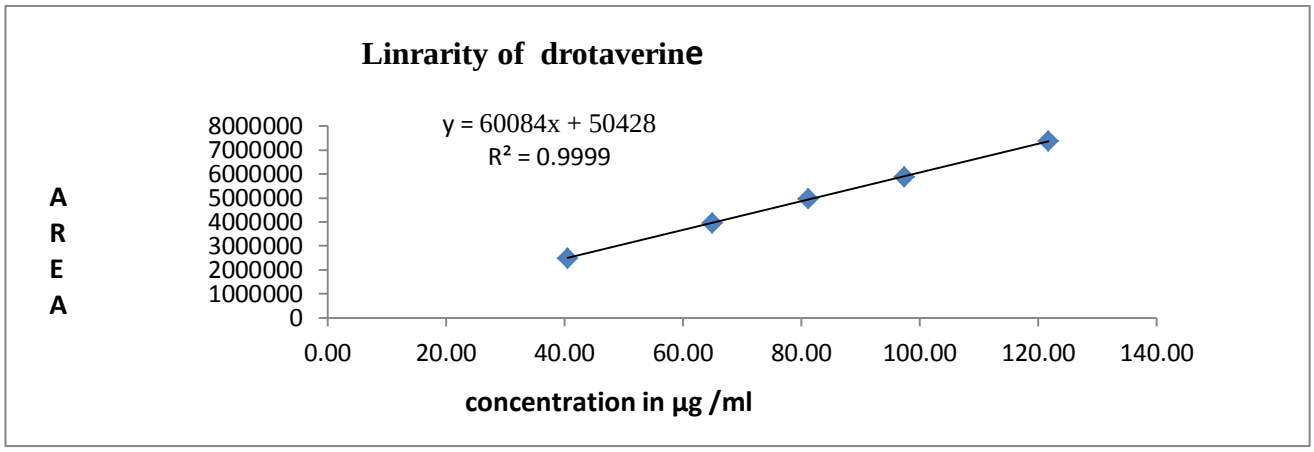

Fig. 4 (b) linearity graph of drotaverine hydrochloride

Accuracy

For the study of accuracy 80 %, 100 % and 120 % levels were used. The accuracy was determined as the percentage recovered by the assay. The results of the percentage recovery are enclosed under table no.3, 4.

Table 3: Accuracy - %Recovery –Aceclofenac

level	Replicate no.	Amount in mg	Peak area	Amount added in µg/ml	Amount recovered in µg /ml	Recovery in percentage	Mean of Recovery
80%	1	10.09	5229438	80.96	79.27	97.91	**97.95**
	2	10.14	5231364	80.96	79.29	97.94	
	3	10.11	5233921	80.96	79.33	97.99	
100%	1	10.08	6677942	101.2	101.22	100.02	**100.12**
	2	10.11	6683221	101.2	101.30	100.10	
	3	10.12	6692157	101.2	101.44	100.23	
120%	1	10.10	7955789	121.44	120.59	99.30	**99.36**
	2	10.12	7959552	121.44	120.65	99.35	
	3	10.11	7966474	121.44	120.75	99.43	
					Mean recovery of all level		**99.14**

Table 4 : Accuracy - %Recovery –Drotaverine hydrochloride

level	Replicate no.	Amount in mg	Peak area	Amount added in µg /ml	Amount recovered in µg /ml	Recovery in percentage	Mean of Recovery
80%	1	8.14	3921154	65.2	64.81	99.40	**99.51**
	2	8.12	3924553	65.2	64.86	99.49	
	3	8.15	3930768	65.2	64.97	99.64	
100%	1	8.11	4996055	81.5	82.57	101.32	**100.91**
	2	8.12	4974101	81.5	82.21	100.87	
	3	8.13	4957453	81.5	81.94	100.54	
120%	1	8.16	5885027	97.8	97.27	99.46	**99.00**
	2	8.15	5850886	97.8	96.70	98.88	
	3	8.14	5838358	97.8	96.50	98.67	
					% Mean recovery		**99.81**

Precision

The study of method precision was carried out in six replicates. The relative standard deviation was calculated and it was in given limits. The results of the same are tabulated in the table no.5(a) ,(b).

Table 5(a) : Statistical evaluation of the data subjected to method precision of aceclofenac

Test	wt of test	Area	% assay
Test-1	10.11	6676042	99.99
Test-2	10.12	6683077	99.99
Test-3	10.09	6695308	100.58
Test-4	10.08	6630662	99.71
Test-5	10.09	6612979	99.34
Test-6	10.07	6602604	99.38
	Mean Assay		**99.83**
	SD		**0.462**
	RSD		**0.463**

Table 5(b) : Statistical evaluation of the data subjected to method precision of Drotaverine

Test	wt of test	Area	% assay
Test-1	4.28	4926950	99.22
Test-2	4.29	4936700	99.18
Test-3	4.31	4954778	99.08
Test-4	4.30	4903302	98.28
Test-5	4.27	4920242	99.31
Test-6	4.32	4927890	98.32
	Mean Assay		**98.90**
	SD		**0.471**
	RSD		**0.476**

Study of robustness

It was carried out by Variation in the flow rate by $\pm$ 0.2 ml /min, mobile phase composition by $\pm$ 2 % and wavelength by$\pm$ 5 nm.

The results are studied of the analysis of the samples From the results it was interpreted the robustness of the method.

Result

The validation of drug by RP-HPLC has great importance in checking the quality of drugs. In reported method, the time of retention for aceclofenac and drotaverine were 4.40 and 6.45 min. The linear range for aceclofenac and drotaverine were 50 - 150 µg / ml and 40-120 µg / ml respectively

The coefficient of co-relation was 0.9999(Table no. 2) hence interference peaks ofdiluent was not observed at the retention time. Hence method has specificity and suitable in the linear range applied. It gives thatangood correlation in the linearity applied. The valuesrelative standard deviations was found to be less than one. The mean recovery was 99.88%. to98.90 %. The study of Variation in the flow rate by $\pm$ 0.2 ml /min, mobile phase composition by $\pm$ 2 % and wavelength by$\pm$ 5 nm has no effect diluents on the drug study.

Discussion

The acceptable limit was found in average recovery and %RSD for assay values of aceclofenac and drotaverine. The stability of the proposed method was confirmed by study of method and system precision. The acceptable limits were followed in robustness parameters like wavelength, flow rate as well as composition of mobile.The relative standard deviationsfor replicateswere within the acceptable limit. The retention times were4.4 and6.4 minutes for aceclofenac and drotaverine respectively. Hence method required less time to complete one sample validation compare the methods suggested in literatures.

Conclusion

The proposed HPLC method is as per ICH guidelines for validation for aceclofenac and drotaverine respectively. It was found to be accurate and precise. The replicate analysis showed repeatability of results. It showed the robustness and system suitability of method. The Value of retention times were 4.4 and 6.4 minutes for aceclofenac and drotaverine hence the method is time saving as compared literature methods. From the above parameters it is observed that the aceclofenac and drotaverine were validated accurately in less time and with more economy by using the proposed RP-HPLC method in formulation and in bulk . Hence it is strongly recommendation for the quality control to adopt such economical and time saving method for assay of drugs in combined dosage as well as individual assay of both drugs in raw material.

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