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Simultaneous Determination of Drotaverine Hydrochloride and Mefenamic Acid by Isocratic RP-HPLC Method

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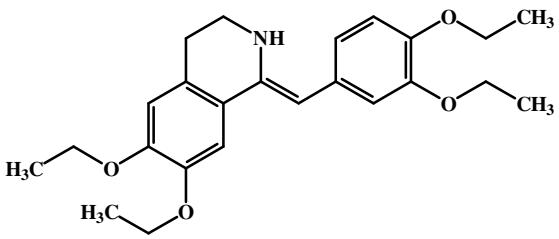
A simple fast, accurate, precise and cost effective isocratic RP-HPLC method is developed for simultaneous determination of drotaverine hydrochloride and mefenamic acid. The retention times of drotaverine hydrochloride and mefenamic acid were found to be 3.05 and 9.52 min, respectively. The method was linear over the range of 16 to 47 ppm with r^2 = 0.9999 for drotaverine hydrochloride and 100 to 300 ppm with r^2 = 0.9999 for mefenamic acid. Mean recovery for drotaverine hydrochloride and mefenamic acid were 101.1 and 101.2, respectively.

Key Words: Mefenamic acid, Drotaverine hydrochloride, RP-HPLC.

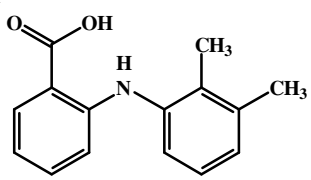
INTRODUCTION

Drotaverine hydrochloride[1,2] (**1**) chemically is 1,2,3,4-tetrahydro-6,7-diethoxy-1-((3,4-diethoxyphenyl)methylene)isoquinoline hydrochloride. It is an antispasmodic drug, structurally related to papaverine. Drotaverine is a selective inhibitor of phosphodiesterase IV and has no antichlolinergic effects.

Mefenamic acid[3,4] (**2**) chemically is 2-[(2,3-dimethyl phenyl)amino]benzoic acid, molecular formula of $C_{15}H_{15}NO_2$ having molecular weight 241.29. Mefenamic acid is white to almost white, micro-crystalline powder. It is odourless and bitter after taste, darkens on prolonged exposure to light, non-hygroscopic, decarboxylates at temperatures above its melting point.



Drotaverine hydrochloride (**2**)



Mefenamic acid (**2**)

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Mefenamic acid is an anthranilic acid derivative[3,4], is a non-steroidal antiinflammatory drug (NSAID) with demonstrated antiinflammatory, analgesic antipyretic activity in laboratory animals. Its mode of action is not completely understood, but it may be related to prostaglandin synthetase inhibition. In animal studies, the drug was found to inhibit prostaglandin synthesis and to complete for binding at the prostaglandin receptor site.

The literature survey revealed that there are no methods reported for simultaneous determination of drotaverine hydrochloride and mefenamic acid by HPLC.

EXPERIMENTAL

Sodium acetate trihydrate, glacial acetic acid, acetonitrile, methanol, all HPLC grade were purchased from Merck.

Chromatographic condition: Column: Inertsil ODS 3V, 250 mm × 4.6 mm, 5 µ; Wavelength: 350 nm; Flow rate: 1.5 mL/min; Injection volume: 20 µL; Column temperature: 25 ºC; Run time: 15 min.

Buffer solution: Dissolved 6.8 g of sodium acetate trihydrate in 1000 mL water, pH adjusted to 4.5 with acetic acid.

Mobile phase: Mixed buffer and acetonitrile in the ratio of 45:55 (v/v) filtered through 0.45 µ membrane filter and sonicated for 5 min.

Diluent: Mixed water and acetonitrile in the ratio of 45:55 (v/v) filtered through 0.45 µ membrane filter and sonicated for 5 min.

Preparation of stock solution: Prepared a solution having the concentration of mefenamic acid 2000 ppm, drotaverine hydrochloride 320 ppm, in acetonitrile.

Mixed standard solution: Transferred 5 mL of above stock solution to a 50 mL volumetric flask and made up to volume with diluent to attain a concentration of mefenamic acid 200 ppm and drotaverine hydrochloride 32 ppm.

Placebo preparation: Approximately 310 mg of placebo was transferred to 250 mL volumetric flask and added 200 mL of diluent, sonicated for 15 min, cooled to room temperature and made up to volume with diluent. Filtered the sample through 0.45 µ Teflon filter. Transferred 5 mL of above solution in 50 mL volumetric flask and made up to volume with diluent, filtered through 0.45 µ Teflon filter.

Sample preparation: Twenty tablets were weighed and crushed finely, powder equivalent to 500 mg of mefenamic acid and 80 mg of drotaverine hydrochloride was transferred to 250 mL volumetric flask and added 200 mL of diluent, sonicated for 15 min, cooled to room temperature and made up to volume with diluent filtered the above sample through 0.45 µ Teflon filter. Transferred 5 mL of above solution in 50 mL volumetric flask and made up to volume with diluent to attain same concentration as of standard, filtered through 0.45 µ Teflon filter.

Injected equal volumes of diluent, placebo preparation, standard preparation separately in six replicates and sample once in equilibrated HPLC system and recorded the chromatograms and measured the response in terms of peak area. System suitability parameters maintained during method validation are theoretical plates not less than 2000, tailing factor not more than 2.0, relative standard deviation for six replicates of standard solution is not more than 2.0 %.

RESULTS AND DISCUSSION

Accuracy was studied at three concentration level at 80, 100 and 120 % level of working concentration. Each level was studied in triplicate. Each preparation was prepared independently by spiking analyte in the placebo. Per cent recovery was calculated by comparing the response obtained in spiked sample with those obtained in standard and obtained results (Tables 1 and 2). The present analytical method found to be well accurate and results obtained are between 98-102 %.

TABLE-1
ACCURACY OF DROTAVERINE HYDROCHLORIDE BY PLACEBO SPIKED RECOVERY METHOD

Accuracy level	Mg added	Test area-1	Test area-2	Average area	Mg recovered	Recovery (%)	Mean % recovery
Level-80 spl-1	66.39	428357	431342	429850	67.01	100.93	
Level-80 spl-2	66.39	429511	422141	425826	66.38	99.99	100.60
Level-80 spl-3	66.39	428202	431134	429668	66.98	100.89	
Level-100 spl-1	82.98	536038	532613	534325	83.29	100.37	
Level-100 spl-2	82.98	538523	537652	538087	83.88	101.08	100.75
Level-100 spl-3	82.98	536187	537154	536670	83.66	100.81	
Level-120 spl-1	99.58	649884	645278	647581	100.95	101.37	
Level-120 spl-2	99.58	647541	648526	648033	101.02	101.44	101.50
Level-120 spl-3	99.58	650549	648812	649680	101.27	101.70	
						Mean	100.95
						S.D	0.48
						% RSD	0.48

TABLE-2
ACCURACY OF MEFENAMIC ACID BY PLACEBO SPIKED RECOVERY METHOD

Accuracy level	Mg added	Test area-1	Test area-2	Average area	Mg recovered	Recovery (%)	Mean % recovery
Level-80 spl-1	401.10	2936541	2924321	2930431	403.05	100.49	
Level-80 spl-2	401.81	2939579	2946541	2943060	404.79	100.74	100.52
Level-80 spl-3	400.78	2930437	2916452	2923445	402.09	100.33	
Level-100 spl-1	500.68	3664472	3659874	3662173	503.69	100.60	
Level-100 spl-2	502.06	3706758	3697643	3702201	509.20	101.42	100.97
Level-100 spl-3	501.30	3672138	3682134	3677136	505.75	100.89	
Level-120 spl-1	602.21	4437274	4443456	4440365	610.73	101.41	
Level-120 spl-2	602.09	4429351	4423167	4426259	608.79	101.11	101.31
Level-120 spl-3	601.82	4441522	4432314	4436918	610.25	101.40	
						Mean	100.93
						S.D	0.40
						% RSD	0.39

Linearity: The standard stock solutions were prepared and diluted linearly and linearity was established, the concentration of the solutions and correlation coefficient was given in Tables 3 and 4.

TABLE-3
LINEARITY DATA OF DROTAVERINE HYDROCHLORIDE

Linearity level	Conc. in ppm	Experimental area (a)	Predicted area (y)	Residuals (b)
			y = mx + c	b = a-y
Level-50 %	5.01	929154	937664	–8510
Level-80 %	7.52	1415671	1397646	18025
Level-90 %	10.02	1836002	1857629	–21627
Level-100 %	12.53	2329220	2317611	11609
Level-110 %	15.04	2772041	2777594	–5553
Level-120 %	12.53	2329220	2317611	11609
Level-150 %	15.04	2772041	2777594	–5553
Correlation	0.999800			
Intercept (c)	17698.42			
Slope (m)	183552.51			

TABLE-4
LINEARITY DATA OF MEFENAMIC ACID

Linearity level	Conc. in ppm	Experimental area (a)	Predicted area (y)	Residuals (b)
			y = mx + c	b = a-y
Level-50 %	100.00	1811359	1819225	–7866
Level-80 %	160.00	2915043	2913982	1061
Level-90 %	180.00	3277421	3278900	–1479
Level-100 %	200.00	3659545	3643819	15726
Level-110 %	220.00	4012059	4008738	3321
Level-120 %	240.00	4369721	4373656	–3935
Level-150 %	300.00	5461585	5468413	–6828
Correlation	0.99998			
Intercept (c)	–5368.33			
Slope (m)	18245.95			

Precision: To check the system precision (repeatability) for peak response obtained with six replicates of standard at limiting concentration. To check repeatability (method precision) of method for independent six different sample preparations were injected and % RSD with six sample preparation found to be within 5.0 %. The obtained results were given in Tables 5 and 6. To demonstrate intermediate precision (ruggedness) of the method by comparing method precision (in terms of absolute difference) on two different days on two different systems.

Solution stability: To demonstrate stability of the standard solution by comparing data of absolute difference in % assay at each interval with respect to initial value. The standard and sample solutions were stored at room temperature and analyzed at initial, about 6, 12 and 24 h. The absolute difference between % assay values should be within 2.0 % with respect to the initial value are considered as stable. From above experimental study, standard and sample solutions are found stable up to 24 h at room temperature.

TABLE-5
PRECISION DATA OF DROTAVERINE HYDROCHLORIDE

Sample condition	Test wt (g)	Avg weight	Test area-1	Test area-2	Avg area	Mg/unit	% Assay
Method precision spl-1	891.97	891.92	523256	525846	524551	80.319	100.40
Method precision spl-2	891.91		523256	522686	522971	80.082	100.10
Method precision spl-3	892.01		518075	515485	516780	79.125	98.910
Method precision spl-4	891.89		527401	520666	524033	80.246	100.31
Method precision spl-5	890.99		523256	525846	524551	80.407	100.51
Method precision spl-6	892.03		518075	517039	517557	79.242	99.05
						AVG	99.88
						SD	0.71
						% RSD	0.71

TABLE-6
PRECISION DATA OF MEFENAMIC ACID

Sample condition	Test wt (g)	Avg weight	Test area-1	Test area-2	Avg area	Mg/unit	% Assay
Method precision spl-1	891.97	891.92	3643294	3663243	3653268	503.60	100.72
Method precision spl-2	891.91		3619719	3590703	3605211	497.01	99.400
Method precision spl-3	892.01		3626973	3663243	3645108	502.45	100.49
Method precision spl-4	891.89		3639305	3645108	3642206	502.12	100.42
Method precision spl-5	890.99		3663243	3645108	3654175	504.28	100.86
Method precision spl-6	892.03		3626973	3619719	3623346	499.44	99.89
						Avg.	100.30
						SD	0.55
						% RSD	0.55

System suitability: The relative standard deviation for the peak areas of drotaverine hydrochloride and mefenamic acid in standard solution was found to be less than 2.0 %.

Ruggedness and robustness: The ruggedness of the method was studied by analyzing the sample on two different days on two different systems and two different columns by same manufacturer, results found to be well with in acceptable limits. Robustness of an analytical method was studied by changing the pH of the buffer and found to acceptable for organic addition, the 5 % change in organic was also not influencing the resolution in between the peaks, hence method validation concludes the method was rugged and robust.

Conclusion

The above developed method was capable to quantify the drotaverine hydrochloride and mefenamic acid present in the drug product. The special quality of the method is well proved as even the presence of mefenamic acid (500 mg) and drotaverine hydrochloride (80 mg) are wide different in the concentration, even though they are well accurate and precisely quantified. The same method was subjected for analytical method validation and found to selective, accurate, precise and linear over the range, the method also found to be rugged.

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