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Simultaneous Estimation of Mefenamic Acid and Drotaverine Hydrochloride in Tablets by High Performance Thin Layer Chromatography

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A simple, rapid, sensitive high performance thin layer chromatographic method was developed and validated for simultaneous estimation of mefenamic acid and drotaverine hydrochloride in tablets. It was performed on TLC plate precoated with silica gel $60GF_{254}$ as a stationary phase and methanol : toluene: triethylamine (1 : 7.5 : 0.2) as mobile phase and the quantitation was carried out at 241 nm in absorbance mode. The R_f values were found to be 0.30 and 0.47 for mefenamic acid and drotaverine hydrochloride, respectively. The results were calculated as percentage of stated amount in each average weight of the tablet in marketed formulation and were found to be 99.45 ± 0.76, 99.29 ± 0.20 by peak height ; nd 100.00 ± 1.18, 101.04 ± 0.77 by peak area. The method was validated in terms of accuracy, precision, specificity, ruggedness and linearity in the range of 3800–8400 ng for mefenamic acid and 1200–2700 ng for drotaverine hydrochloride. The recovery studies were carried out by standard addition method and % recovery was found to be 100.39% ± 1.30 and 99.50% ± 1.25 for mefenamic acid and 99.08% ± 0.61 and 100.86% ± 0.66 for drotaverine hydrochloride, respectively.

Key Words: Mefenamic acid, Drotaverine hydrochloride, High performance thin layer chromatography.

INTRODUCTION

Mefenamic acid (MFA) is a non-steroidal antiinflammatory and analgesic drug. Chemically, it is 2-[(2,3-dimethylphenyl)amino] benzoic acid[1] and is official in IP, BP and USP. Drotaverine hydrochloride (DTH) is used as antispasmodic and chemically it is 1-[(3,4-diethoxyphenyl)methylene]-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline[2] and is not official in any pharmacopoeia.

Literature survey revealed that a few methods like colorimetric[3, 4] spectrophotometric[5–9], HPTLC[10] and HPLC[11, 12, 13] are reported for estimation of MFA alone and with other drugs in combination. Similarly, various spectrophotometric[14, 15] and HPLC[16, 17, 18] methods are reported for determination of DTH.

The objective of the present work was to develop a sensitive, rapid and reproducible HPTLC method for the estimation of MFA and DTH in combined dosage form.

EXPERIMENTAL

All chemicals and reagents used throughout the project work were AR/HPLC grade. Marketed tablets Drotin M labelled to contain MFA 250 mg and DTH 80 mg per tablet were used.

The instrument used in the present study was CAMA-HPTLC system comprising CAMAG LINOMAT IV automatic sample applicator, CAMAG TLC Scanner III with CATS 4 software, CAMAG twin trough glass chambers.

Standard solution

Standard solution of MFA (1 mg/mL) and DTH (0.32 mg/mL) was prepared in methanol.

Experimental chromatographic conditions

Standard experimental conditions were critically established and kept constant throughout the experimental study. Stationary phase-silica gel 60 GF_{254} TLC precoated aluminium foiled plates with thickness-200 µm, mobile phase : methanol : toluene : Triethylamine 1 : 7.5 : 0.2, saturation time 10 min, sample application with constant rate of 0.16 µL/s and scanning speed 10 mm/s were employed with 6 mm band and slit dimension of 5 × 0.45 mm, development technique: ascending, temperature: 20 ± 5°C, relative humidity: 50–60%, migration distance: 70 mm scanning mode: absorbance/reflectance, detection wavelength: 241 nm. The detection wavelength was selected from overlain spectra of both the drugs (Fig. 1).

Calibration curve response: MFA and DTH solutions ranging from 3800–

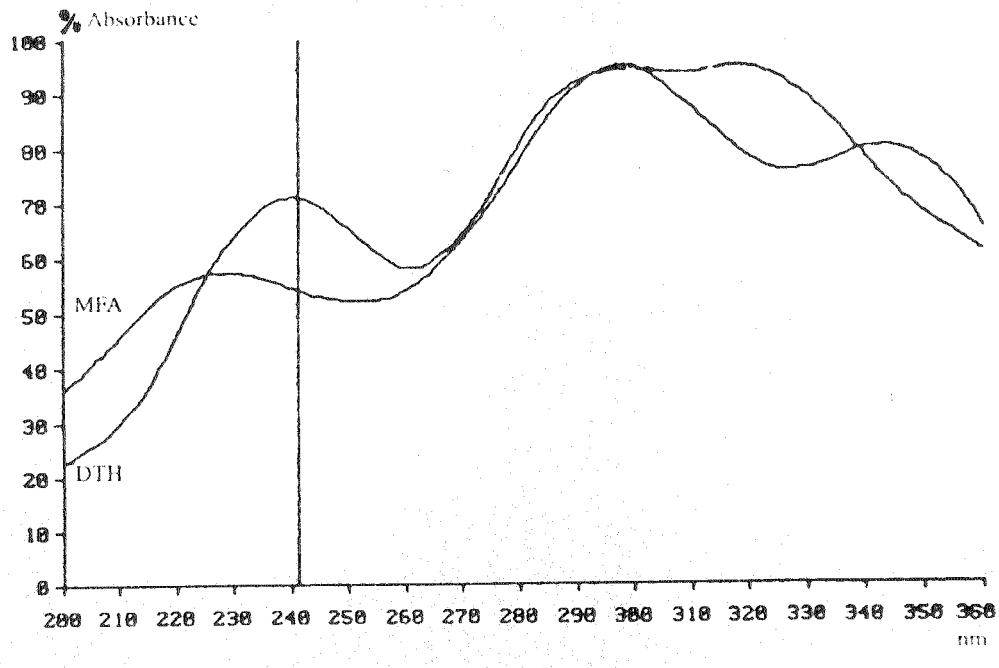

Fig. 1. Overlain spectra of mefenamic acid and drotaverine hydrochloride

8400 ng (1.0 μg/μL) and 1200–2700 ng (0.32 μg/μL) were applied on TLC plates by microlitre syringe with the help of automatic sample applicator. The plates were developed, dried and densitometrically scanned at 241 nm. Peak height and area were recorded for each concentration of drugs and calibration curves (concentration *vs.* peak height/area) were constructed.

Standard laboratory mixtures: Five different laboratory mixtures were prepared in the same manner as that of a standard preparation to get the final concentration of about MFA 1.0 mg/mL and DTH 0.32 mg/mL. Each standard solution (6.0 μL) (two spots) and laboratory mixture (six spots) were applied on TLC plates as 6.0 mm band. Plates were then developed in presaturated twin trough chamber with mobile phase. After development the plates were dried with the help of hot air dryer and evaluated densitometrically at a wavelength of 241 nm.

Assay: Twenty tablets were weighed, thoroughly powdered and mixed. An accurately weighed quantity of tablet powder equivalent to 25 mg of MFA was shaken with 10.0 mL of methanol for about 15 min and the volume was made up to 25.0 mL with methanol. The solution was then filtered through Whatman No. 1 filter paper and the filtrate was used. The same procedure was followed as described under laboratory mixture.

The per cent labelled claim of drug estimated in marketed formulation was calculated by using the formula

$$\% \text{ Labelled claim} = \frac{\text{Amount estimated} \times \text{Average weight} \times \text{Dilution factor}}{\text{Amount applied} \times \text{Labelled claim} \times \text{Weight taken}} \times 100$$

Validation of proposed method

The proposed method was validated for the following parameters:

Accuracy: The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. The per cent recovery was calculated by using the following formula:

$$\% \text{ Recovery} = \frac{A - B}{C} \times 100$$

where A = total drug estimated, B = amount contributed by tablet powder (as per proposed method), C = amount of pure drug added.

Precision: Precision of an analytical method was expressed as S.D. or R.S.D. of series of measurement by replicate estimation of drug by proposed method.

Specificity: The stability indicating ability of proposed method was investigated by exposing the sample to different stress conditions to detect the presence of matrix components. The sample solution was stored for 24 h under different stress conditions like 1.0 mL of 0.1 N of HCl (acid), 1.0 mL of 0.1 N of NaOH (alkali), 1.0 mL of 3% of H_2O_2 (oxidation), at 60°C (heat) and in UV-chamber. After 24 h, the contents of flask were shaken with methanol for 15 min and volume was made up to 25.0 mL, filtered, diluted and analyzed as previously described.

Linearity detector response: The study was performed by application of different volumes of standard solution and response was obtained densitometrically.

Ruggedness: Ruggedness was carried out for two parameters, *i.e.*, for different days and by different analysts.

RESULTS AND DISCUSSION

The proposed HPTLC method is simple, accurate, reproducible and economical as evidenced from the results. After some trials, the mobile phase comprising of methanol : toluene : triethylamine in the ratio of 1 : 7.5 : 0.2 v/v was found to be suitable giving good separation of MFA and DTH with R_f values 0.30 and 0.47, respectively. The construction of calibration curves for both the drugs shows good lincarity ranging from 3800–8400 ng for MFA and 1200–2700 ng for DTH with coefficient of correlation values 0.9959, 0.9967 and 0.9916, 0.9991 for MFA and DTH respectively (Table-3).

TABLE-1
PER CENT ESTIMATION OF MFA AND DTH FROM LABORATORY MIXTURE, MARKETED FORMULATION AND RECOVERY STUDIES

Sr. No.	Sample	Statistics	% Estimation of labelled claim*				% Recovery*			
			MFA	DTH	MFA	DTH	MFA	DTH	MFA	DTH
			By Height	By Area	By Height	By Area	By Height	By Area	By Height	By Area
1	Standard	Mean	99.70	100.22	99.42	100.44	—	—	—	—
	laboratory	±S.D.	1.06	1.16	0.87	1.09	—	—	—	—
	mixture	C.V.	1.06	1.15	0.88	1.08	—	—	—	—
2	Marketed	Mean	99.45	100.00	99.29	101.04	100.39	99.50	99.08	100.86
	formulation	±S.D.	0.76	1.18	0.20	0.77	1.30	1.25	0.61	0.66
		C.V.	0.76	1.18	0.21	0.76	1.30	1.25	0.62	0.65

*Each reading is the mean of five observations.

The results for per cent estimation of drug (by peak height and peak area) in marketed formulation were found to be 99.45 ± 0.76, 100.00 ± 1.18 and 99.29 ± 0.20, 101.04 ± 0.77 for MFA and DTH, respectively. These results (Table-2) show that the method is accurate and precise.

TABLE-2
RESULT OF SPECIFICITY STUDY

Sr. No.	Sample	% Label claim*			
		MFA		DTH	
		By height	By area	By height	By area
1	Acid	101.06	99.74	98.22	99.45
2	Alkali	99.56	100.29	71.91	77.04
3	Oxide	96.86	94.21	65.39	77.53
4	Heat	98.91	99.79	100.21	99.17
5	UV	99.21	99.86	99.61	100.82

*Each reading is the mean of five observations.

Further the accuracy of the method was ascertained by the recovery studies of MFA and DTH. The average value was found between the prescribed limits of 98–102%, which shows that the method is free from interference of excipients present in formulation (Table-3). S.D. and C.V. values are within the prescribed limit of 2% indicating the repeatability of the method (Table-4).

The above results shows that the proposed method is simple, accurate and rapid and can be used for the routine analysis of MFA and DTH in their combined dosage form.

TABLE-3
RESULTS OF LINEARITY STUDIES

Drug	Linearity range (ng)	Coefficient of correlation		Slope		Y-intercept	
		By height	By area	By height	By area	By height	By area
MFA	3800-8400	0.9959	0.9967	17.12	1181.75	538.46	6846.34
DTH	1200-2700	0.9916	0.9991	80.82	761.98	309.91	1528.32

TABLE-4
RESULTS OF RUGGEDNESS STUDY

S. No.	Parameters	Statistics	% Labelled claim*			
			MFA	DTH	MFA	DTH
			By height	By area	By height	By area
1	Different days	Mean	99.88	100.02	99.54	100.67
		±S.D.	0.49	0.65	0.10	0.18
		C.V.	0.49	0.65	0.10	0.18
2	Different analysts	Mean	99.44	99.81	99.88	100.61
		±S.D.	0.44	0.75	0.68	0.43
		C.V.	0.45	0.75	0.68	0.43

*Each reading is the mean of three observations.

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