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SIMULTANEOUS ESTIMATION OF MEFENAMIC ACID AND DICYCLOMINE HYDROCHLORIDE BY SPECTROSCOPIC METHODS

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ABSTRACT

A novel, simple, accurate, sensitive, reproducible, economical spectroscopic method was developed and validated for the determination of Mefenamic acid and Dicyclomine hydrochloride in combined dosage form. Three different analytical methods, Absorption correction method, Differential derivative method, Simultaneous equation method were developed for estimation of Dicyclomine hydrochloride(10mg) and Mefenamic acid (250mg) in tablet dosage form. wavelength for estimation was 223nm for Dicyclomine hydrochloride and 308.60nm for Mefenamic acid in absorption correction method. 211.60nm was Zero crossing point of Mefenamic acid and 308.80nm was Zero crossing point of Dicyclomine hydrochloride which can estimate in differential derivative method. Simultaneous equation method was developed in NaOH which was linear in the range of 1-6µg/ml for Dicyclomine hydrochloride and 25-150µg/ml for Mefenamic acid, the correlation coefficient obtained was nearer to one. The method was validated for linearity, accuracy and precision as per ICH guidelines. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form.

INTRODUCTION: Mefenamic acid [*N*-(2, 3-xylyl) anthranilic acid] is an Aminobenzoate, a subclass of analgesic with Non steroidal anti-Inflammatory properties [1]. It acts by binds the prostaglandin synthetase receptors COX-1 and COX-2, inhibiting the action of prostaglandin synthetase [2, 3]. It is used for the treatment of rheumatoid arthritis, osteoarthritis, dysmenorrhea, and mild to moderate pain, inflammation, and fever [4].

Dicyclomine (bicyclohexyl]-1-carboxylic acid is an antispasmodic and anticholinergic (antimuscarinic) agent [5]. It Action is achieved via a dual mechanism: a specific anticholinergic effect (antimuscarinic) at the acetylcholine-receptor sites, a direct effect upon smooth muscle (musculotropic) [5].

It is used to treat a certain type of intestinal problem called irritable bowel syndrome. It helps to reduce the symptoms of stomach and intestinal cramping. This medication works by slowing the natural movements of the gut and by relaxing the muscles in the stomach and intestines [6, 7]. Combination of Mefenamic Acid and Dicyclomine Hydrochloride has a Synergistic effect.

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This Combination is a highly effective and used in the treatment of spasmodic dysmenorrhoea**,** intestinal colic, biliary colic, ureteric colic [8]. Mefenamic Acid is a medication that helps in reducing the pain in the body.Antipyretic is a drug that helps in reducing the fever by reducing the temperature of the body to the normal temperature. Anti-inflammatory is a kind of medication that is mainly for reducing the inflammation by controlling the pain. Lastly Dicyclomine HCl helps in offering relief to the cramps of the stomach, bladder and intestine [8].

Both the drugs are official in Indian pharmacopoeia 2010 [11], United State Pharmacopoeia [12] and British Pharmacopoeia [13]. Literature survey reveals that RP-HPLC [14], Liquid Chromatography [15], Voltametry [18], UV-Visible Spectrophotometry [20, 21], methods were reported for the estimation of Mefenamic Acid alone or in combination with other drugs except Dicyclomine Hydrochloride and Capillary Gas-Chromatography [30], Stability indicating gas-liquid chromatography [30], HPTLC[31], methods were reported for the estimation of Dicyclomine Hydrochloride alone or in combination with other drugs except Mefenamic Acid.

As per literature survey, no analytical method has been reported for simultaneous estimation of Mefenamic Acid and Dicyclomine Hydrochloride in pharmaceutical dosage forms. Therefore the present research work, our aim is to develop a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Mefenamic Acid & Dicyclomine Hydrochloride in their combined dosage form in routine analysis.

MATERIALS AND METHODS:

Reagents and Chemicals: Methanol (AR Grade) and NaOH (S.D. fine Chemicals) were used as solvent. Pure Standard gift sample of Mefenamic Acid (MEF) and Dicyclomine Hydrochloride (DICY) provided by Shree Dhanvantary Pharmaceutical Analysis & Research Centre, (Kim) and Mercury labs Ltd., Vadodara. Tablets of MEFTAL-SPAS (Mefenamic Acid- 250 mg, Dicyclomine Hydrochloride- 10 mg) were purchased from local market.

Instruments: Shimadzu UV/Vis-2450 and UV/Vis-1800 double beam UV/Vis spectrophotometer with a fixed slit width of 2 nm, 1 cm quartz cells was used for recording derivative spectra of standard and test samples. Sartorius CD2250 balance was used for weighing the samples. Class 'A' volumetric glassware were used.

Preparation of standard solution:

1. **Absorption Correction and Differential Derivative Method:**

   a. **Preparation of stock solution of MEF:** Weighed accurately about 10mg of MEF & transferred into 100 mL volumetric flask add 20mL of methanol and sonicated for about 15 min than diluted up to the mark with methanol to get a stock solution having strength 100 µg/mL.

   b. **Preparation of working Standard solution of MEF:** 100 µg/mL of MEF solution was prepared by diluting stock solution to 10mL with Methanol: NaOH (50:50) and NaOH. This solution was diluted further to get the concentration range of 10, 25, 30, 40, 50, 75 µg/mL of MEF.

   c. **Preparation of stock solution of DICY:** Weighed accurately about 10mg of DICY & transferred into 100 mL volumetric flask add 20mL of methanol and sonicated for about 15 min than diluted up to the mark with methanol to get a stock solution having strength 100µg/mL.

   d. **Preparation of Working Standard Solution of DICY:** 100 µg/mL of DICY solution was prepared by diluting stock solution to 10 mL with Methanol: NaOH (50:50) and NaOH. This solution was diluted further to get the concentration range of 1, 2, 3, 4, 5, 6 µg/ml of DICY.

2. **Simultaneous Equation method:** An accurately weighed quantity of MEF (50 mg) and DICY (10 mg) were transferred to a separate 100 mL volumetric flask, respectively and dissolved and diluted to the mark with Methanol to obtain standard solution having concentration of MEF (500µg/mL) and DICY (100 µg/mL). Further dilution with NaOH as a solvent.

Procedure for Determination of Wavelength for Measurement:

1. **Absorption Correction and Differential Derivative Method:** 0.1 mL of working standard solution of Dicyclomine HCl(100µg/mL) and 2.5 mL of working standard solution of Mefenamic acid(2500µg/mL) was pipetted out in two different 10 mL volumetric flask to have a concentration of 10µg/mL and 250µg/mL for DICY and MEF respectively. One flask dilute up to make with 0.1N NaOH and labelled as sample cell & Second flask were dilute up to mark with mixture of Methanol: NaOH (50:50) and labelled as reference cell. Prepared solutions were scanned in the range of 200-400 nm on Shimadzu double beam UV visible spectrophotometer using Reference compartment solution in reference cell & sample compartment in sample cell. Wavelength estimation was selected from scan spectra. According to same procedure the derivative spectra were recorded by using digital differentiation (Convolution method) with a derivative wavelength difference (Δλ (N)) of 2 nm in the range of 200-400 nm.

2. **Simultaneous equation Method:** By appropriate dilution of two standard drug stock solutions with methanol, solutions containing 500 µg/mL of MEF and 100 µg/mL of DICY were scanned separately in the range of 200-400 nm to determine the wavelength of maximum absorption for both the drugs. MEF showed absorbance maxima at 335 nm and DICY at 218.40 nm.

Validation: Sample preparation for validation was given in following manner but dilutions were made according to solvent required for differential & simultaneous equation method.

The methods were validated with respect to linearity, precision, accuracy, robustness, LOD & LOQ and assay.

Linearity: Standard stock solutions were prepared by dissolving 25 mg MEF and 10 mg of DICY in 100 ml volumetric flasks in 25 mL Methanol and the volume was made up with Methanol to get a concentration of 250 µg/mL of MEF and 100 µg/mL of DICY. From this, suitable dilutions were made in NaOH to get the working standard solutions of 10-75 µg/mL for MEF and 1-6 µg/mL for DICY. Absorbance of each solution measured at 308.60nm for MEF, 223nm for DICY in Absorption Correction Method. The absorbance of the derivatised spectra was measured at 308.80 nm and 211.60 nm for MEF and DICY, respectively in Differential Derivative Method. Several aliquots of standard stock solutions were taken in different 10mL volumetric flask and diluted up to mark with NaOH, such that the final linearity concentration of MEF and DICY were 25-150 µg/mL and 1-6 µg/mL in Simultaneous Method. Five replicate analyses were carried out. Plot the calibration curve of absorbance Vs respective concentration for MEF and DICY. Find out correlation coefficient and regression line equations for MEF and DICY.

Precision: The precision of the developed method was assessed in terms of repeatability (intra-day) and intermediate precision (inter-day) by analyzing three replicate of standard stock solutions at three levels that cover the calibration ranges for MEF and DICY. The % RSD value of the results corresponding to the absorbance was expressed for intra-day precision and on 3 days for intermediate (inter-day) precision.

Accuracy: Accuracy of the method was calculated by recovery studies at three levels (80%, 100% and 120%) by standard addition method. Twenty tablets were weighed and average weight was calculated. Twenty tablets were weighed and average weight was calculated. The tablets were crushed to obtain fine powder. Tablet powder equivalent to 125 mg MEF was transferred to 50 mL volumetric flask. 25 mL Methanol was added to dissolve the drugs and then volume was made up to the mark and sonicated for 15 minutes. The solution was then filtered through a Whatman filter paper (No. 41). From the filtrate 0.1 mL was transferred to three 10.0 mL volumetric flasks and add 0.08 mL (Flask 1), 0.1 mL (Flask 2), and 0.12 mL(Flask 3) of stock solution of API and then made up to the mark with suitable solvent to made them 80%, 100% and 120% spiking.

Robustness: The robustness of an analytical procedure is the measure of its capacity to remain unaffected by small, but deliberate, variations in method parameters and provides an indication of its reliability during normal usage. The parameters were change of volumetric flasks (10 mL, 50 mL and 100 mL) and

Change in instrument (UV-Vis Spectrophotometer model no. 1800 and 2450). Three replicates were made for the same conc. (25µg/mL of MEF and 1µg/mL of DICY) in 10 mL, 50 mL and 100 mL volumetric flasks and the recording of absorbances were done on both the UV-Vis spectrophotometer. The result is expressed in Percentage RSD.

Limit of detection (LOD) and limit of quantization (LOQ): LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the ten replicate determinations of same conc. (25 µg/mL of MEF and 1 µg/mL of DICY), standard deviation (SD) of the responses was calculated. From these values, the parameters Limit of Detection (LOD) and Limit of Quantitation were determined on the basis of standard deviation and slope of the regression equation.

LOD = (3.3 x SD) / Slope

LOQ = (10 x SD) / Slope

Assay: Twenty tablets were weighed and finely powdered. The average weight of tablets is determined with the help of weight of 20 tablets. A portion of powder equivalent to the weight of 125 mg of MEF was accurately weighed into 50 mL A-grade volumetric flask and 25 mL Methanol was added. The volumetric flask was sonicated for 15 min to effect complete dissolution of the DICY and MEF, the solution was then made up to volume with Methanol: NaOH & NaOH. The solution was filtered through Whatman filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 1µg/mL of DICY and 25 µg/mL of MEF. The % assay of the drugs was calculated.

RESULTS AND DISCUSSION:

1. **Selection of Wavelength for Simultaneous Estimation of Mefenamic Acid and Dicyclomine HCl: (Absorption Correction and Differential Derivative Method):** From the overlain spectrum of MEF and DICY in Methanol-NaOH and NaOH (**Figure 1**), it was observed that Dicyclomine has zero absorbance at 308.60nm; where as Mefenamic Acid has substantial absorbance. Thus, MEF was estimated directly at 308.40 nm with no interference of DICY. For the estimation of DICY, the absorbance of MEF was deducted from the total absorbance of sample mixture at 223 nm. The calculated absorbance was called as Corrected absorbance for DICY (eq. 2). The Concentration of DICY was determined from calibration curve at 223 nm using the corrected absorbance (**Figure 1**).

$$C_{MEF} = A_1 / a_{x1} \quad \text{.....(1)}$$

$$C_{DICY} = \frac{A_2 - a_{X2}\, C_{MEF}}{a_{Y2}} \quad \text{.....(2)}$$

Where, A_1 and A_2 are the absorbances of mixture at 308.60 nm and 223 nm respectively, a_{x1} and a_{x2} are absorptivities of MEF and DICY at 308.60 nm and 223 nm respectively; a_{y2} is absorptivity of DICY at 223 nm; C_{MEF} is concentration of Mefenamic Acid (MEF); C_{DICY} is concentration of Dicyclomine HCl (DICY).

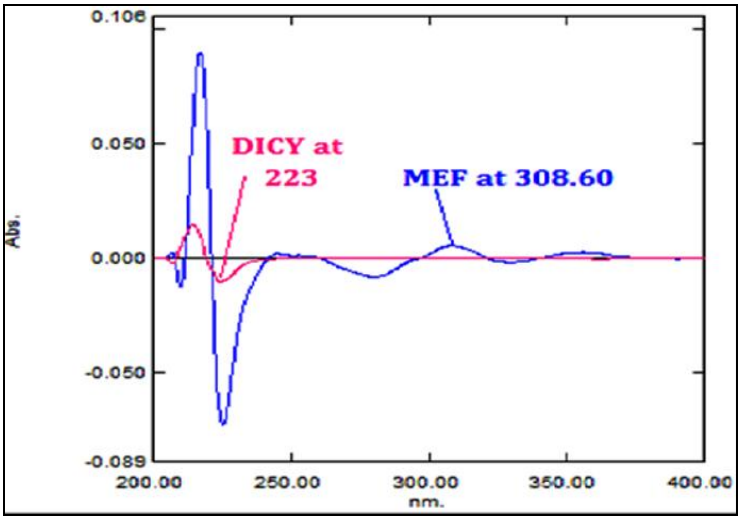


FIG. 1: OVERLAIN SPECTRA OF MEF AND DICY

In Differential Derivative Method, First order derivative spectrum for DICY showed zero crossing points: 211.60 nm. The wavelength selected for estimation of MEF was 210.59 nm because it showed adequate absorbance at this wavelength in mixture. Similarly, first order derivative spectrum for MEF was taken and it showed zero crossing point: 308.80 nm. The wavelength selected for estimation of DICY was 308.80 nm because it showed adequate absorbance at this wavelength in mixture (**Figure 2**).

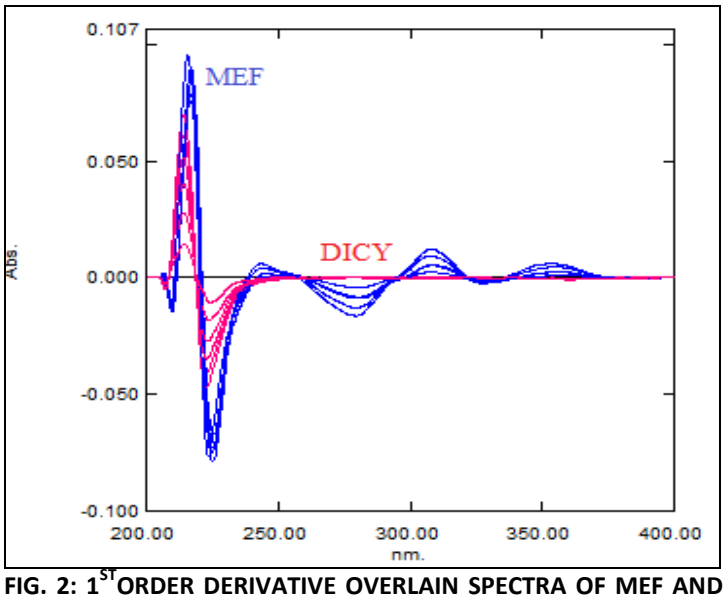


FIG. 2: 1ST ORDER DERIVATIVE OVERLAIN SPECTRA OF MEF AND DICY

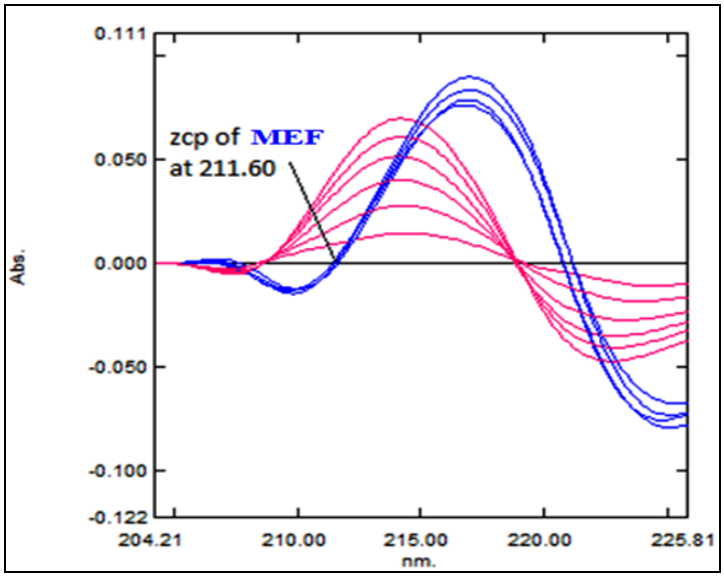


FIG. 3: ZERO CROSSING POINT OF DICY

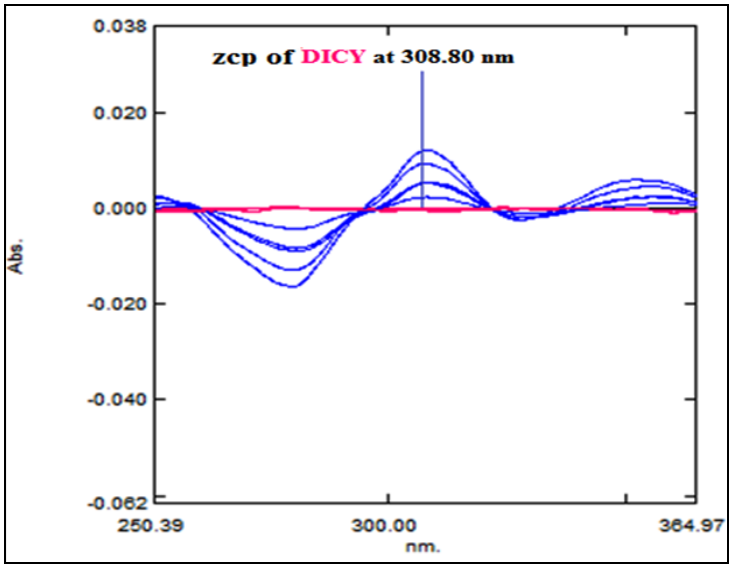


FIG. 4: ZERO CROSSING POINT OF MEF

2. The proposed Simultaneous method provides a rapid, convenient and accurate way for routine quantitative determination of MEF and DICY. The stock solutions, working standards were prepared in methanol. The λ_{max} the both the drugs for analysis were determine by separately scan both the drugs in the range of 200-400 nm, wavelength selected for quantitation were 335.0 nm (λ_{max} of MEF) and 218.40 nm (λ_{max} of DICY) (Figure. 5). Two simultaneous equations (in two variables C_x and C_y) were formed using these absorptivity coefficient values.

$$A_1 = 1.275\ C_x + 0.0510\ C_y \quad \text{..... (1)}$$
$$A_2 = 0.490\ C_x + 0.0197\ C_Y \quad \text{..... (2)}$$

Where, A_1 and A_2 are the absorbance of mixture at 335.0 (λ_1) nm and 218.40 (λ_2) nm wavelength respectively, C_x and C_y are the concentration of MEF and DICY measured in µg/mL, in sample solutions respectively, ax_1 and ax_2 are absorptivities of MEF at λ_1 and λ_2 respectively, ay1 and ay_2 are absorptivities of DICY at λ_1 and λ_2 respectively. By applying the Cramer's rule to equation 1 and 2, the concentration of C_{MEF} and C_{DICY}, can be obtained as follows,

$$C_{DIC} = A_2\,(0.0510) - A_1\,(0.0197)/-0.00013$$
$$C_{MEF} = A_1\,(0.490) - A_2\,(1.275)/-0.00013$$

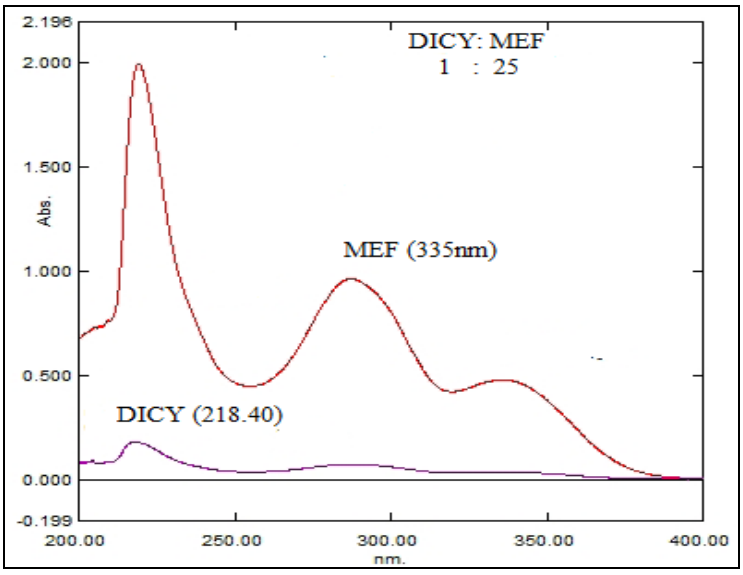
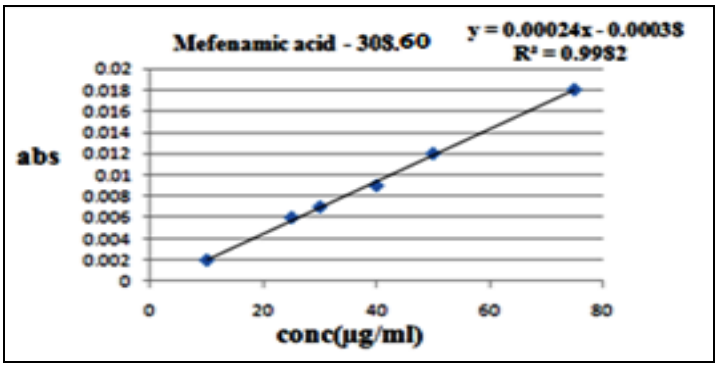
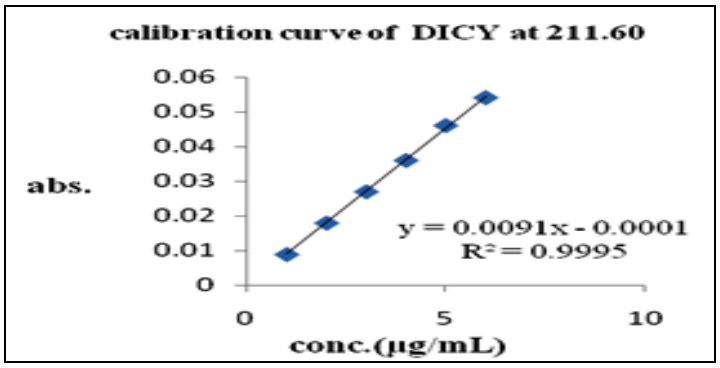


FIG. 5: ZERO ORDER OVERLAIN SPECTRA OF DICY AND MEF

RESULTS:

1. Absorption Correction and Differential Derivative Method:





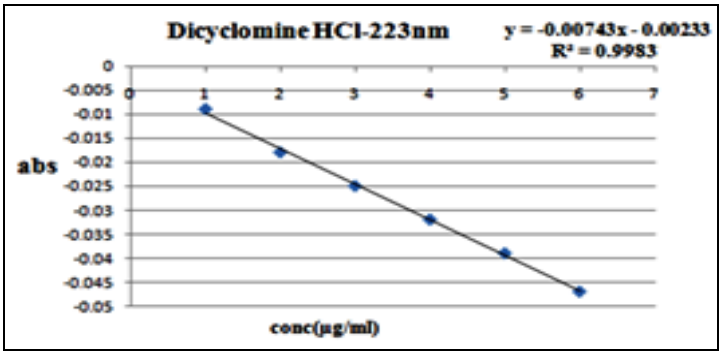


FIG. 6: CALIBRATION CURVE OF MEF AND DICY. (ABSORPTION CORRECTION METHOD)

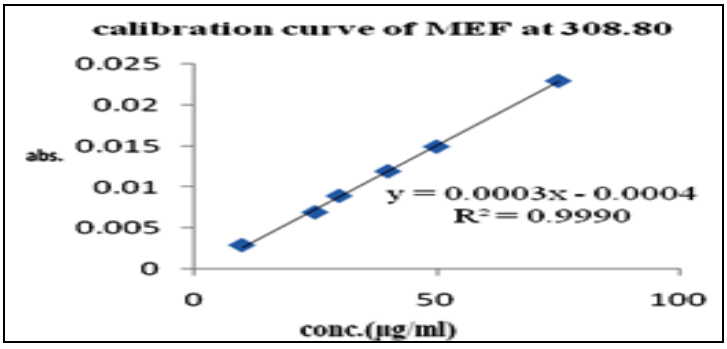


FIG. 7: CALIBRATION CURVE OF DICY AND MEF (DIFFERENTIAL DERIVATIVE METHOD)

TABLE 1: LINEARITY (n=5)

Sr. no	Conc. (µg/mL)		Absorption correction method		Differential derivative method	
	MEF	DICY	Abs. (308.60)	Abs. (223)	Abs. (308.80)	Abs. (211.60)
1	10	1	0.002	-0.009	0.003	0.009
2	25	2	0.006	-0.018	0.007	0.018
3	30	3	0.007	-0.025	0.009	0.027
4	40	4	0.009	-0.032	0.012	0.036
5	50	5	0.012	-0.039	0.015	0.046
6	75	6	0.018	-0.047	0.023	0.054

TABLE 2: PRECISION (n=3)

Conc. (µg/mL) (DICY: MEF)	Absorption correction method				Differential derivative method			
	Dicyclomine HCl		Mefenamic Acid		Dicyclomine HCl		Mefenamic Acid	
	Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD
Repeatability								
1.0 : 25.0	0.87	0.1385	24.57	0.1428	1.04	0.0029	25.59	0.0028
2.0 : 50	2.05	0.0777	50.01	0.1666	1.92	0.0003	49.24	0.0007
3.0 : 75.0	2.92	0.0234	74.25	0.0326	3.02	0.0005	74.25	0.0006
Intra Day								
1.0 : 25.0	0.92	0.6662	25.77	0.1428	1.22	0.0009	25.59	0.0028
2.0 : 50	2.05	0.0362	51.53	0.0911	1.95	0.0006	51.39	0.0007
3.0 : 75.0	2.92	0.0234	74.25	0.0368	2.91	0.0002	73.97	0.0002
Inter Day								
1.0 : 25.0	0.81	0.1237	25.77	0.2474	1.15	0.0014	24.51	0.0014
2.0 : 50	2.09	0.0063	51.53	0.0455	1.92	0.0008	51.39	0.0007
3.0 : 75.0	2.96	0.0234	75.77	0.05556	2.98	0.0003	75.05	0.00025

TABLE 3: ASSAY (n=3)

Conc(µg/mL) (DICY:MEF)	ABSORPTION CORRECTION METHOD				DIFFERENTIAL DERIVATIVE METHOD			
	Dicyclomine HCl		Mefenamic Acid		Dicyclomine HCl		Mefenamic Acid	
	Conc. (µg/mL) ± SD	%Assay	Conc. (µg/mL) ± SD	%Assay	Conc. (µg/mL) ± SD	%Assay	Conc. (µg/mL) ± SD	%Assay
2:50	1.98±0.0057	99	50.9±0.1793	101.8	2.1±0.0070	105	50.1±0.1414	100.2
	2.01±0,0152	100.5	49.9±0.3055	99.8	2.0±0.1098	104.94	51.3±0.9388	102.6

TABLE 4: ACCURACY (RECOVERY STUDY)

Absorption correction method:

Formulation (MEFTAL-SPAS)	(%API) DICY+MEF (µg/mL)	Amt. recovered (DICY)		Amt. recovered (MEF)	
		Conc. (µg/mL) ± SD	%Recovery	Conc. (µg/mL) ± SD	%Recovery
1:25	(80) (1.8 + 45)	1.80±0.1492	100.33	44.85±0.42857	99.67
	(100) (2 + 50)	2.014±0.1492	100.74	50.03±0.64150	100.07
	(120) (2.2 + 55)	2.19±0.39186	99.54	54.48±0.64150	99.05

Differential derivative method:

formulation (MEFTAL-SPAS)	(%API) DICY+MEF (µg/mL)	Amt. recovered (DICY)		Amt. recovered (MEF)	
		Conc. (µg/mL) ± SD	%Recovery	Conc. (µg/mL) ± SD	%Recovery
2:50	(80) (3.6 +90)	3.66±0.1414	100.15	90.11±1.924	100.12
	(100) (4 + 100)	4.066±0.1154	101.66	102.3±3.333	102.3
	(120) (4.4 + 110)	4.46±0.1154	101.51	112.3±3.333	102.2

TABLE 5: LOD and LOQ

Parameter	Absorption correction method		Differential derivative method	
	DICY(µg/mL)	MEF(µg/mL)	DICY(µg/mL)	MEF(µg/mL)
LOD	0.009	0.0015	0.0015	0.0015
LOQ	3.281	0.0046	2.1295	0.0046

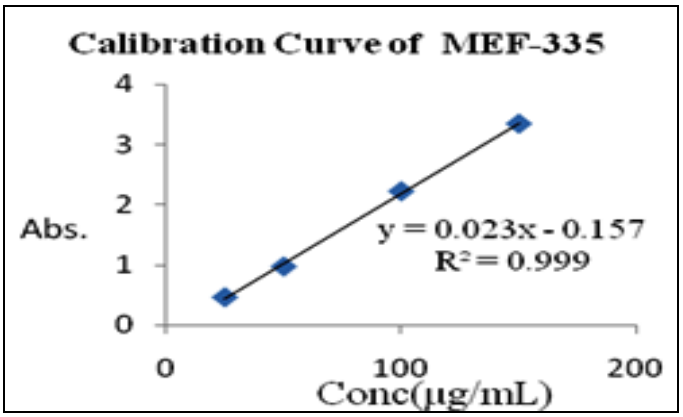
TABLE 6: RUGGEDNESS & ROBUSTNESS

Conc. (µg/ml) DICY:MEF	Change in Condition	Absorption correction method				Differential derivative method			
		Dicyclomine HCl		Mefenamic Acid		Dicyclomine HCl		Mefenamic Acid	
		Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD	Conc. (µg/mL)	%RSD
1:25	**Volumetric Flask**								
	10++	1.01	0.1414	23	0.2824	1.16	0.0673	24.6	0.202
	50++	1.14	0.1571	25.5	0.1285	1.06	0.202	26.3	0.4714
	100++	1.07	0.0074	25.5	0.1285	1.38	0.0565	24.6	0.202
	Analyst								
	1#	1.2	0.0673	25.5	0.0902	1.21	0.0673	25.2	0.0902
	2#	1.14	0.1414	25.25	0.1064	1.14	0.1414	25	0.1064
	UV-Vis Spectrophotometer model								
	U.V 1800**	1.07	0.0744	24.5	0.1573	1.03	0.0744	24.6	0.202
	U.V 2450**	1.00	0.1571	24.75	0.1398	1.00	0.1571	24.7	0.1398

TABLE 7: SUMMARY OF VALIDATION PARAMETERS FOR PROPOSED METHOD

Parameters	Absorption correction method		Differential derivative method	
	Mefenamic acid	Dicyclomine HCl	Mefenamic acid	Dicyclomine HCl
Concentration range (µg/mL)	10-75	1-6	10-75	1-6
Regression equation	Y= 0.00024x-0.00038	Y= -0.00743x-0.00233	Y= 0.0003x-0.0004	Y= 0.0091x-0.0001
Correlation Coefficient(r^2)	0.9982	0.9983	0.9990	0.9995
Accuracy(%Recovery)	99.05-100.07	99.54-100.74	100.12-102.3	100.15-101.51
Intraday precision(%RSD)	0.0368-0.1428	0.0234-0.6662	0.0002-0.0028	0.0002-0.0009
Interday precision(%RSD)	0.0455-0.2474	0.0063-0.1237	0.0002-0.0014	0.0003-0.0014
Ruggedness and Robustness	0.0902-0.2824	0.0074-0.1571	0.0902-0.4714	0.0565-0.1571
LOD(µg/mL)	0.0015	0.009	0.0015	0.0015
LOQ((µg/mL)	0.0046	3.281	0.0046	2.1295
% Assay	99-101.8	99-100.5	100.2-102.6	104.94-105

2. Simultaneous equation method:



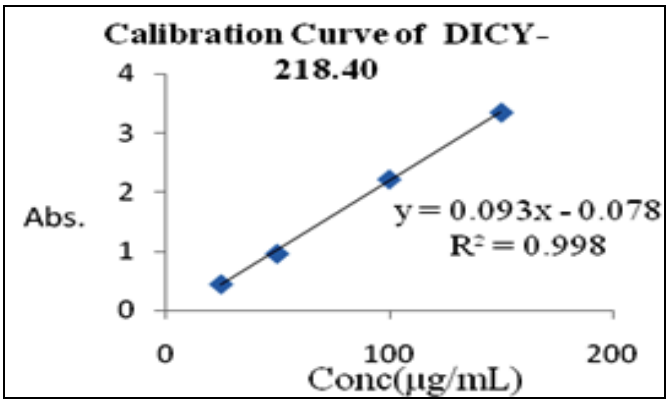


FIG. 8: CALIBRATION CURVE OF MEF AND DICY

TABLE 8 : LINEARITY DATA FOR MEF AND DICY* (n=6)

Sr. No	Concentration (DICY:MEF) (µg/mL)	Avg.abs. (218.40nm)±SD*	Avg.abs. (335 nm)±SD*
1	1:25	0.021±0.0016	0.453±0.0011
2	2:50	0.096±0.0124	0.966±0.0013
3	4:100	0.300±0.0021	2.219.±0.0035
4	6:150	0.478±0.0018	3.345±0.0027

TABLE 9: PRECISION (REPEATABILITY) *(n=3)

Conc. (µg/mL) (Dic: Mef)	Dicyclomine HCl		Mefenamic Acid	
	Conc. (µg/mL) ± SD	%RSD	Conc. (µg/mL) ±SD	%RSD
Intra Day				
2.0 : 50	1.99±0.00057	0.1877	50.12±0.00057	0.06133
4.0 : 100	4.02±0.00264	0.0777	100.80±0.00251	0.13215
6.0 : 150	5.99±0.002081	0.0559	150.85±0.003511	0.122993
Inter Day				
2.0 : 50	1.993±0.00264	0.086	50.49±0.004725	0.4983
4.0 : 100	4.0±0.00152	0.0449	100.98±0.00152	0.08007
6.0 : 150	6.11±0.030512	0.8151	150.64±0.01050	0.36838

TABLE 10: ACCURACY

formulation (Meftal-spas)	(%API) Dic+Mef (µg/mL)	Amt. recovered	Dicyclomine HCl	Amt. recovered	Mefenamic Acid
		Conc. (µg/mL) ± SD	%Recovery	Conc. (µg/mL) ± SD	%Recovery
02:50	(80) (3.6 +90)	3.59±0.00294	99.83	91.57±0.42857	101.7
	(100) (4 + 100)	4.011±0.00169	100.26	100.4±0.02749	100.4
	(120) (4.4 + 110)	4.38±0.01619	99.77	109.8±0.07273	99.89

TABLE 11: ASSAY

Conc. (µg/mL) (Dic: Mef) (1:25)	Dicyclomine HCl	%Assay	Mefenamic Acid	%Assay
Meftal spas	2.04±0.007071	102	49.1±0.14142	98.2
Dysmen	2.08±0.007071	104	49.96±0.01414	99.92
Spasmonil plus	2.02±0.056569	101	50.97±0.01414	101.94

TABLE 11: LOD & LOQ

Parameter	DICY (µg/mL)	MEF (µg/mL)
LOD	0.01259	0.04143
LOQ	0.03823	0.1255

TABLE 12: RUGGEDNESS & ROBUSTNES

Conc. (µg/mL) (DICY:MEF	Change in Condition	Dicyclomine HCl		Mefenamic Acid	
		Conc. (µg/mL) ± SD	%RSD	Conc. (µg/mL) ± SD	%RSD
1:25	**Volumetric Flask**				
	10	0.77±0.02121	0.7456	26.65±0.0007	0.1335
	50	0.80±0.04171	0.4635	25.24±0.0008	0.1688
	100	0.81±0.02474	0.8676	26.28±0.0021	0.4059
	Analyst				
	1	0.81±0.01414	0.0503	0.53±000707	0.13354
	2	2.85±0.00707	0.0248	0.51±0.00849	0.16888
	UV-Vis Spectrophotometer model				
	U.V 1800	0.83±0.0077	0.2733	25.6±0.0063	0.1362
	U.V 2450	0.81±0.0077	0.0247	26.1±0.0063	0.4091

TABLE 13: SUMMARY OF VALIDATION PARAMETERS FOR PROPOSED METHOD

Parameters	Simultaneous equation method	
	Mefenamic acid	Dicyclomine HCl
Concentration range (µg/mL)	25-150	1- 6
Regression equation	0.023x-0.157	0.093x-0.078
Correlation Coefficient(r^2)	0.999	0.998
Accuracy(%Recovery)	99.89-101.7	99.77-100.26
Intraday precision(%RSD)	0.0613-0.1321	0.0559-0.1877
Interday precision(%RSD)	0.0800-0.4983	0.0449-0.8151
Ruggedness and Robustness	0.1335-0.4091	0.0247-0.8676

CONCLUSION: The developed method was novel, simple, accurate, precise reproducible, economical, which would be used to estimate MEF & DICY in their combined dosage form in routine analysis.

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