Derivative spectrophotometric method for estimation of Chlorphinramine Maleat in pure and its formulation Phenadone

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Key Words : First and second Derivative Spectrophotometry , Chlorpheniramine Maleat , phenadone.

Received (June) **, Accepted** (June).

ABSTRACT

A simple, sensitive, economic, accurate and precise first (D1) and second (D2) derivative spectrophotometric method has been developed for determination of Chlorpheniramine maleate in pure and syrup form . The method obeys Beer's Law in concentration range of 5-45µg/ml employed for evaluation . The quantitative determination of the drug was carried out using the first and second derivative values by measured ; peak to base line at (274 nm and 236 nm for first derivative) and (282 nm , 262 nm and 241 nm for second derivative) , peak to peak at (274 nm – 263 nm for first derivative) and (282 nm-262 nm and 262 nm – 241 nm for second derivative) and peak area at (290 nm – 264 nm and 264 nm – 226 nm for first derivative) and at (304 nm -274 nm , 274 nm – 254 nm , 254 nm – 232 nm for second derivative) in 1:10% absolute ethanol: distal water . The results of analysis were validated by recovery studies . The recovery and RSD was (102.916-95.555 %) and (0.751 – 0.006 %) respectively . The proposed method can be used for the routine quality control testing of the marketed formulations .

تقدير عقار الكلورفينيرامين ماليت باستخدام مطيافية المشتقة بصورته النقية وفي المستحضر الصيدلاني فينادون

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الكلمات المفتاحية : طيف المشتقة الاولى والثانية ، الكلورفينرامين ماليت ، الفينادون

الخلاصة

تم تطوير طريقة طيفية بسيطة وحساسة واقتصادية وذات دقة وتوافق لتقدير عقار كلورفينرامين ماليت (CM) في صورته النقية وفي مستحضره الصيدلاني باستخدام مطيافية المشتقة الاولى والثانية . المدى الخطي للاستجابة يتراوح بين 5-45 مكغم.مل$^{-1}$. التقدير الكمي للعقار تم بقياس ارتفاع القمة -خط القاعدة عند (274nm و 236 للمشتقة الاولى) و (282 nm و262 و241 للمشتقه الثانية) وارتفاع قمة الى ارتفاع قمة عند (274 nm - 236 nm للمشتقة الأولى) و (282 - 262 nm و 262 nm – 241 nm للمشتقة الثانية) فضلا عن مساحه القمة عند (290 nm -264 nm وnm 246- 226 nm للمشتقة الاولى) و(304 nm- 274 nm و274 nm- 254 nm و - 232 nm 254 للمشتقة الثانية) في 10:1% ماء مقطر : الايثانول المطلق . وكانت النتائج التي تم الحصول عليها من تحليل المركب قيد الدراســة متوافقة ودقيقة حيث تراوحت قيمة RSD% بين-0.751)

(0.006 وقيمة Rec% بين (102.916_95.555%) للـ(CM) كما وأمكـن تطبيق الطريقة لتقديـــر(CM) بنجاح في المستحضر الصيدلاني فينادون .

INTRODUCTION

Chlorpheniramine maleate (CM) [(RS)-3-(4-chlorophenyl)-3- (2-pyridyl) propylmethylamine hydrogen maleate] is a histamine H1 antagonist used in allergic reactions, hay fever, rhinitis, urticaria, and asthma [1,2] . It is also effective against nausea and motion sickness, with its primary mechanism of action being its ability to reduce acetylcholine levels in the brain [3]. It has been determined alone or in combination using Spectrophotometric [4,5,6] , chromatographic [7,8,9] and electrochemical techniques [10] . Despite its wide use as an anti –allergy drug but it is very bitter and as yet no mouth dissolving/disintegrating taste-masked preparation that might be useful for pediatric and geriatric patients is available in the market [11].

Fig. 1 shows CM structure .

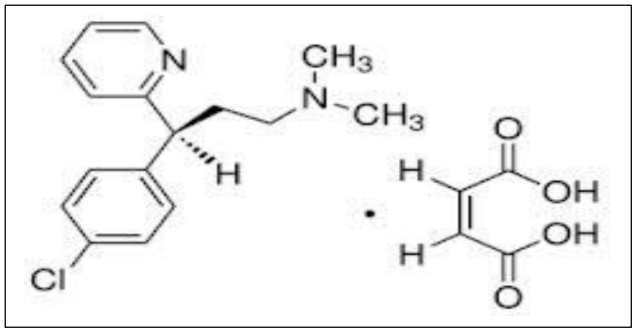


Fig. 1 :Structure of Chlorpheniramine maleate (CM)

Instrumentation

1- Sensitive Balance
2- A SHIMADZU UV-Visible-1650–Japan double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements .

Preparation of Standard Stock Solutions

3- 1000µg/ml Chlorpheniramine maleate (CM) Standard Stock Solution

Accurately weighed CM (0.1 gm) was transferred to 100 ml volumetric flask, dissolved in 1:10 % ml of absolute ethanol and distal water respectively and volume was made up-to 100 ml to obtain stock solution of 1000µg/ml .

4- 100µg/ml Pharmaceutical Solution (Phenadone)

2.5 ml was transferred from Phenadone (every 100 ml containing 40 mg CM equivalent to 400 µg/ml) to 10 ml volumetric flask, diluted in 1 ml of absolute ethanol and volume was made up-to 10 ml with distal water to obtain pharmaceutical solution of 100µg/ml according of dilution equation :

$N_1V_1=N_2V_2$

$400*2.5=N_2 * 10 = 100$ µg/ml

Preparation of calibration curve for CM

From standard stock solution of CM , 5-45µg/ml solutions was prepared in a series of 10 ml volumetric flasks of drug . Calibration curve was constructed from absorbance measure at 190-400 nm against 1:10 from absolute ethanol and distal water as blank for standard containing 5-45 µg/ml of CM shown in Fig. 2

METHOD VALIDATION

Linearity

A calibration curve was constructed using first and second derivative values versus concentration in the range of 5-45 µg/ml . The absorbance was measured versus blank at 190-400nm .

Precision

Solutions of Phenadone were prepared at two concentration levels 12,18 (µg/ml) each in triplicate. These solutions were analyzed respectively , the results are reported in terms of relative standard deviation (RSD) .

Accuracy

The accuracy of the method was assessed, based on recovery study. The technique of standard addition was used to assess accuracy of the method. From the pharmaceutical solution (Phenadone) (100µg/ml) , 1 ml was transferred to six series 10 ml volumetric flask .To these six flasks 0-2.5 ml of standard stock solution of 100µg/ml CM was added and volume was made up to 10 ml volumetric flasks diluted in the same solvent (1:10% absolute ethanol and distal water) . The absorbances of the samples matrix and after standard addition were measured . The results are reported in terms of % recovery .

RESULTS AND DISCUSSION

The CM was soluble in 1:10 ethanol : distal water , so it was used in this method as solvent . The response for CM was found to be linear in the concentration range of 5-45 µg/ml. The optical characteristics of the method and regression analysis of the calibration curve are shown in Table 1. The recovery of CM was found to be satisfactory. Excipients used in the specificity study did not interfere with response of the drug at its analytical wavelength. Hence, the method is specific and robust for of determination CM . The proposed spectrophotometric methods were applied to the estimation of CM in its pharmaceutical formulations **.**

CM Absorbance Spectra

Spectra were measured for zero , first and second derivative, as shown in Figures 3 , 4

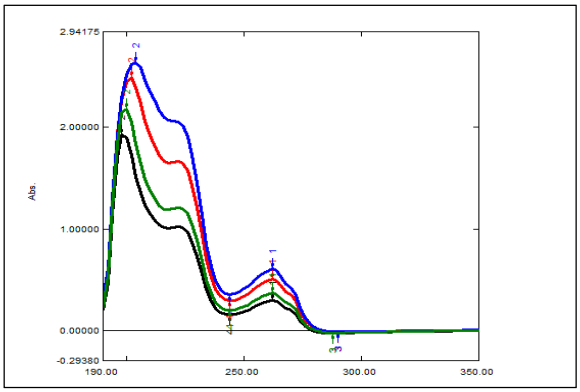
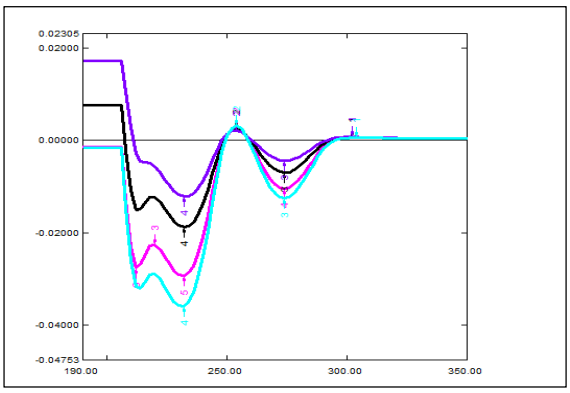


Fig. 2 Overlay spectra of zero order derivative for CM (30-45 µg/ml)



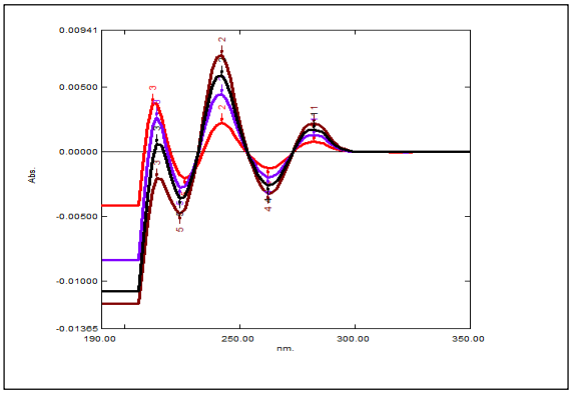


Fig. 3 : Overlay spectra of first order derivative for CM (10-35 µg/ml)

Fig. 4 : Overlay spectra of second order derivative for CM (10-35 µg/ml)

Calibration Curves for First and Second Derivative Spectra

Under the optimum operating conditions , it was used of multiple measurements conducted on the spectra of the first and second derivative recorded for measuring spectroscope ; peak to base line , peak to peak and area under peak for CM quantitative analysis in its samples . Details curves shown in the Fig. 5-10 below

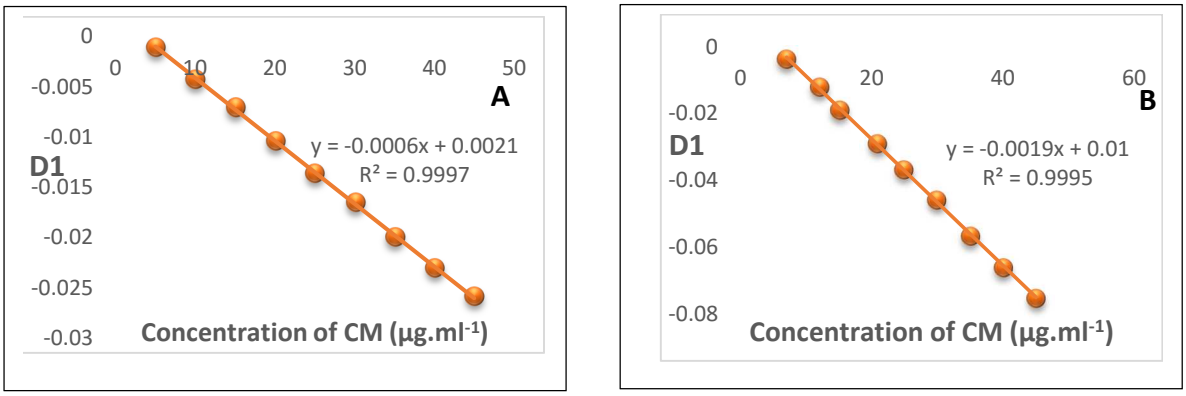


Fig. 5 : Peak to base line calibration curves for first derivative spectra for CM (A) at 274nm and (B) at 236nm

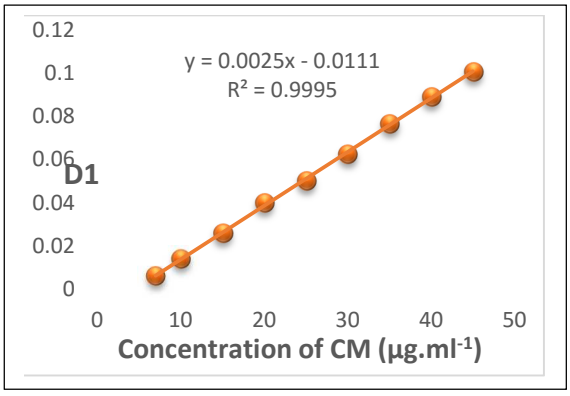


Fig. 6 : Peak to peak calibration curves for first derivative spectra for CM at 274 - 236nm

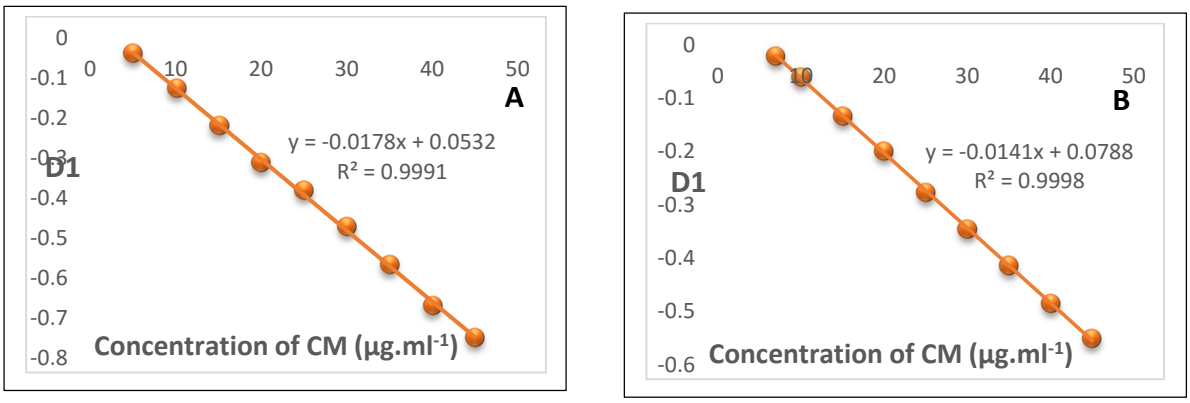


Fig. 7 : Peak area calibration curves for first derivative spectra for CM (A) at 290 - 264nm and (B) at 246-226nm

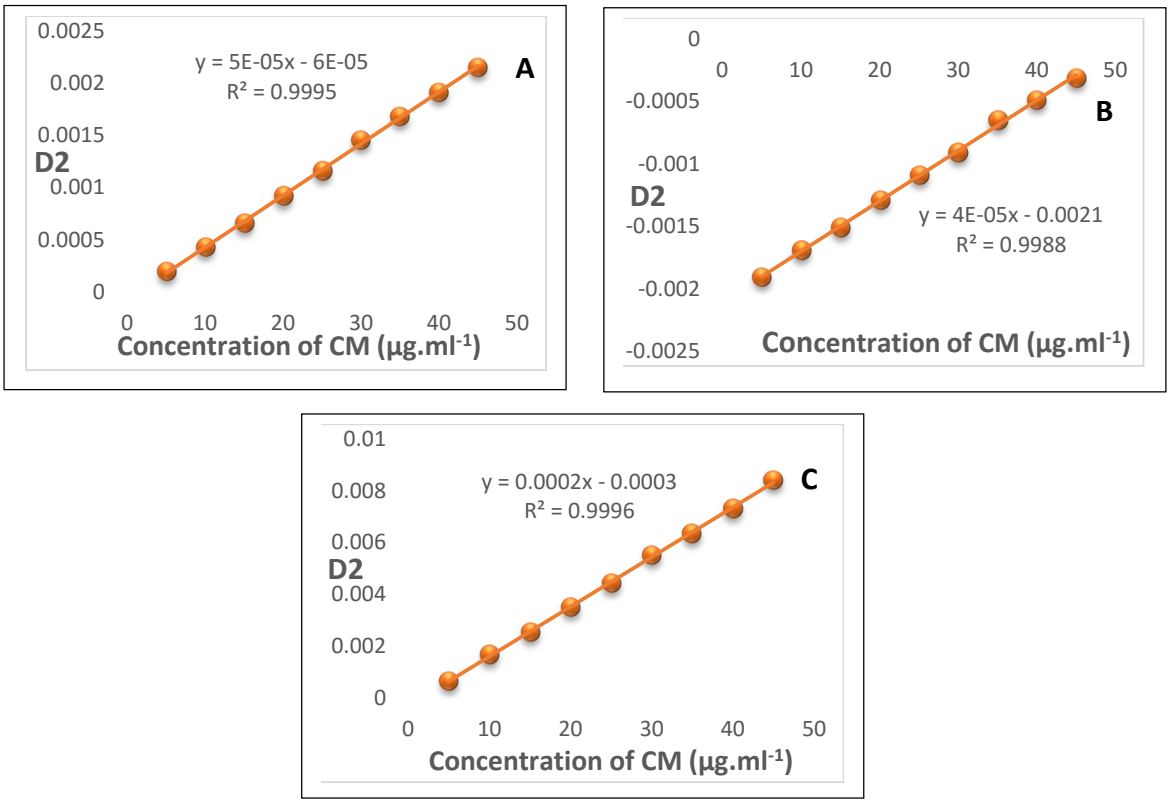
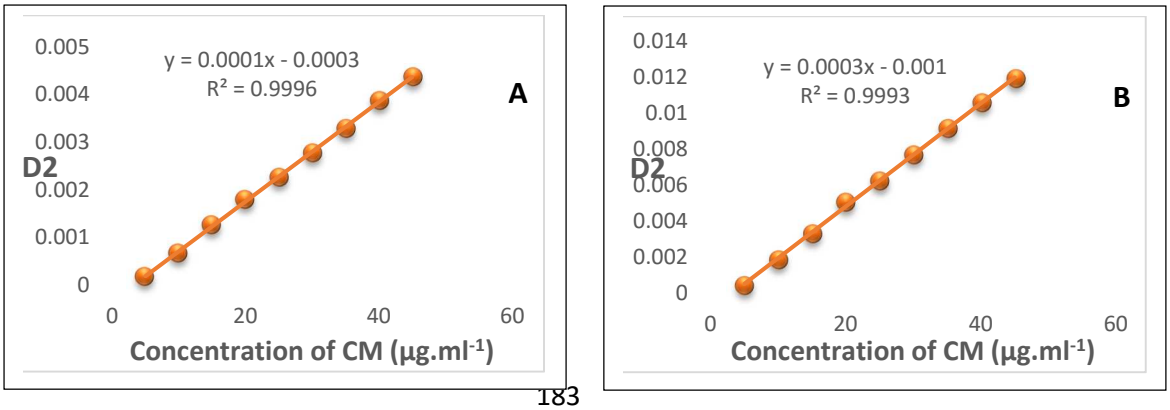


Fig. 8 : Peak to base line calibration curves for second derivative spectra for CM (A) at 282nm , (B) at 262nm and (C) at 241nm



for

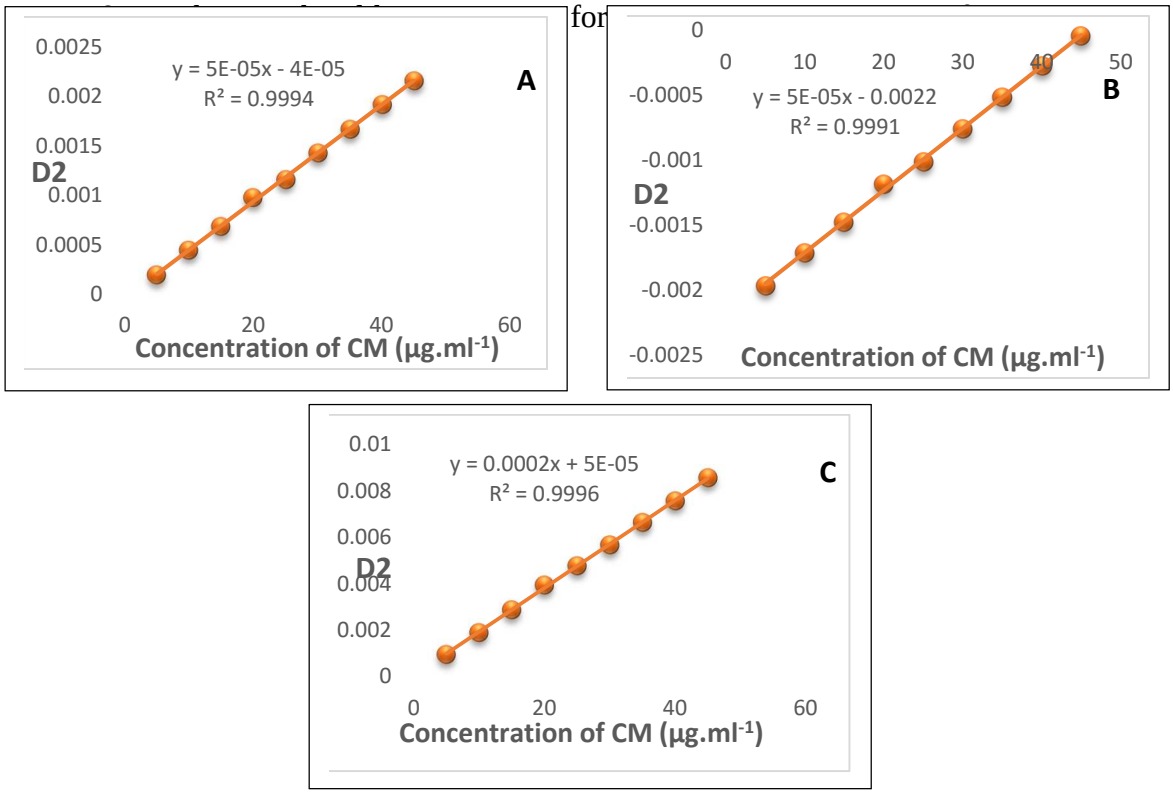


Fig. 10 : Peak area calibration curves for second derivative spectra for CM (A) at 304 -274nm , (B) at 274-254nm and (C) at 254-232nm

Table 1 : Optical Regression Characteristics and Validation Parameters of CM

Order of derivative	Mode of analysis	λ(nm)	Regression equation	R^2	Slope	Drug conc. (µg.ml^{-1})		Rec.%	R.S.D*%
						Taken	Found		
First	Peak to base line	274	Y= -0.0006x+0.0021	0.9997	-0.0006	12	12.083	100.694	0.194
						18	18.500	100.520	0.055
		236	Y= -0.0019x+0.01	0.9995	-0.0019	12	11.615	96.798	0.082
						18	17.842	99.122	0.072
	Peak to peak	274-236	Y=0.0025x-0.0111	0.9995	0.0025	12	11.844	98.700	0.054
						18	17.600	98.777	0.526
	Peak area	290-264	Y=-0.0178x+0.0532	0.9991	-0.0178	12	11.793	98.280	0.087
						18	18.347	101.930	0.055
		246-226	Y=-0.0141x+0.0788	0.9998	-0.0141	12	12.039	100.332	0.006
						18	18.157	100.873	0.309
Second	Peak to base line	282	Y=5E-05 x-6E-05	0.9995	5E-05	12	11.600	96. 666	0.068
						18	18.400	102.222	0.039
		262	Y=4E-05x-0.0021	0.9988	4E-05	12	11.750	97.916	0.085
						18	18.500	102.777	0.046
		241	Y=0.0002x-0.0003	0.9996	0.0002	12	12.350	102.916	0.100
						18	17.950	99.722	0.051
	Peak to peak	262-282	Y=0.0001x-0.0003	0.9996	0.0001	12	12.200	101.666	0.087
						18	18.400	102.222	0.060
		241-262	Y=0.0003x-0.001	0.9993	0.0003	12	12.033	100.277	0.081
						18	17.766	98.730	0.067
	Peak area	274-304	Y= 5E-05x-4E-05	0.9994	5E-05	12	11.800	98.333	0.751
						18	17.600	97.777	0.591

		254-274	Y= 5E-05x-0.0022	0.9991	5E-05	12	11.600	96.666	0.069
						18	17.200	95.555	0.041
		232-254	Y=0.0002x+5E-05	0.9996	0.0002	12	12.300	102.500	0.098
						18	17.700	98.333	0.074

*** Three replicated**

Estimating of pharmaceutical samples for CM

The drug has been estimate in pharmaceutical formulation (Phenadone) by standard additions method . Table 2 Shows the efficiency and success of the method used . The recovery values was 105-96.29 and RSD values was -5-3.703 . Details curves shown in the Fig. (11-16) below .

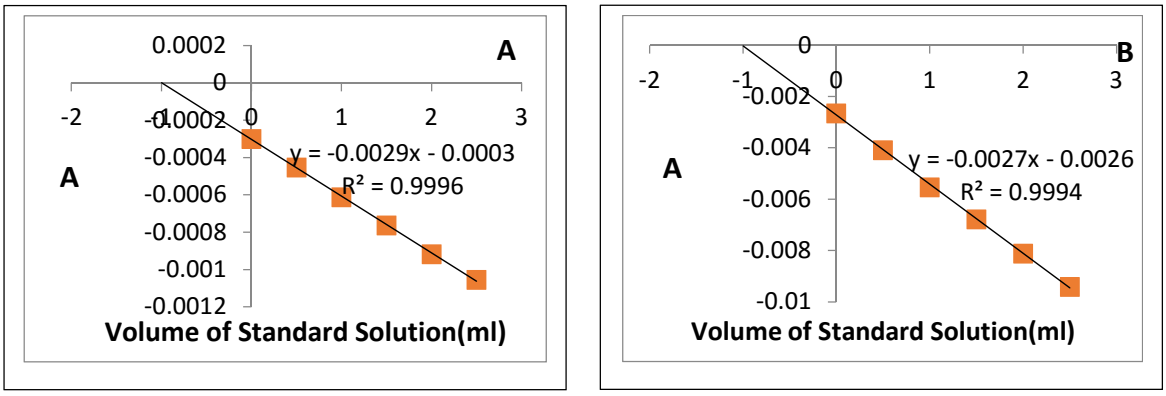


Fig. 11 : Estimation of CM by standard additions method depended on first derivative spectra for peak to base line measurement (A) at 274nm and (B) at 236nm

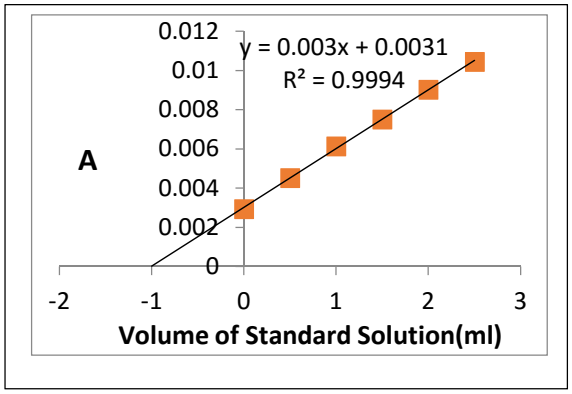


Fig. 12 : Estimation of CM by standard additions method depended on first derivative spectra for peak to peak measurement at 274 - 236nm

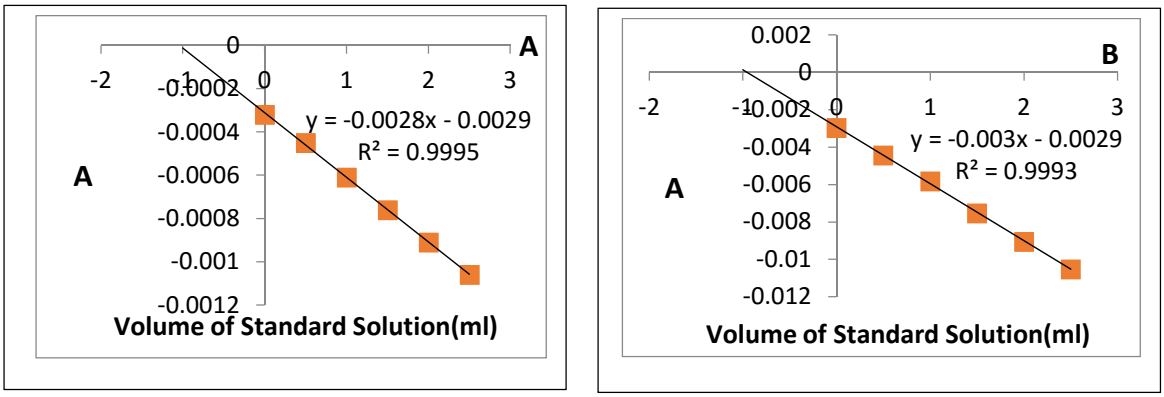


Fig. 13 : Estimation of CM by standard additions method depended on first derivative spectra for peak area measurement (A) at 290-264nm and (B) at 246-226nm

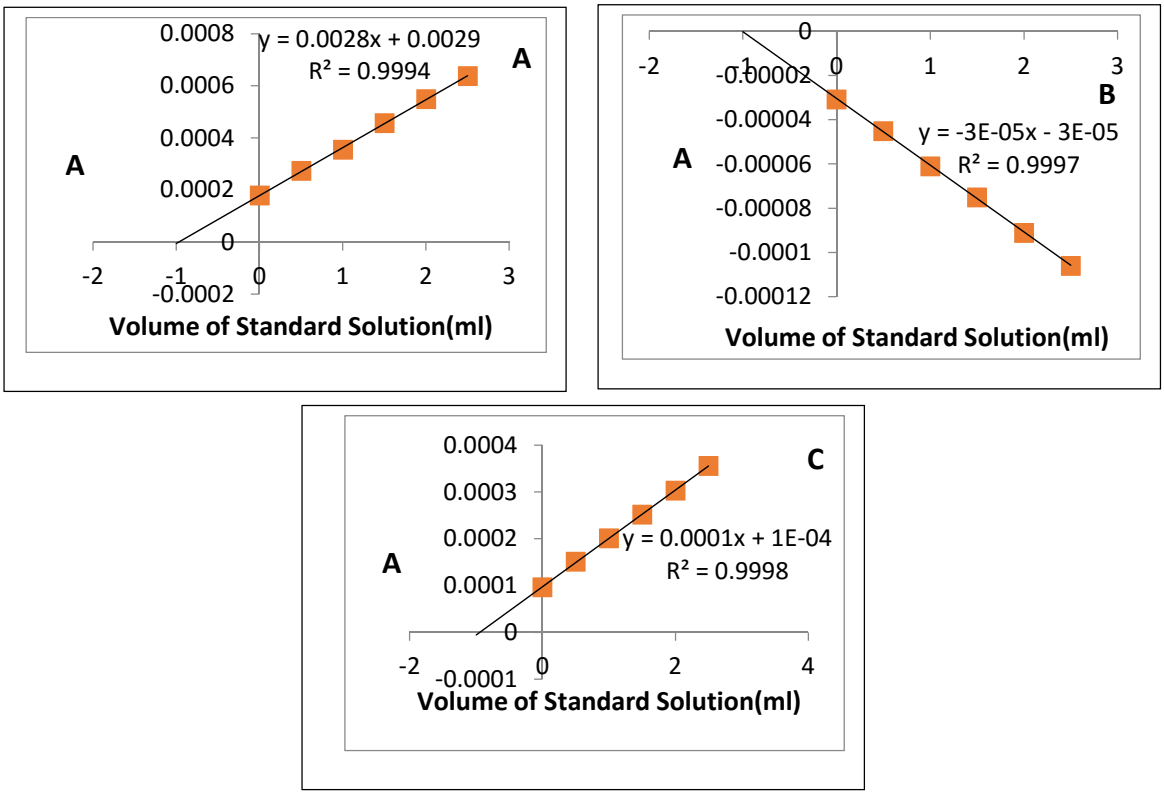


Fig. 14 : Estimation of CM by standard additions method depended on second derivative spectra for peak to base line measurement (A) at 282nm , (B) at 262nm and (C) at 241nm

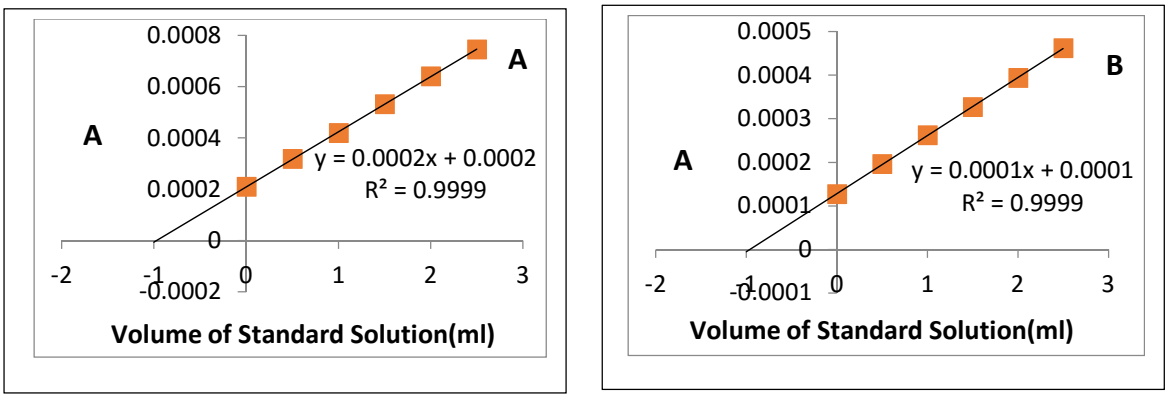
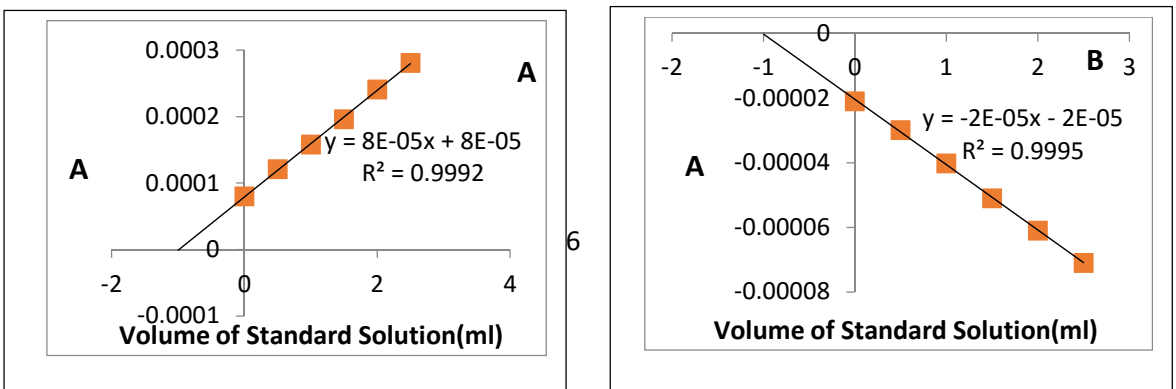


Fig.15 : Estimation of CM by standard additions method depended on second derivative spectra for peak to peak measurement (A) at 282-262nm and (B) at 262-241nm



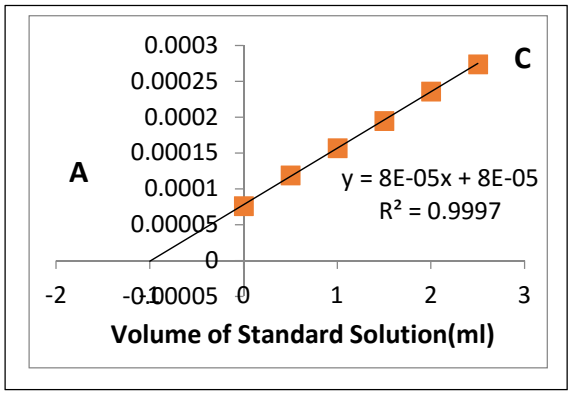


Fig. 16 : Estimation of CM by standard additions method depended on second derivative spectra for peak area measurement (A) at 304-274nm , (B) at 274-254nm and (C) at 254-232nm

Table 2 : Estimation of CM in Phenadone by the proposed method

Sample	Order	Mode of analysis	λ(nm)	Drug Conc.(μg.ml^{-1})		Rec%	ER%
				Taken	Found		
Phenadone (40 mg)	First	Peak to base line	274	10	10.3	103.00	−3.000
			236	10	9.629	96.29	3.703
		Peak to Peak	247−236	10	10.333	100.33	−3.333
		Peak area	264−290	10	10.357	103.35	−3.571
			226−246	10	9.666	96.66	3.333
Phenadone (40 mg)	Second	Peak to base line	282	10	10.357	103.35	−3.571
			262	10	10	100.00	0.000
			241	10	10	100.00	0.000
		Peak to Peak	282−262	10	10.5	105.00	−5.000
			262−241	10	10	100.00	0.000
		Peak area	304−274	10	10	100.00	0.000
			274−245	10	10	100.00	0.000

Methods for the determination of Chlorpheniramine maleate

Official Methods

Chlorpheniramine maleate is official in Indian pharmacopoeia, British Pharmacopoeia and United state pharmacopoeia.

Table 3: Summary of Compendia Methods

Pharmacopoeia	Method
IP	Potentiometry:[12] : Weigh accurately 0.2 g and dissolve in 20 mI anhydrous glacial acetic acid. Titrate with 0.1 M perchloric acid, determining the. end-point potentiometrically. Carry out a blank titration. I mI of 0.1 M perchloric acid is equivalent to 0.01954 g of $C_{20}H_{23}ClN_2O_4$.

USP	Titrimetry:[13] Dissolve 500 mg in 20 ml anhydrous acetic acid. Add 2 drops of crystal violet. Titrate with 0.1 M perchloric acid. Perform a blank determination. 1 ml of 0.1 M perchloric acid is equivalent to 19.54 mg of $C_{20}H_{23}ClN_2O_4$
BP	. Potentiometry:[14] Dissolve 0.15 g in 25 ml anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determining the end-point potentiometrically. 1 ml of 0.1 M perchloric acid is equivalent to 19.54 mg of $C_{20}H_{23}ClN_2O_4$.

Reported Methods

Analytical Methods for Single Drug

Table 4: Summary of Analytical Methods for Single Drug

Ref.	Method	Title & Abstract
[15]	HPLC	**Rapid Quantitative Analysis of Chlorpheniramine in Plasma, Saliva and Urine by High-Performance Liquid Chromatography.** A method was developed for the rapid quantitative analysis of Chlorpheniramine in plasma, saliva and urine using HPLC. A diethyl ether or hexane extract of the alkalinized biological samples was extracted with dilute acid which was chromatographed on a reversed-phase column. Ultraviolet absorption at 254 nm was monitored for the detection and brompheniramine was employed as the internal standard for the quantitation. The effects of buffer, pH, and acetonitrile concentration in the mobile phase on the chromatographic separation were investigated. A mobile phase 20% acetonitrile in 0.0075 *M* phosphate buffer at a flow-rate of 2 ml/min was used for the assays of plasma and saliva and urine samples.
[16]	TLC-Densitometry	**Rapid Identification and Quantification of Chlorpheniramine maleate or Pheniramine maleate in Pharmaceutical Preparations by Thin-Layer Chromatography-Densitometry.** Thin-layer chromatography (TLC)-densitometry was used to separate, identify, and quantitate Chlorpheniramine maleate (CPM) or Pheniramine maleate (PM) when present in combination with other drugs in pharmaceutical preparations of tablets, syrups, eye and ear drops, etc. CPM or PM was extracted (tablets, capsules, etc.) or diluted (liquid preparations, if needed) with 80% ethanol and isolated from other ingredients by TLC on silica gel G using cyclohexane– chloroform–methanol–diethylamine (4.5 + 4.0 + 0.5 + 1.0, v/v) as the mobile phase. Separated CPM and PM were detected under shortwave ultraviolet light and quantitated by scanning densitometry at 260 nm.
[17]	RIA	**Subnanogram Quantitation of Chlorpheniramine in Plasma by a New Radioimmunoassay and Comparison with a Liquid Chromatographic Method.** A new Radio Immuno Assay allows the determination of Chlorpheniramine levels up to 96 h after oral administration of a single 4-mg tablet to healthy volunteers. This procedure was sensitive to a 156-pg/mL plasma concentration when a 100-µL plasma sample was used. The mean coefficient of variation over the linear range from 0.156 to 20 ng/ml was 3.79%. The specificity of the assay was investigated, and the antisera showed 7% cross-reactivity with the *N,N*-didemethyl analogue and 17% cross-reactivity with the *N*-demethyl analogue. This high degree of specificity was also evident from the findings that the plasma

		concentrations determined by this newly described RIA procedure gave a strong correlation (r^2 = 0.88) with values obtained by an HPLC-UV procedure. The antiserum cross-reacted 100% with brompheniramine and, thus, can be used for its analysis in plasma

Analytical Methods For Combined Dosage Forms

Table 5: Summary of Analytical Methods for Combined Dosage Forms

Ref.	Method	Title & Abstract
[18]	Spectro-photometry	**Simultaneous Estimation & Validation of Paracetamol, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in Tablets by Spectrophotometric Method.** This method utilize 0.1N NaOH as solvent. Simultaneous equation develops using 256.8 nm, 236.8 nm and 222.4 nm as the λ_{max} of Paracetamol, Phenylephrine HCl and Chlorpheniramine maleate respectively. Calibration curves were linear over the concentration ranges of 0-35 g/ml for all drugs. The results demonstrated that the procedure is accurate, precise and reproducible (relative standard deviation < 1 %), while being simple, cheap and less time consuming, and hence can be suitably applied for simultaneous determination of three drugs in laboratory prepared mixtures and in commercial tablet preparation.
[19]	Spectro-photometry	**A Validated UV Spectrophotometric Method for the Simultaneous Estimation of Dextromethorphan Hydrobromide and Chlorpheniramine Maleate in Syrup Formulation.** A novel spectrophotometric method for the simultaneous estimation of Dextromethorphan HBr and Chlorpheniramine Maleate in combined liquid dosage form,(i.e., syrup). This is a derivative spectroscopic method. Dextromethorphan HBr has absorbance maxima at 289.2nm and Chlorpheniramine maleate has absorbance maxima at 262.6nm in methanol. The proposed method was validated in terms of Linearity, Accuracy, Specificity, Precision, Ruggedness, Beer's Law was obeyed in the concentration range of 10-70 µg/ml for both the drugs. r^2 = 0.999.
[20]	HPLC	**Simple HPLC Method for Simultaneous Determination of Acetaminophen, Caffeine and Chlorpheniramine maleate in Tablet Formulations.** A simple, rapid and accurate, routine-HPLC method is described for simultaneous determination of Acetaminophen, Caffeine and Chlorpheniramine maleate in a new tablet formulation Chromatographic separation of the three pharmaceuticals was achieved on a Hypersil CN column (150 × 5.0 mm, 5 µm) using a mobile phase comprising a mixture of acetonitrile, an ion-pair solution and tetrahydrofuran (13:14:87, v/v,pH4.5). The flow-rate

		was changed from 1.0 ml/min(in 0≈7.5 min) to 1.8 ml/min (after 3.5 min). was complete in <10 min.

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