RP-HPLC Method for Simultaneous Estimation of Diclofenac sodium ,Chlorphenaramine malate and Paracetamol in Tablets

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KEY WORDS: Estimation of Diclofenac sodium ,Chlorphenaramine malate and Paracetamol using Rp-HPLC.

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ABSTRACT:

A simple, selective, accurate reverse phase high Performance Liquid Chromatographic (Rp-HPLC) method was developed and validated for the analysis of. Diclofenac sodium ,Chlorphenaramine malate and Paracetamol in tablets .Chromatographic separation achieved isocratically on a C18 column [Use Inertsil C18, 5m , 25 mm x 4.6 mm] utilizing a mobile phase of acetonitrile/phosphate buffer (55:45, v/v, pH 6.0) at a flow rate of 1.2 ml/min with UV detection at 262 nm. The retention time of Diclofenac sodium, Chlorpheniramine maleate and Paracetamol were 2.115, 2.567 and 3.021 min respectively. The developed method was validated in terms of accuracy, precision, linearity for three drugs was found in the range of 2-60 µg/ml , 2-40 µg/ml and 2-40 µg/ml for Diclofenac sodium , Chlorphenaramine malate and Paracetamol respectively. The limit of detection for Diclofenac sodium , Chlorphenaramine malate and Paracetamol was found to be 0.8957 µg/mL , 0.935 µg/mL and 0.4033 µg/mL respectively whereas, the limit of quantification was found to be 2.7229 µg /mL, 2.8335 µg /mL and 1.2220 µg /mL respectively. The average recovery was found to be 99.482%, 100.932% and 98.303% for Diclofenac sodium , Chlorphenaramine malate and Paracetamol respectively.

The proposed method was found to be accurate, precise and rapid for the simultaneous determination of Diclofenac sodium , Chlorphenaramine malate and Paracetamol.

استخدام كروماتوغرافيا الطور العكوس للتقدير الكمي لعقاقير الدايكلوفنك صوديوم والكلوروفينرامين ماليت والبارسيتول في اشكالها الخام والصيدلانية.

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الملخص:

في هذه الدراسة تم تطوير طريقة جديدة بسيطة وانتقائية وحساسة واقتصادية ومباشرة لنقدير كمي ونوعي لخليط من عقاقير الفولتارين والكلوروفينرامين ماليت والبارسيتول في اشكالها الخام والصيدلانية باستخدام تقنية الكروماتوغرافيا السائلة عالية الاداء- الطور العكوس وعلى العمود C18 ذو ابعاد (Inertsil C18, 5m , 25 mm x 4.6 mm) وقد تم الاسترداد بطريقة الايزوكراتك وعلى الطور المتحرك المكون من الاسيتونتريل-

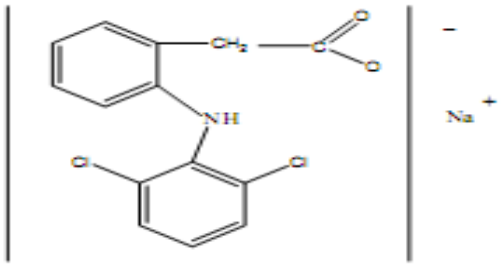
محلول الفوسفات المنظم (55:45, v/v, pH 6.0) وبمعدل جريان 1.2 مل/ دقيقة وباستخدام كاشف الاشعة فوق البنفسجية وعند طول موجي 262 nm .

وكان معدل ازمان الاحتباس لعقاقير الفولتارين والكلوروفينرامين ماليت والبارسيتول(2.115, 2.567 3.021 دقيقة) على التوالي. وقد تم تقيم الطريقة احصائيا فكان معدل الخطية للعقاقير اعلاه (2-40 , 2-60 µg/ml µg/ml,2-40) على التوالي . امامعدل حدود الكشف العام والكمي فكان (2.7229 µg/ml, 0.8957 µg/ml) لعقار الفولتارين) و (2.8335 µg/ml,0.9350 µg/ml) لعقار الكلوروفينرامين ماليت (1.222 µg/ml, 0.4033 µg/ml) لعقار البارسيتول . وكان معدل الاسيعادية لعقاقير الفولتارين والكلوروفينرامين ماليت والبارسيتول 99.482%, and و 100.932% و 98.303% على التوالي.

وقد تم تطبيق الطريقة لتقدير خليط من العقاقير الثلاثة على احد التركيبات الصيدلانية بهيئة اقراص فكانت الطريقة حساسة وموثوقة ودقيقة وخالية من التداخلات.

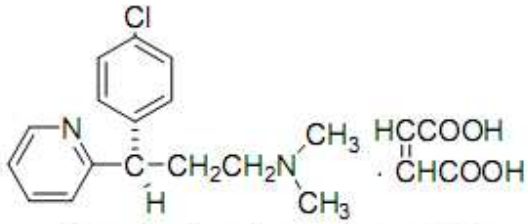
INTRODUCTION:

Diclofenac sodium or Sodium [O-(2,6-dichlorophenyl)-amino-phenyl]acetate[D.S] is a non-steroidal antiinflammatory analgesic with potent cycloxygenase inhibition activity [(1)] This drug is commonly used for pain control and treatment of rheumatic diseases. Diclofenac is well absorbed after oral administration with hepatic metabolism[(1)].



Diclofenac sodium

Chlorpheniramine maleate[CM] chemically, 3-(4-chlorophenyl)-N, N-dimethyl-3-pyridin-2-ylpropan-1-amine is an antihistamine drug that is widely used in pharmaceutical preparations for symptomatic relief of common cold and allergic diseases[(1)].

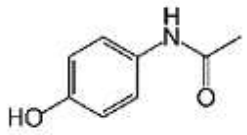


Chlorpheniramine maleate (CM)

Paracetamol or 4-hydroxyacetanilide; N-(4-hydroxy-phenyl)acetamide[P]

It is a para-aminophenol derivative having analgesic and antipyretic properties and does not possess anti-inflammatory activity. The mechanism of action of paracetamol is due to its inhibition of the cyclooxygenase enzyme and the prostaglandin synthesis in the

central nervous system and its direct activity on the centre for the body temperature regulation in the hypothalamus [1]. Many methods have been described in the literature for the determination of paracetamol with other drugs individually and in combination.



Paracetamol

Diclofenac sodium[DS] alone or in combination with other drugs is reported to be estimated by spectrophotometric method[2-5], GC [6] HPLC[7-11] , and Micellar electrokinetic chromatographic method.

For estimation of Paracetamol alone or in combination with other drugs, spectrophotometric method[12-20], Fluorimetry[21] , amperometric determination[22] HPTLC[23],HPLC[24-25] and Micellar electrokinetic chromatographic method.

Literature survey revealed that spectrophotometry[26-28] , electrochemical [29,30] , HPTLC[31] RP-HPLC[32-38] , methods have been reported for the estimation of Chlorpheniramine maleate[CM] in pharmaceutical formulations.

Although there are a number of methods reported for the determination of both the drugs separately or in combination with other drugs, no method is reported for the simultaneous determination of these drugs using RP-HPLC in pharmaceutical formulations. The aim of this study was to develop a sensitive, accurate and specific RP-HPLC method for the simultaneous determination of these drugs in pharmaceutical dosage formulations.

The present work describes a simple reverse phase HPLC method for the determination of diclofenac sodium ,Chlorphenaramine malate and Paracetamol in tablets.

EXPERIMENTAL

Materials and Reagents:

HPLC grade Sodium dihydrogen phosphate Na_2HPO_4), acetonitrile procured and phosphoric acid from Merck . Water HPLC grade was obtained from Merk. Reference standards of Diclofenac sodium , Chlorphenaramine malate and paracetamol were procured from S.D.I Iraq.

Stock and Standard Solutions:

Standard stock solution (100mg) of Diclofenac sodium ,Chlorphenaramine malate and Paracetamol were prepared by dissolving in volumetric flask 100 ml with mobile phase , separately. The solutions were suitably diluted with mobile phase to get mixed standard solution containing 20.00 µg/ml of Diclofenac sodium , 20 .00 µg/ml Chlorphenaramine malate and 15.00 µg/ml of Paracetamol.

Sample Solution:

Twenty tablets (Pain Leave, Ajanta pharma limited, India) each containing 50 mg of Diclofenac sodium , 4 .00mg of Chlorphenaramine malate and 500.00mg Paracetamol were powdered and weighed equivalent to 25.00 mg of drug was weighed accurately and taken into 25 ml volumetric flask. The drugs were extracted using acetonitrile, volume was adjusted to 25 ml the same solvent , vortexed and then filtered through 0.45 µ millipore filter before used and degassed in an ultrasonic bath. From this solution, further dilutions were made using mobile phase to get a final concentration of 25 µg/ml of Diclofenac sodium , 20 µg/ml of Chlorphenaramine malateand, 30 µg/ml of Paracetamol and this solution was used for the estimation.

Chromatographic Conditions:

A High Performance Liquid Chromatograph system, with LC solutions data handling system (Shimadzu-LC2010) , rheodyne injector with a 20 µl loop, vacuum degasser ,UV-Visible detector were used for the analysis. The data was recorded using LC 2010 solutions software. The determination performed on a stainless steel column 250 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5mm diameter (Inertsil C18, 5m , 250 mm x 4.6 mm, make: Shimadzu ltd, Japan) with isocratic mobile phase containing acetonitrile and phosphate buffer in the ratio of 55:45 (v/v pH 5.2) at ambient temperature. Flow rate was kept at 1.2 ml/min and the elution was monitored at 262 nm and injection volume were 20µl.

With the optimized chromatographic conditions, a steady baseline was recorded. The retention times of Diclofenac sodium , Chlorphenaramine malate and Paracetamol were found to be 2.115, 2.567 and 3.021 min respectively. The assay procedure was repeated for six times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated.

The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

Linearity:

Linearity is investigated to determine the range over which analyte response is a linear function of concentration. This study was performed by preparing standard solutions at different concentrations and analyses were performed in triplicate. The responses were measured as peak area. The calibration curves were obtained by plotting peak area against concentration and the obtained data were represented figures 2,3,4.

Plotting of Calibration curves:

In a series of 10 ml volumetric flask several dilutions of Diclofenac sodium (1.00 - 60 .00µg/mL),Chlorphenaramine malate(2.00 -40.00 µg/mL) and Paracetamol(1.00 - 50.00 µg/mL) were prepared in the mobile phase . Each solution was injected and a chromatogram was recorded . The peak area of Diclofenac sodium , Chlorphenaramine malate and Paracetamol were calculate and respective calibration curves were plotted against ratio of area under curve and concentration of drug. The linearity was observed in the concentration range of 2.00-40.00 µg/mL, 2.00-40.00 µg/mL and 2.00-60.00 µg/mL for Diclofenac sodium , Chlorphenaramine malate and Paracetamol respectively and the obtained data were represented in figures 2,3 and 4.

Limits of Detection and Limit t of Quantitation:

The LOD and LOQ were separately determined on the basis of standard calibration curve. The residual standard deviation of the regression line or the standard deviation of y-intercepts of regression lines was used to calculate LOD and LOQ. Following formulae were used; LOD= 3.3×D/S and LOQ= 10×D/S, where, D is the standard deviation of the y-intercepts of regression line and S is the slope of the calibration curve.

Table 1. Linearity Results, Limit of Detection (LOD) and Limit of Quantification

Drug	Equation	R^2	RSD%	intercept	LOQ µg/ml	LOD µg/ml
Diclofenac sodium	**y = 4.2944x + 0.2849**	**0.9999**	1.6220	0.2849	2.7229	0.8957
Chlorphenaramine malate	y = 15.723x + 1.8979	0.9989	0.7919	1.8979	2.8335	0.935
Paracetamol	**y = 14.574x + 1.3014**	**0.999**	0.8254	**1.3014**	1.2222	0.4033

Y= Area under peak $x10^4$

Precision:

The precision of an analytical method(within-day variations of replicate determination) is the closeness of replicate results obtained from analysis of the same homogeneous sample. The precision of the method was expressed as the RSD% at the LOQ level was 20.00 ,8 .00 and 30 µg/ml for Diclofenac sodium, Chlorphenaramine malate and Paracetamol respectively . The repeatability and intermediate precision, in accordance with ICH(International Conference on Harmonization) recommendations. Results from determination of repeatability and intermediate precision were expressed RSD.

Table 2: The precision of DS,CP and PA (n=3)

Compound	Peak Area $x10^4$	RSD %
Diclofenac sodium 20 µg/mL	85.37636	1.2000
Chlorphenaramine malate 8 µg/mL	128.6031	0.8312
Paracetamol 30 µg/mL	440x75096	0.8145

Accuracy:

The accuracy of an analytical method is the closeness of results obtained by that method to the true value for the sample. It is expressed as recovery (%), which is determined by the standard addition method. The experiment was performed in triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

Table 3: The accuracy of DS,CP and PA (n=3)

Drug	Spiked Concentration µg/mL	Measurement Concentration µg/mL mean	RSD %	Recovery %
Diclofenac sodium	10.00	10.107	1.2500	101.07
Chlorphenaramine malate	10.00	10.010	0.7880	100.10
Paracetamol	10.00	09.820	0.8341	98.20

RESULTS AND DISCUSSION:

A reversed-phase column procedure was proposed as a suitable method for the simultaneous determination of Diclofenac sodium, Chlorphenaramine malate and Paracetamol in combined dosage form. The chromatographic conditions were optimized by changing the mobile phase composition, pH, and buffers used in the mobile phase. Different ratios were experimented to optimize the mobile phase. Finally a mixture of Acetonitrile and di sodium hydrogen phosphate buffer (pH-5.2) in the ratio of 55:45 was used. A typical chromatogram obtained by using the aforementioned mobile phase from 20 µL of the assay preparation is illustrated in Fig. 1. The retention times of DS , CP and PA were 2.115 , 2.567 and 3.021 min, respectively.

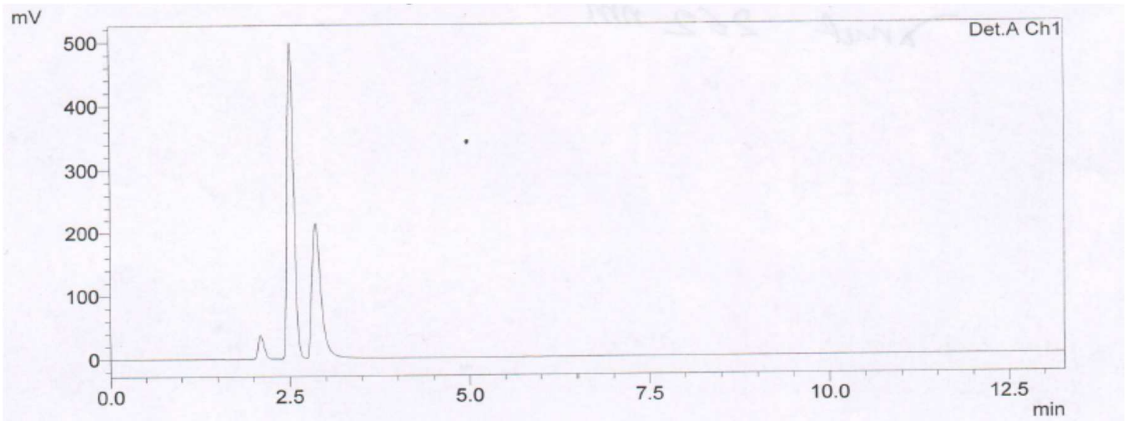


Fig. 1 : A typical chromatogram showing the peaks of DS **(2.115 min),CP(2.567min) and PA (3.021 min) in the mixture of pure forms of 20ppm ,10ppm ,30ppm of three drugs respectively .**

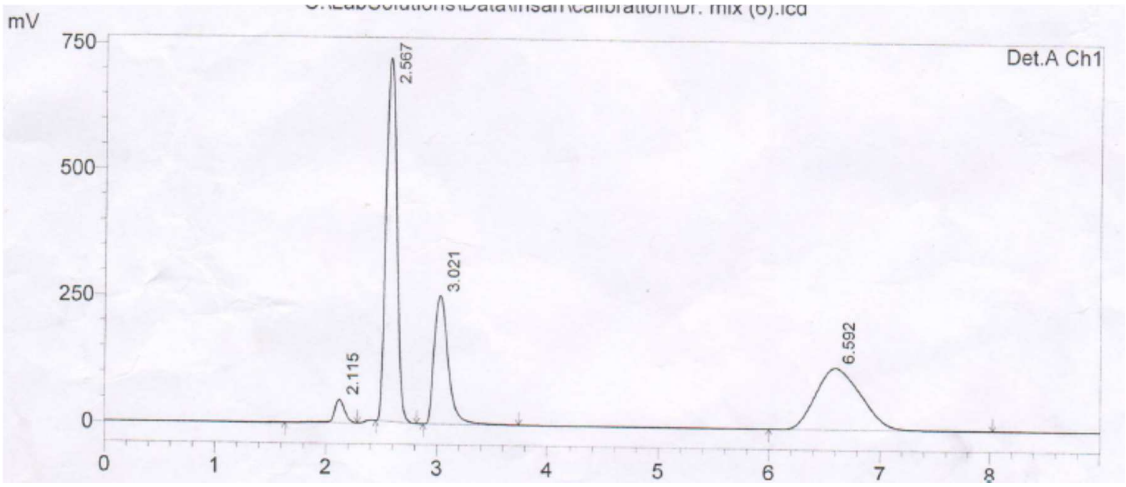


Fig. 2 : A typical chromatogram showing the peaks of DS **(2.115 min),CP(2.567min) and PA (3.021 min) in pharmaceutical dosage forms.**

The linearity was observed in the concentration range of 1-40 µg/ml , 2-40 µg/ml and -1 50 µg/ml for Diclofenac sodium , Chlorphenaramine malate and Paracetamol respectively. . Linearity solutions were injected in triplicate and the calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/ml. In the simultaneous determination, the calibration graphs were found to be linear for both the analytes in the mentioned concentrations and the correlation coefficients for the regression line were 0.9999 , 0.9989 and 0.999 for Diclofenac sodium , Chlorphenaramine malate and Paracetamol respectively.

The accuracy of the method was studied by recovery experiments. The recovery experiments were performed by adding known amounts of the drug to the placebo. The recovery was determined at three levels, viz. 80%, 100%, and 120% of the selected concentrations.

Table 4: The accuracy of DS,CP and PA (n=3)

Compound	**t_R(n=8,mean)**	**Area(n=8,mean)**	**K^-**	**R_s**	**α**	**T**
D.S	**2.115**	**256200.7**	**0.175**	**-----**	**2.434**	**1**
Cp	**2.567**	**4822580.6**	**0.426**	**1.29**	**1.591**	**0.5**
PA	**3.021**	**2189320.2**	**0.678**	**1.0**		**1**

Tm= 1.8 min

Table 5. Recovery Results for Diclofenac sodium , Chlorophenaramine malate and Paracetamol in synthetic mixture. Where **K^- Capacity factor ,R (Resolution) , α Selective factor ,(T) Tailing factor .**

Mixture	D .S			CM			PA		
Mixture	added µg	Found µg	Recovery %	added µg	Found µg	Recovery %	Added µg	Found µg	Recovery %
1	**6.000**	5.854	**97.571**	2.000	1.994	**99.719**	**0.600**	**0.597**	**99.546**
2	**8.000**	7.972	**99.660**	6.000	6.014	**100.234**	**1.000**	**0.984**	**98.461**
3	**10.000**	10.107	**101.069**	8.000	8.058	**100.732**	**2.000**	**1.978**	**98.876**
4	**15.000**	15.176	**101.173**	10.000	10.103	**101.034**	**6.000**	**5.816**	**96.929**
5	**20.000**	19.815	**99.072**	15.000	15.279	**101.860**	**8.000**	**7.784**	**99.546**

Three samples were prepared for each recovery level. The recovery values for DS ,CP and PA ranged from 98.6-99.02% , 96.6-99.7%,and98.6-100% respectively (Table 1). The precision (repeatability and intermediate precision) of the method was determined from one lot of combined dosage form. Intra and Inter day studies were performed by taking six replicates of three concentrations. The results are shown in (Table 2). The limit of detection (LOD) and limit of quantitation (LOQ) for DS, CP,PA was 0.003 µg/ml, 0.0061 µg/ml and 0.01 µg/ml, 0.02 µg/ml, respectively. To determine the robustness of the developed method experimental conditions were purposely altered and RSD of the

peak areas of DS,CP and PA were found not greater than 2.0 illustrate the robustness of the method.

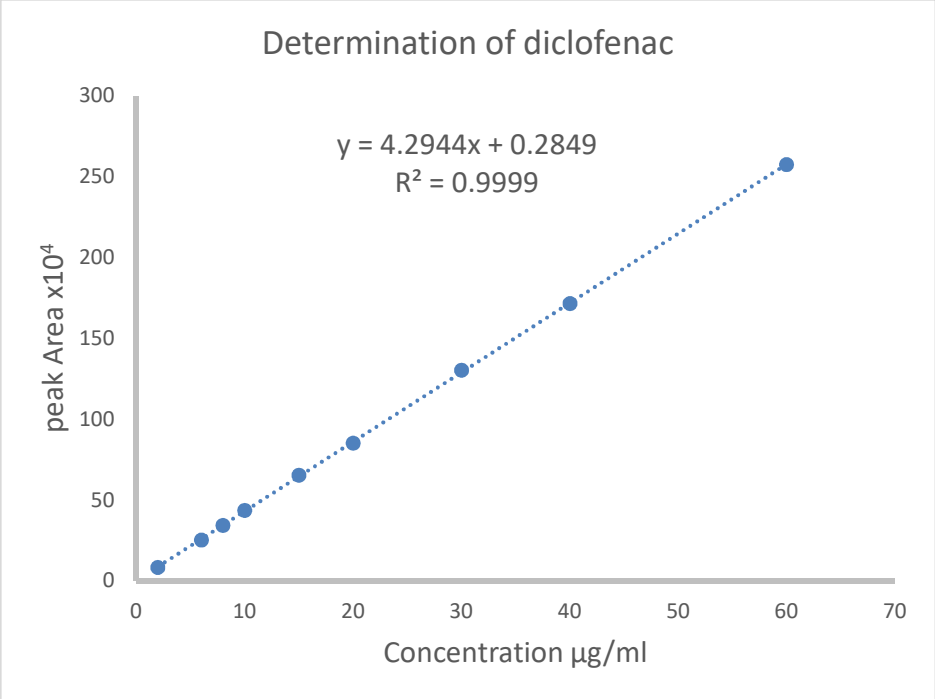


Fig 2. Calibration curves of diclofenac sodium.

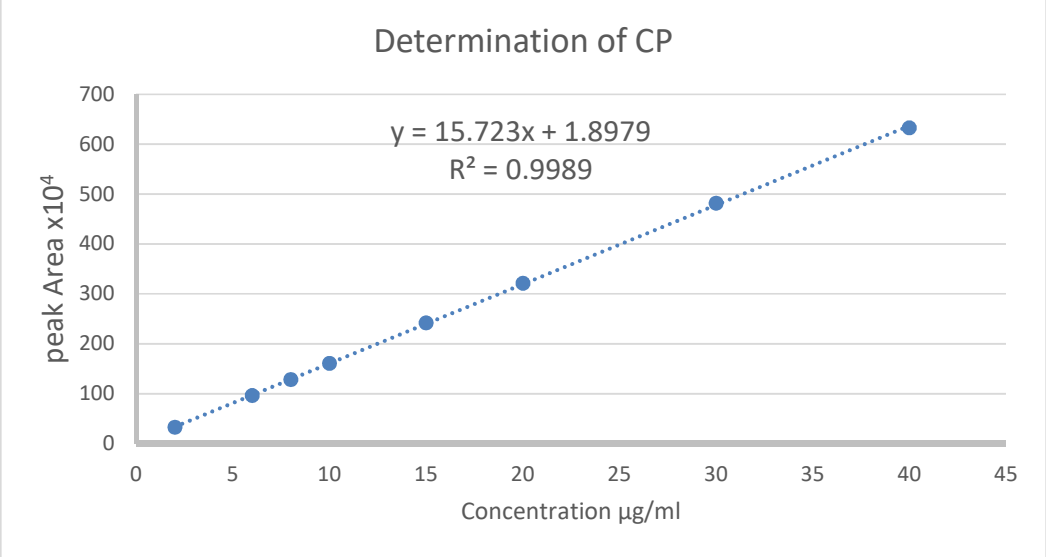


Fig 3. Calibration curves of Chlorphenaramine malate**.**

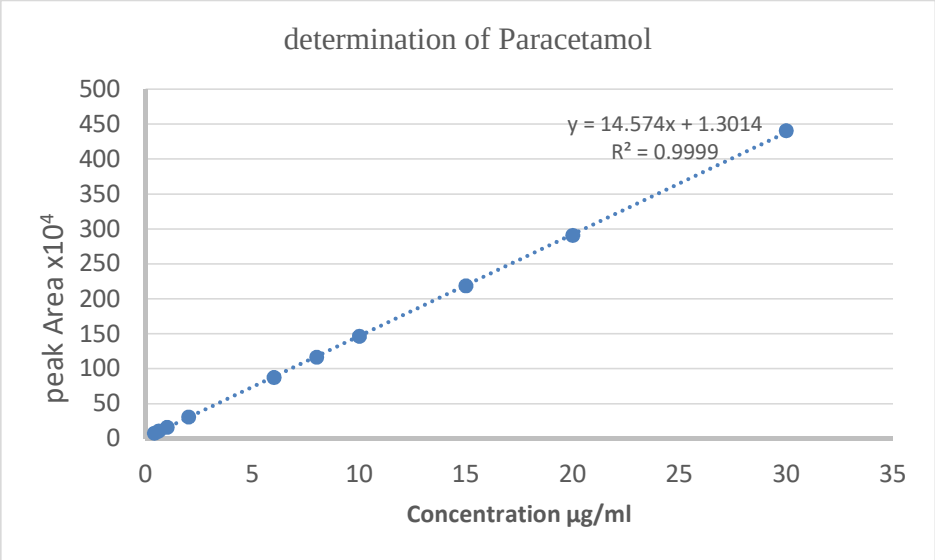


Fig 4. Calibration curves of paracetamol.

Application of the method to pharmaceutical dosage forms:

The method is sensitive and specific for the quantitative determination of DS,CP and PA and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Tablets from **Ajanta** manufacturer (Pain Leave PA 500 mg, DS 50 mg and CP 4 mg were evaluated for the amount of PA,DS and CP present in the formulations.
Each sample was analyzed in triplicate after extracting the drug as mentioned above in experimental section. The amount of paracetamol,diclofenac sodium and Chlorphenaramine malate was found to be within the range of 95%-105%. None of the tablet excipients were found to interfere with the analyte peak and the results were shown in Table 7.

Table 7 : Results of the determination of paracetamol,diclofenac sodium and Chlorphenaramine malate in Tablets (n=6)

Company	Drug	Labeled amount(mg)	Amount(mg)		Assay	
			Taken	Found	%RSD	%w/w
Ajanta	PA	500	500	496.2	1.45	99.2
	DS	50	50	48.45	1.088	96.9
	CP	4	4	4.01	1.0341	100.25

CONCLUSION:

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Diclofenac sodium , Chlorphenaramine malate and Paracetamol from pure and in pharmaceutical dosage forms.

The mobile phase is simple to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Diclofenac sodium , Chlorphenaramine malate and Paracetamol in combined dosage forms and can also be used for dissolution or similar studies.

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