SIMULTANEOUS DETERMINATION OF PHENYLEPHERINE, CHLORPHENERAMINE, PARACETAMOL AND DEXTROMETHORPHAN IN BULK AND MARKETED FORMULATION WITH THEIR DEGRADATION PARAMETERS

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ABSTRACT:

Objective: a new method has been developed for simultaneous estimation of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan in bulk and marketed formulations. Method: Chromatography was performed using C8 column of dimension 250*4.6mm and particle size 5µ. the best results were obtained by phosphate buffer (20 Mm) pH 6.6 adjusted with diluted orthophosphoric acid and Acetonitrile(70:30) taken in Gradient Programme at flow rate of 1ml/m with detection at 225 nm. Retention time was less than 6mins for all the four drugs. According to ICH guidelines the method was proven to be linear, precise and accurate.

Results: The linearity ranges from 0.5-487.5 µg/ml. LOQ was found to be 0.02,0.01,3.92 and 0.06. The sample solution injected after 24 hr did not show any appreciable change. Recovery was found to be 100.38-100.53.

Conclusion: The developed method was simple, specific and sensitive as the excipients have no interference in the determination of main components. The proposed method can be used for routine analysis of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan in combined dosage form which are present in variable concentrations.

KEY WORDS: Phenylephrine, Chlorpheneramine, Paracetamol, Dextromethorphan, Reverse phase HPLC, PDA Detector.

I. INTRODUCTION:

Drugs for cold and cough are available in various combinations like antipyretic, antihistamine, and decongestant and expectorant. Formulations marketed contain combination of more than two or three and analytical estimation becomes difficult due to presence of various additives like coloring agent, flavouring agent and preservatives. lierature review reveals that there is no single method developed for estimation of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan in combination[1-12].

Phenylepherine *N*-(4-hydroxyphenyl)ethanamide *N*-(4-hydroxyphenyl)acetamide used as an alternative for pseudoephedrine in decongestant and used for constriction of vascular smooth muscle and is often used in the treatment of hemorrhoids. Chlorpheneramine 3-(4-chlorophenyl)-N-N-dimethyl-3-pyridin-2-yl-propan-1-amine acts antihistamine by blocking H1 receptor. Paracetamol *N*-(4-hydroxyphenyl)ethanamide *N*-(4-hydroxyphenyl)acetamide is commonly used for fever and pains by inhibiting cyclooxygenase. it is widely used antipyretics and analgesics. Dextromethorphan is a cough suppressant which blocks the trigger zone for cough in the brain.

Aim of the work was to develop new, simple and accurate RP-HPLC method for the simultaneous determination of Phenylephrine, Chlorpheneramine, Paracetamol and Dextromethorphan in bulk and marketed formulation. The method was validated as per ICH guidelines.

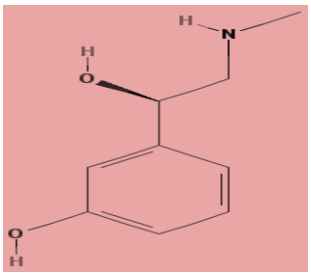

Fig 1.Structure of phenylephrine

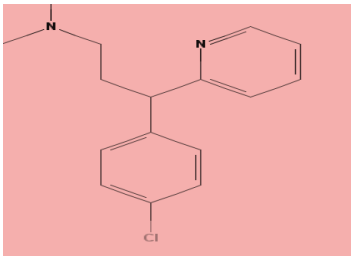

Fig 2.Structure of Chlorpheneramine

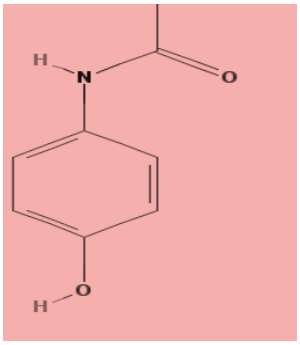

Fig 3. Structure of Paracetamol

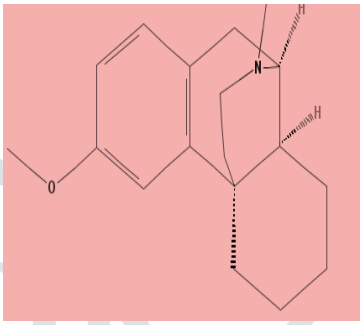

Fig 4. Structure of Dextromethorphan

II. MATERIALS AND METHODS:

III. INSTRUMENTATION:

Chromatography was performed with Water's 2695 HPLC system provided with Hamilton Syringe, auto sampler and 2996 Photodiode array detector. Online degasser was equipped within the HPLC system which degasses the mobile phase and prevents the pressure fluctuations; along with this a column compartment was present in order to control the temperature. The HPLC system operates with Empower2 software and it is used for analysis, data acquisition followed by reporting the data.

IV. MATERIALS AND CHEMICALS:

Pharmaceutically pure sample of Phenylephrine, Chlorpheneramine, Paracetamol and Dextromethorphan were obtained from spectrum pharma research solutions, Hyderabad as gift samples along with their analytical reports. The chemicals required for the preparation of mobile phase i.e.orthophosphoric acid, HPLC grade acetonitrile, methanol were obtained from Merck, Mumbai. Millipore water (HPLC grade) used for the preparation of buffer and solutions was obtained from Milli-Q water purification system. Commercial formulation syrup obtained from local pharmacy store.

***Preparation of Buffer*: (0.1%OPA)**

1ML of Ortho phosphoric acid solution was taken in a 1000ml of volumetric flask and volume was made up to 1000 ml with milli-Q water

***Preparation of Mobile phase*:**

Mobile phase was prepared by mixing 0.1% OPA and acetonitrile in the ratio of 70:30

***Preparation of standard stock solution*:**

Accurately weighed and transferred 5mg&2mg&10mg of Phenylephrine, chlorpheneramine, Dextromethorphan. working Standards into 100ml clean dry volumetric flask, add 70ml of diluent, and 32.5mg of paracetamol working Standards into10ml clean dry volumetric flask add 7ml of diluent sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solutions 1ml was pipeted out in to a 10ml Volumetric flask and then make up to the final volume with diluent. (5µg/ml Phenylephrine & 2µg/ml chlorpheneramine, 10µg/ml Dextromethorphan and 325 µg/ml paracetamol).

Preparation of working standard solutions:

Aliquot of 0.25 ml, 0.5 ml, 0.75 ml, 1 ml, 1.25 ml and 1.5 ml were pipette out from stock solution into 10 ml volumetric flask separate and volume was made up to 10 ml with diluent. This gives the solutions of 81.25 µg/ml, 162.5 µg/ml, 243.75 µg/ml, 325 µg/ml, 406.25 µg/ml and 487.5 µg/ml respectively for Paracetamol, 1.25 µg/ml, 2.5 µg/ml, 3.75 µg/ml, 5 µg/ml, 6.25 µg/ml and

7.5 µg/ml respectively for Phenylephrine ,0.5 µg/ml, 1 µg/ml, 1.5 µg/ml, 2 µg/ml, 2.5 µg/ml and 3 µg/ml respectively for Chlorpheneramine and 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 12.5 µg/ml and 15 µg/ml respectively for Dextromethorphan.

Preparation of sample solution:

5ml syrup was transferred into a 100 mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent.

V. RESULT AND DISCUSSION:

VI. *Linearity*

By appropriate aliquots of the standard phenylephrine, chlorpheneramine, paracetamol and dextromethorphan solutions with the mobile phase, six working solutions ranging between 1.25-7.5 µg/mL, 0.5-3 µg/mL,81.25-487.5 µg/mL and 2.5-15 µg/mL were prepared and injected (n=3). The peak areas were plotted against the concentration of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan to obtain the calibration curve and the results were shown Fig 5. The linearity were represented by a linear regression equation as follows: y (phenylephrine) = 14436.x + 632.6 (r^2=0.999), y (chlorpheneramine)= 27148.x +310.8 (r^2=0.999), y (paracetamol)= 14096.x + 68443.5 (r^2=0.999) and y(dextromethorphan)= 14240.x + 360.9 (r^2=0.999). Fig 5 shows the calibration curves of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

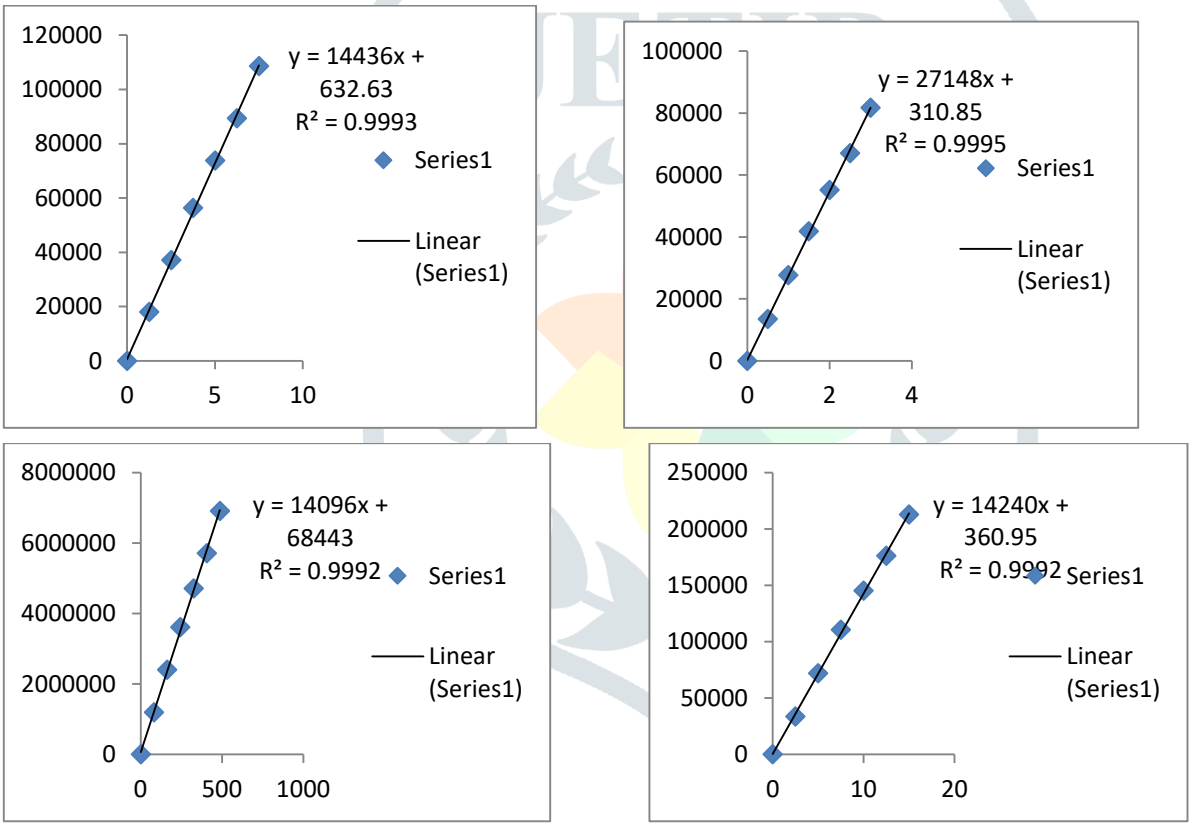

Fig.5 Calibration Curve for phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

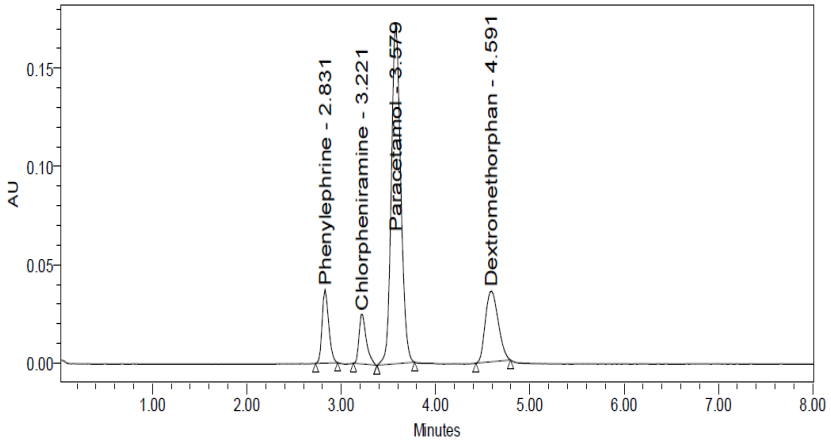

Fig.5.1 Linearity 25% chromatogram of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

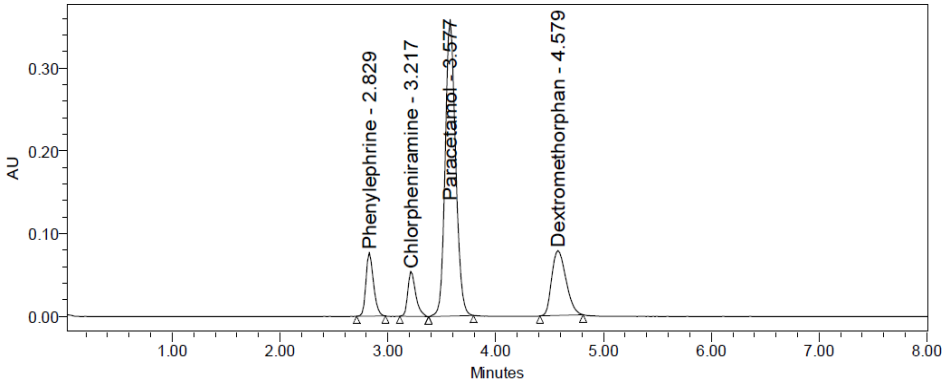

Fig.5.2 Linearity 50% chromatogram of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

VII. System Precision:

System precision table of Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol

Table :1 System Precision

SNo	Area of Phenylephrine	Area of chlorpheneramine	Area of Dextromethorphan	Area
1.	73902	55909	4720724	144056
2.	73075	55708	4714060	143939
3.	73004	55303	4723920	143627
4.	73346	55344	4693510	143945
5.	73564	55508	4728424	143284
6.	73723	55186	4709102	144031
Mean	73436	55493	4714957	143814
S.D	358.0	272.8	12569.9	301.4
%RSD	0.5	0.5	0.3	0.2

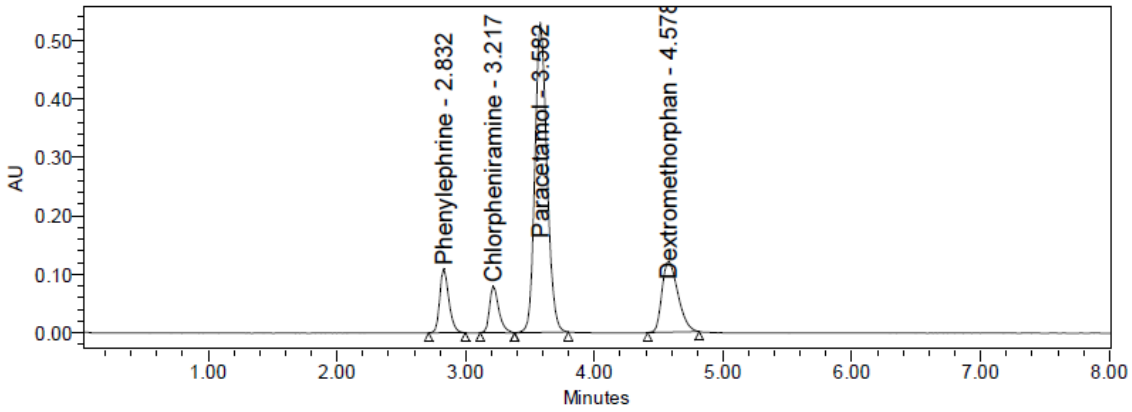

Fig 6 System precision chromatogram

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.5%, 0.5%, 0.3%, and 0.2% respectively for chlorpheneramine, Dextromethorphan, and paracetamol..As the limit of Precision was less than "2" the system precision was passed in this method.

VIII. Repeatability:

Table :2

Repeatability table of Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol

S. No	Area of Phenylephrine	Area of chlorpheneramine	Area of Dextromethorphan	Area of paracetamol
1.	72896	55815	145873	4687002
2.	73970	55386	144859	4715880
3.	73766	55040	145705	4709283
4.	73830	55234	145084	4792443
5.	73715	55136	145173	4697848
6.	73915	55298	143074	4696521
Mean	73682	55318	144961	4716496
S.D	396.3	271.8	1001.7	38566.8
%RSD	0.5	0.5	0.7	0.8

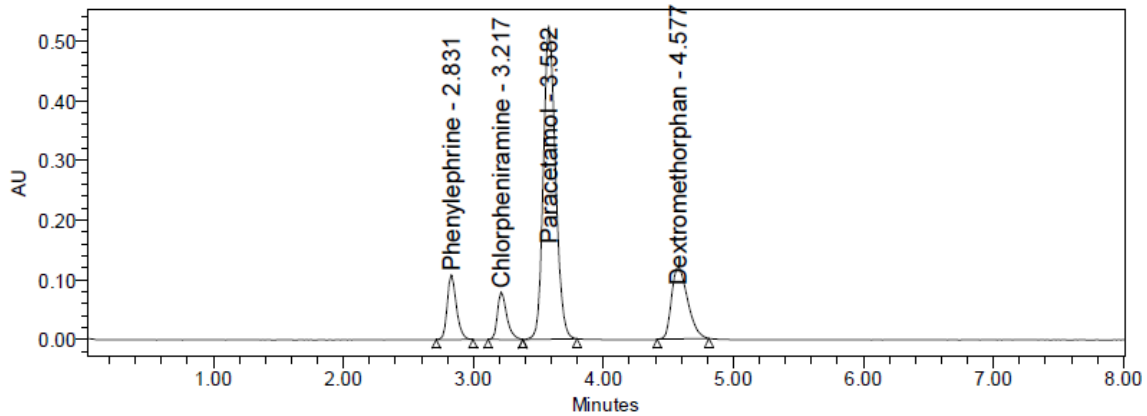

Fig No. 7 Repeatability chromatogram

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas were mentioned in the above table.

Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7%, 0.2% , 0.7%, and 0.8% respectively for Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol. As the limit of Precision was less than "2" the system precision was passed in this method.

IX. Intermediate precision (Day_ Day Precision):

Table 3 Intermediate precision table of Abacavir and Lamivudine

S. No	Area of Phenylephrine	Area of chlorpheneramine	Area of Dextromethorphan	Area of paracetamol
1.	77829	57186	156075	4949720
2.	76818	57563	154879	4897603
3.	77759	57534	154358	4887392
4.	76833	56915	156110	4949606
5.	77092	56766	154056	4908830
6.	76401	57466	154787	4870945
Mean	77122	57238	155044	4910683
S.D	566.1	339.1	864.8	32664.9
%RSDD	0.7	0.6	0.6	0.7

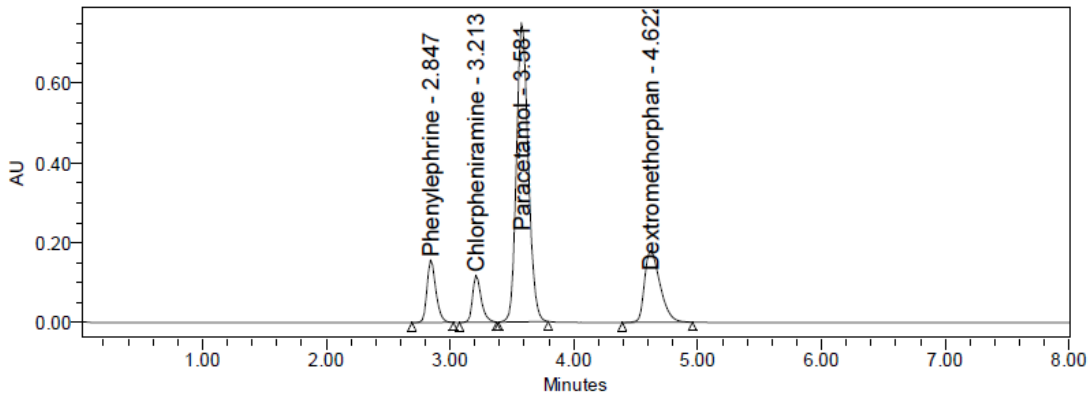

Fig: 7 Inter Day precision Chromatogram

Discussion: Multiple sampling from a sample stock solution was done and six working sample solutions of same concentrations were prepared, each injection from each working sample solution was given on the next day of the sample preparation and obtained areas were mentioned in the above table. Average area, standard deviation and % RSD were calculated for two drugs and obtained as 0.7% , 0.6%, 0.6%and 0.7% respectively for Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol. As the limit of Precision was less than "2" the system precision was passed in this method.

X. 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. In the present study standard addition method was followed to determine the % recovery. And it is determined by. Accuracy was assessed by spiking the active ingredients at different concentrations 50%, 100% and 150% each of the labelled claim and injected in developed chromatographic conditions in triplicate. The recovery was found to be between 100.38 – 100.53 for all the four drugs and it is shown in Table 3 .

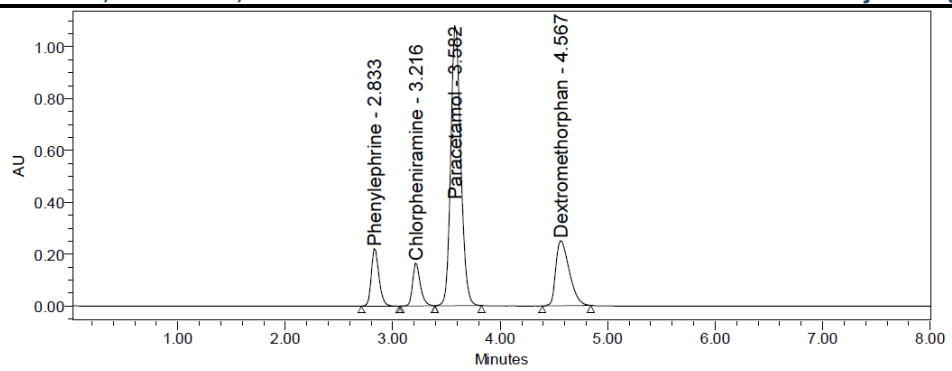

Fig.8 Accuracy 50% chromatogram of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

Table 3: Accuracy studies of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

Recovery level (%)	Standard added	Amount added (mg)	Mean recovery(mg) (n=3)	Mean % Recovery
50	phenylephrine	2.5	2.51	100.38
	chlorpheneramine	1	1.00	100.14
	paracetamol	162.5	163.5	100.63
	dextromethorphan	5	5.01	100.21
100	phenylephrine	5	4.98	99.57
	chlorpheneramine	2	2.01	100.29
	paracetamol	325	324.20	99.75
	dextromethorphan	10	10.10	101.02
150	phenylephrine	7.5	7.471	99.61
	chlorpheneramine	3	3.001	100.03
	paracetamol	487.5	486.62	99.82
	dextromethorphan	15	15.08	100.53

XI. ROBUSTNESS:

The robustness of the method was performed by changing the chromatographic conditions. The change in the % organic strength (±5%), column temperature (±5^{0}c) and the flow rate (±0.1mL) did not bring any significant changes in the chromatography pattern. and the %RSD were also within the acceptance limits, showing that the method is robust and results were shown in Table 4.

Table 4: Robustness studies of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

* n=6 for each parameter meter

Phenylephrine meters		%RSD of peak area response				Mean tailing factor				Mean retention time in min			
		PHENYLEP	CHLORP	PARA	DEXT	PHENYLEP	CHLORP	PARACE	DEXTRO	PHENYL	CHLO	PARA	DEXT
flow rate	+0.1	0.8	0.4	0.4	0.2	1.22	1.29	1.16	1.39	2.84	3.21	3.5	4.5
	std	0.5	0.5	0.3	0.2	1.22	1.34	1.14	1.33	2.8	3.2	3.5	4.5
	-0.1	0.8	1.1	0.9	0.5	1.24	1.32	1.15	1.40	2.8	3.2	3.6	4.5
% Organic phase	+5	0.3	0.5	0.6	0.5	1.21	1.07	1.05	1.50	4.3	6.4	7.6	8.7
	std	0.5	0.5	0.3	0.2	1.22	1.34	1.14	1.33	2.8	3.2	3.5	4.5
	-5	0.2	0.3	0.3	0.4	1.22	1.34	1.16	1.37	2.8	3.1	3.5	4.2
Column Temperature	+5	0.4	0.5	0.4	0.4	1.20	1.31	1.15	1.42	2.8	3.2	3.6	4.6
	std	0.5	0.5	0.3	0.2	1.22	1.34	1.14	1.33	2.8	3.2	3.5	4.5
	-5	0.5	0.6	0.5	0.6	1.22	1.31	1.15	1.42	2.8	3.2	3.6	4.6

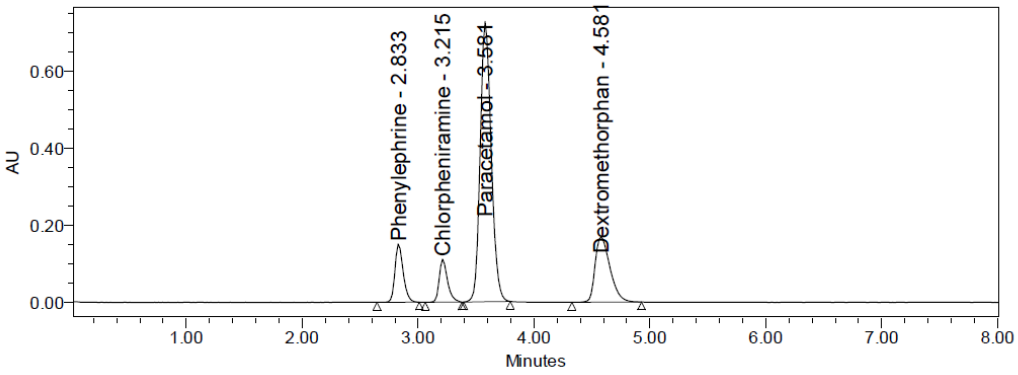

Fig.9. Robustness (Temperature Plus) chromatogram of phenylephrine, chlorpheneramine, paracetamol and dextromethorpha

XII. LOD and LOQ:

Limit of detection (LOD) and limit of quantification (LOD) of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan were determined by calibration curve method. Solutions of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan were prepared in linearity range and injected (n=3). The graph was plotted against average peak areas and concentration. The LOD and LOQ were calculated by using the equations, LOD = (3.3 ×Syx)/b and LOQ= (10.0×Syx)/b, Where Syx is residual variance due to regression; b is slope. LOD and LOQ of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan were determined by calibration curve method and the results were shown in the Table 5.

Table 5: LOD and LOQ values of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

Drug	LOD	LOQ
phenylephrine	0.01	0.02
chlorpheneramine	0.004	0.01
paracetamol	1.29	3.92
dextromethorphan	0.02	0.06

XIII. ASSAY

The content of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan was found in the syrup by the proposed method and results were shown in Table 6.

Table 6: Analysis of marketed formulation by proposed method

Marketed formulation	Ingredients	Amount Found%
Syrup	phenylephrine	99.85
	chlorpheneramine	100.15
	paracetamol	100.07
	dextromethorphan	100.59

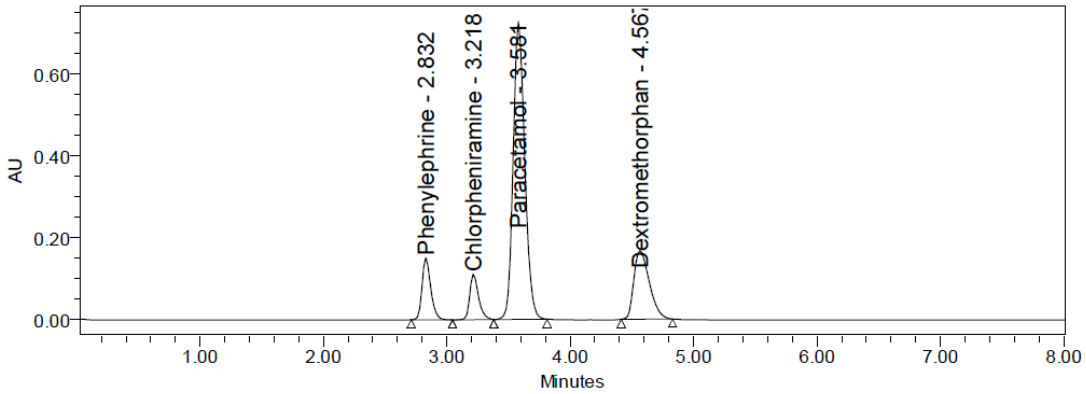

Fig.10.Assay chromatogram of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan

XIV. Degradation studies:

Oxidation:

To 1 ml of stock solution of Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol. 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 5µg/ml, 2 µg/ml, 10µg/ml and 325µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock ssolution Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol.,1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 5µg/ml, 2 µg/ml, 10µg/ml and 325µg/ml of all components and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Phenylephrine, chlorpheneramine, Dextromethorphan, and paracetamol., 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 5µg/ml, 2 µg/ml, 10µg/ml and 325µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 5µg/ml, 2 µg/ml, 10µg/ml and 325µg/ml of all components and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies:

The photochemical stability of the drug was also studied by exposing the 50µg/ml&20µg/ml&100µg/ml and 3250µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 5µg/ml, 2 µg/ml, 10µg/ml and 325µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

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Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to obtain 5µg/ml, 2 µg/ml, 10µg/ml and 325µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stabilit**.**

Degradation studies of Phenylephrine

s.n	Sample	Standard area	Sample area	% assay	% degradation
1	Acid	73902	70976	96.46	3.54
2	Base	73075	71751	97.51	2.49
3	Peroxide	73004	71549	97.24	2.76

4	Thermal	73346	72350	98.32	1.68
5	Uv	73564	73033	99.25	0.75
6	Water	73723	73891	100.42	0.0

Degradation studies of chlorpheneramine

s.n	Sample	Standard area	Sample area	% assay	% degradation
1	Acid	55909	53713	96.60	3.40
2	Base	55708	54181	97.44	2.56
3	Peroxide	55303	53675	96.53	3.47
4	Thermal	55344	54179	97.44	2.56
5	Uv	55508	54792	98.54	1.46
6	Water	55186	54548	98.10	1.90

Degradation studies of Dextromethorphan

	Sample	Standard area	Sample area	% assay	% degradation
	id	144056	139076	96.32	3.68
	se	143939	140342	97.20	2.80
	Peroxide	143627	140237	97.12	2.88
	Thermal	143945	142356	98.59	1.41
		143284	143397	99.31	0.69
	ater	144031	143565	99.43	0.57

Degradation studies of Paracetamol

	mple	Standard area	Sample area	% assay	% degradation
	id	472072	454799	96.17	3.83
	se	471406	461997	97.69	2.31
	Peroxide	472392	462836	97.87	2.13
	Thermal	469351	468427	99.05	0.95

		472842	473128	100.05	0.0
	ater	470910	472611	99.94	0.06

XV. CONCLUSION:

A novel RP-HPLC-PDA method has been developed for the simultaneous estimation of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan in bulk and syrup in which the active agents are present in variable concentrations. Because of the wide variability among the drugs, their polarities and also there concentrations in the dosage form it became a tough task to optimise the method which gave good resolution for all the four drugs with a short run time (8 min). The developed method was validated according to ICH guidelines. The developed method was simple, specific as the excipients have no interference in the determination of main components, precise, accurate, and sensitive. The proposed method can be used for routine analysis of phenylephrine, chlorpheneramine, paracetamol and dextromethorphan in combined dosage form which are present in variable concentrations. It can be also applied in the quality control of bulk manufacturing of presented API's

XVI. BIBLIOGRAPHY

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