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Simultaneous estimation of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in pharmaceutical dosage form using High Performance Liquid Chromatography

Gurunath V. Pandit*, Shailendra V. Dalvi, Gunesh G. Gundi, Shivani G. Gaonkar

Quantitative Quality Chemistry, Chikitsak Samuha's Patkar-Varde College, Goregaon, Mumbai - 400061, Maharashtra, India

BSTRACT

A simple, precise and accurate HPLC method was developed for the simultaneous estimation of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in tablet dosage form. Chromatographic separation of the four pharmaceuticals were performed on a COSMOSIL MSII ODS column (C-18, 250mm x 4.6mm x 5µm) with mobile phase containing 0.1% 1- Octane Sulphonic Acid sodium salt (pH 3.0 with Orthophosphoric acid) and Methanol in gradient mode with column oven temperature maintained at 40 °C, at flow rate of 1.0 ml/min. UV detection was performed at 272 nm with Rt of 3.68 mins, 4.81 mins, 9.45 mins, 24.45 mins for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate respectively. Separation was completed within 25 mins. The method was validated for linearity, accuracy, precision, robustness, recovery and solution stability. Linearity was found to be acceptable over ranges (32.5 to 97.5 µg/ml) for Paracetamol, (75.0 to 225.0 µg/ml) for Caffeine, (25.0 to 75.0 µg/ml) for Phenylephrine Hydrochloride and (20.0 to 60.0 µg/ml) for Chlorpheniramine Maleate.

Keywords

Paracetamol, Caffeine, Phenylephrine Hydrochloride, Chlorpheniramine Maleate, HPLC, Simultaneous estimation, Tablet dosage

INTRODUCTION

Drug combinations are single preparations containing two or more active pharmaceutical ingredients (APIs) for the purpose of their concurrent administration as a fixed dose mixture combinations drug. Most multi-component drug formulations usually contain two or more active ingredients which are responsible for a combined therapeutic activity of the drug. This concept is beneficial when the selective

agents have different mechanisms of action that provide additive or synergistic efficacy.[1] There is increased production of multi component drugs formulation due to increased efficacy, increased resistance of microorganisms to single component formulations and dependency and/or tolerance, and this has further led to increased drug counterfeiting and adulteration.[2]

However, monographs in most official pharmacopoeia are for single component drugs, hence local Pharmaceutical manufacturing companies in the analysis of multi-component drug formulations use methods that involve multiple and repeated extractions to extract each active component before their quantification using spectrophotometry or titrimetry. Such methods are thus laborious and cumbersome. This has led to researchers developing various methods to help facilitate easy and quick analysis of multi-component drugs. With HPLC being a method of choice, many researchers have worked at developing various RP-HPLC methods for the simultaneous estimation of various active components in multi-component drugs.[3,4,5,6] Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate belongs to a class of medication called 'cough and cold medications' primarily used to treat the common cold and allergic symptoms like sneezing watery eyes, or itchy/watery nose and throat. Paracetamol (PARA) chemically referred to as N-(4-hydroxyphenyl) acetamide is the most common drug used all over the world for its analgesic and antipyretic properties.[7,8] Paracetamol is available in different dosage forms: tablet, capsules, drops, elixirs, suspensions and suppositories. Dosage forms of paracetamol and its combinations with other drugs have been listed in various pharmacopoeias.[9,10] Due to its minor side effects, it is conveniently used by both young and elderly people for the relief of minor aches, pain, and fever.[11,12] The therapeutic dose of paracetamol does not cause any negative side effects for human health; however, overdose consumption causes damages to the liver and kidneys.[13,14] The drug is partially soluble in cold water, more soluble in hot water and alcohols.[15] Phenylephrine hydrochloride (PHE; 3-[1-hydroxy-2-(methyl amino) ethyl] phenol hydrochloride) acts as nasal and sinus decongestant. Phenylephrine is a medication primarily used as a decongestant, to dilate the pupil, to increase blood pressure, and to relieve hemorrhoids.[16,17] It was long maintained that when taken orally as a decongestant, it relieves nasal congestion due to colds and hay fever.[16] Chlorpheniramine Maleate 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.[18] Caffeine (CAF; 1,3,7-trimethyl-3,7- dihydro-1H-purine-2,6-dione) is an addictive stimulant that elevates heart rate and respiration, confers psychotropic properties, and acts as a mild diuretic. Various analytical methods, including spectroscopy[19] and high performance liquid chromatography (HPLC)[20,21], have been utilized for the estimation of CPM alone[19,20,21] in various dosage forms. Similarly, diverse analytical methods are available for the determination of PARA[22,23], PHE[24,25], and CAF[26,27] from different dosage forms. However, no single method is available for the simultaneous determination of all four active components in a combination form.

EXPERIMENTAL

IP Reference standards for Paracetamol, Caffeine, Phenylephrine hydrochloride, and Chlorpheniramine maleate were procured from Indian Pharmacopoeia Commission (Ghaziabad, Uttar Pradesh). Fixed dose combination tablet Sinus 77 (Dales laboratories pvt ltd.) containing 325 mg Paracetamol, 15 mg Caffeine, 5 mg Phenylephrine Hydrochloride, and 4 mg Chlorpheniramine maleate was purchased from local market, Goregaon, Maharashtra, India. Methanol, 1- Octane Sulphonic Acid-sodium salt and Orthophosphoric acid used were HPLC grade, purchased from Merck Chemicals. All dilutions were performed in standard volumetric flasks.

Instrumentation and Chromatographic Conditions: Chromatography was performed with a Shimadzu high-performance liquid chromatograph comprising a LC20AD pump, equipped with 20-µL loop, and a Shimadzu SPD20A detector. A double-beam spectrophotometer was use for scanning and selecting the detection wavelength. Chromatograms and data were recorded by means of Lab Solutions software.

A COSMOSIL MSII ODS column (C-18, 250mm x 4.6mm x 5µm) was used for the analysis. The mobile phase was 0.1% 1- Octane Sulphonic Acid-sodium salt (pH 3.0 with Orthophosphoric acid) and Methanol in gradient mode with column oven temperature maintained at 40 °C and elution monitored by a UV detector at 272 nm.

All noted measurements were performed with an injection volume of 40 µl of samples dissolved in a diluent consist of Milli Q water and Methanol in the ratio of 1:1, (pH 3.0 with Orthophosphoric acid). During development of bio-analytical procedure, diluent was changed accordingly.

Mobile phase A:

Weighed accurately and transferred 1.0 g of 1- Octane Sulphonic Acid sodium salt to 1 litre bottle containing 1000 ml of water. Sonicated the mixture to dissolve and adjusted the pH to 3.0 with Orthophosphoric acid. Filtered the mobile phase.

Mobile phase B:

Methanol

Table 1.0

Gradient pattern for co-elution of selected actives in optimized HPLC conditions.

Time (Mins)	Composition	
	Mobile phase A (%)	Mobile phase B (%)
0.01	60	40
5.00	60	40
7.00	50	50
15.00	50	50
17.00	30	70
24.00	30	70
28.00	60	40
32.00	60	40

Preparation of solutions:

Paracetamol Standard Stock Preparation

Weighed accurately 65 mg of Paracetamol Reference standard, transferred to 100 ml volumetric flask added sufficient amount of diluent, sonicated to dissolve and made up the volume with diluent and mixed.

Caffeine Standard Stock Preparation

Weighed accurately 20 mg of Caffeine Reference standard transferred to 20 ml volumetric flask added sufficient amount of diluent, sonicated to dissolve and made up the volume with diluent and mixed.

Phenylephrine Hydrochloride Standard Stock Preparation

Weighed accurately 20 mg of Phenylephrine hydrochloride Reference standard transferred to 20 ml volumetric flask added sufficient amount of diluent, sonicated to dissolve and made up volume with diluent and mixed.

Chlorpheniramine Maleate Standard Stock Preparation

Weighed accurately 20 mg of Chlorpheniramine Maleate Reference standard transferred to 20 ml volumetric flask added sufficient amount of diluent, sonicated to dissolve and made up volume with diluent and mixed.

Standard Preparation Mixture A

From the above prepared Stock solutions, pipetted out 10 ml of Paracetamol standard, 15 ml of Caffeine standard, 5 ml of Phenylephrine Hydrochloride standard and 4 ml of Chlorpheniramine Maleate and transferred to 100 ml volumetric flask. Added diluent to made up the volume and mixed well.

Standard Preparation Mixture B

From the above prepared Stock solutions, pipetted out 10 ml of Paracetamol standard, 15 ml of Caffeine standard, 5 ml of Phenylephrine Hydrochloride standard and 4 ml of Chlorpheniramine Maleate and transferred to 100 ml volumetric flask. added diluent to made up the volume and mixed well.

Sample Preparation

Weighed 20 tablets determined the average weight of the tablet. Weighed accurately and transferred powder equivalent to 325 mg Paracetamol, 15 mg Caffeine, 5 mg Phenylephrine Hydrochloride, and 4 mg Chlorpheniramine Maleate into 100 ml volumetric flask. Added about 50 ml diluent sonicated for 20 minutes with intermittent shaking, cooled to room temperature. Made up the volume with diluent and filtered the solution through 0.45µ PVDF (Injected for Caffeine, Phenylephrine hydrochloride and Chlorpheniramine Maleate). Pipetted out 1 ml of the filtered solution and diluted to 50 ml (Injected for Paracetamol).

Method validation:

Linearity: Linearity of the method was studied by injecting seven concentrations of the drug prepared in the diluent in triplicate into the HPLC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs. For peak shapes refer **Fig 5,6,7**. The plots were linear for Paracetamol in the range 32.5 to 97.5 µg/ml, for Caffeine in the range 75.0 to 225.0 µg/ml, for Phenylephrine Hydrochloride in the range 25.0 to 75.0 µg/ml, for Chlorpheniramine Maleate in the range
20.0 to 60.0 µg/ml **Fig 1,2,3,4**. The data were analyzed by linear regression least-squares fitting. The statistical data obtained is given in **Table 2.0**.Table 2.0

Analysis Performance Data of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate

	Paracetamol	**Caffeine**	**Phenylephrine Hydrochloride**	**Chlorpheniramine Maleate**
Linear working range	32.5 to 97.5 µg/ml	75.0 to 225.0 µg/ml	25.0 to 75.0 µg/ml	20.0 to 60.0 µg/ml
Slope	48997.32	119421.06	1115749.00	754428.16
Intercept	61871.45	1239341.41	-47717.90	-44608.78
Correlation coefficient	0.9999	0.9991	0.9997	0.9992

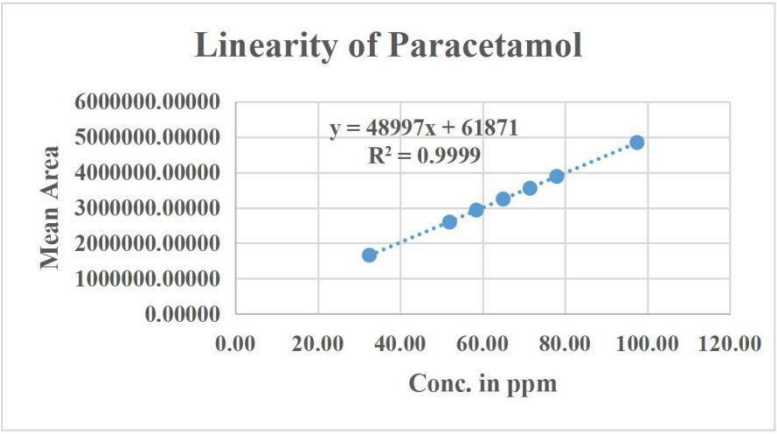

Fig 1. Linearity plot for Paracetamol

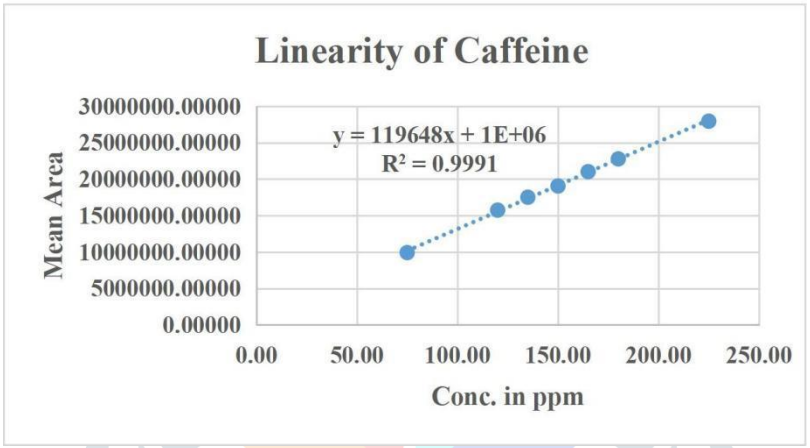

Fig 2. Linearity plot for Caffeine

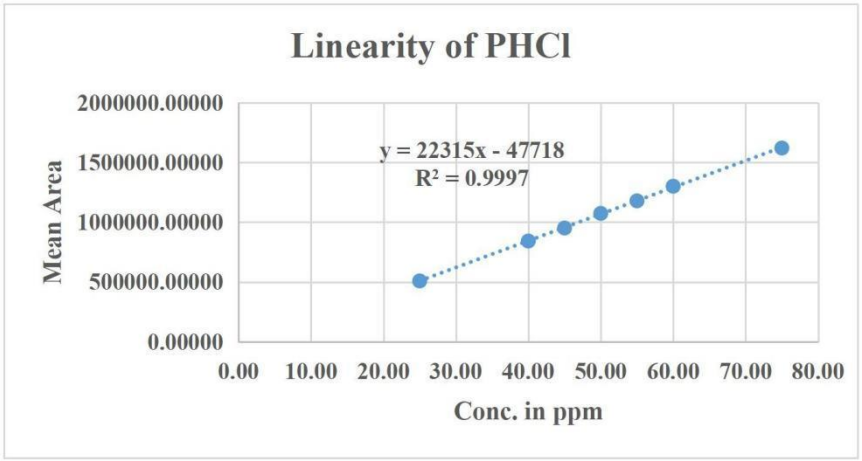

Fig 3. Linearity plot for Phenylephrine Hydrochloride

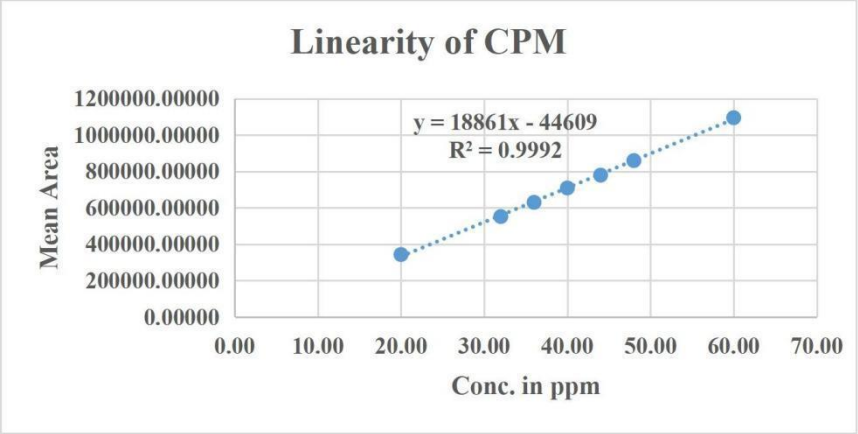

Fig 4. Linearity plot for Chlorpheniramine Maleate

Accuracy: Accuracy was determined by applying the method to synthetic mixtures of drug product components to which known quantities of each drug substance corresponding to 50%, 100%, and 150% of the label claim of each drug was added. The accuracy was expressed as the percentage of analytes recovered by the assay. Mean recoveries for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate from the specific formulations are shown in **Table 3.0**. The results indicate the method is highly accurate for simultaneous determination of four drugs.

Table 3.0

Accuracy of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate

Accuracy Level		**Paracetamol**	**Caffeine**	**Phenylephrine Hydrochloride**	**Chlorpheniramine Maleate**
50	Mean	99.0	100.5	99.4	98.9
100		100.2	100.2	99.8	99.7
150		100.7	100.0	100.2	99.8
50	SD	1.2214	0.7355	1.3960	0.3808
100		1.2932	0.6551	0.6039	1.5949
150		0.1223	0.5836	1.6320	1.1397
50	% RSD	1.2	0.7	1.4	0.4
100		1.3	0.7	0.6	1.6
150		0.1	0.6	1.6	1.1

Precision: The precision of the method was demonstrated by inter day and intraday variation studies. In the inter day and intraday studies, six replicate injections of the mixed standard solutions and sample solutions were made and the percentage RSD were calculated. From the data obtained, the developed HPLC method was found to be precise. Refer **Table 4.0** and **Table 5.0**

Table 4.0

Assay Results of Spotting repeatability/Instrument precision for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate

Sample	Paracetamol (% Assay)	Caffeine (% Assay)	Phenylephrine Hydrochloride (% Assay)	Chlorpheniramine Maleate (% Assay)
Sample-1	99.2	99.3	99.6	100.2
Sample-2	101.3	98.8	98.7	99.2
Sample-3	100.3	98.9	100.7	99.1
Sample-4	99.2	98.0	98.1	100.9
Sample-5	98.5	97.7	100.8	101.0
Sample-6	98.2	99.4	99.6	101.7
Mean	**99.7**	**98.5**	**99.6**	**100.1**
STD DEV	**1.1727**	**0.7158**	**1.0727**	**1.0413**
% RSD	**1.2**	**0.7**	**1.1**	**1.0**

Table 5.0

Assay Results of Intra-Assay / Within day precision for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate

Sample	Paracetamol (% Assay)	Caffeine (% Assay)	Phenylephrine Hydrochloride (% Assay)	Chlorpheniramine Maleate (% Assay)
Sample-1	98.6	99.2	100.0	99.7
Sample-2	98.8	100.8	99.5	101.3
Sample-3	100.2	100.7	99.2	99.8
Sample-4	98.5	101.3	99.0	99.4
Sample-5	99.3	101.0	99.6	99.6
Sample-6	98.6	100.5	99.3	100.7
Mean	**99.1**	**100.6**	**99.5**	**99.9**
STD DEV	**0.6609**	**0.7084**	**0.3621**	**0.7409**
% RSD	**0.7**	**0.7**	**0.4**	**0.7**

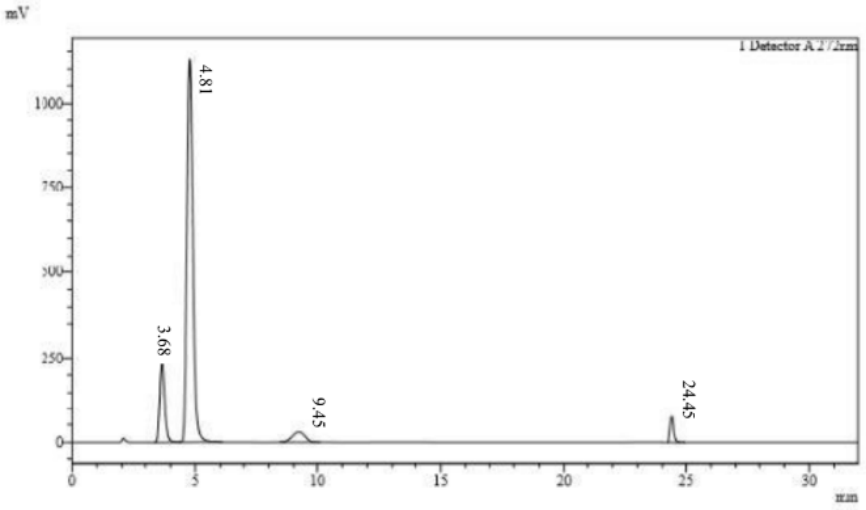

Fig 5. Typical Chromatogram of Standard for Paracetamol, Caffeine, Phenylephrine Hydrochloride, Chlorpheniramine Maleate

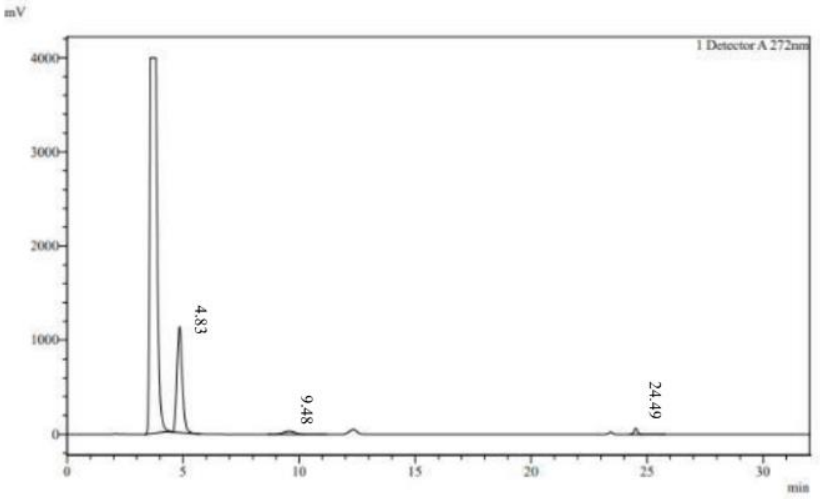

Fig 6. Typical Chromatogram of Sample for Caffeine, Phenylephrine Hydrochloride, Chlorpheniramine Maleate

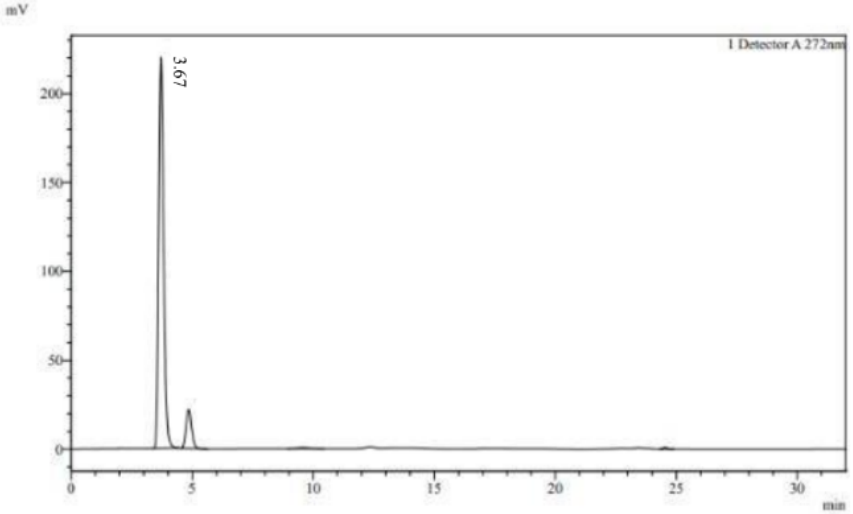

Fig 7. Typical Chromatogram of Sample for Paracetamol

Content uniformity:

The content uniformity was determined by weight variation method in which 10 tablets were weighed individually and transferred into 100 ml volumetric flask, added about 50 ml diluent sonicated for 20 minutes with intermittent shaking, cooled to room temperature and made up volume with diluent. For content uniformity studies, six replicate injections of the mixed standard solutions and 10 injections of sample solutions were made and the content were calculated for Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate **Table 6.0**. The content uniformity of Paracetamol is not determined as the label claim is greater than 25 mg.

Table 6.0

Content uniformity of Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate

Sample	Caffeine (% Assay)	Phenylephrine Hydrochloride (% Assay)	Chlorpheniramine Maleate (% Assay)
Sample-1	101.2	100.8	100.3
Sample-2	99.9	100.6	99.2
Sample-3	101.7	99.1	98.8
Sample-4	100.6	99.6	98.4
Sample-5	102.0	98.9	98.6
Sample-6	101.0	98.7	100.7
Sample-7	99.7	98.9	101.3
Sample-8	101.9	98.3	99.4
Sample-9	98.8	100.5	99.0
Sample-10	98.1	99.8	100.6
Mean	**100.8**	**99.5**	**99.6**
STD DEV	**1.3369**	**0.8804**	**1.0097**
% RSD	**1.3**	**0.9**	**1.0**

Stability studies:

The stability experiments were aimed at testing all possible conditions that the samples might experience after collecting and prior the analysis. The stability of the drug spiked for short term bench top (at room temperature for 0 h, 1 h, 2 h, 4 h, 8 h, 12 h, 16 h and 24 h), were evaluated. Stability of all analytes in analytical solution was observed at room temperature for period of 24 h.

Robustness:

The Robustness of the proposed method was evaluated by altering the temperature of the column oven and by variation in the flow rate. The column oven temperature was changed by ±5 ^{0}C (35 ^{0}C and 45 ^{0}C) and the flow rate was changed by ±0.1 mL/min (9.0 mL/min and 1.1 mL/min). At both the ends of the column oven temperature variations and change in flow rate, there was no significant difference in the

peak resolution and retention time of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate. The results indicated that the separation of four active substances is achievable under the given conditions using the method developed, which is satisfactory for the simultaneous determination of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in tablet dosage form.

RESULTS AND DISCUSSION

Use of COSMOSIL MSII ODS column (C-18, 250mm x 4.6mm x 5µm) with mobile phase containing 0.1% 1- Octane Sulphonic Acid sodium salt (pH 3.0 with Orthophosphoric acid) and Methanol in gradient mode resulted in good separation of all the four drug substances and the reference standards.

Regression analysis of the calibration data for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate showed that the dependent variable (peak area) and the independent variable (concentration) were represented by the equation **Y = 0.0000x - 1.2575** for Paracetamol, **Y = 0.000x - 10.194** for Caffeine, **Y = 0.0000x - 2.1506** for Phenylephrine Hydrochloride, **Y = 0.000x - 2.396** for Chlorpheniramine Maleate. The correlation of coefficient obtained was 0.9999, 0.9991, 0.9997, 0.9992 for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate respectively. The system suitability experiment was carried out before the determination of all four active substances in unknown sample. The coefficient of variation was less than 2 % for replicate measurements of the same sample. This shows that the method and the system both are suitable for the determination of unknown sample. The precision studies including the instrument precision, intra-assay precision and intermediate precision was carried out to evaluate the precision of the method. The intermediate precision included analysis on a different day and by a different analyst. The values of standard deviation and coefficient of variation were calculated. The coefficient of variation for Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate for intra-assay precision was 1.2, 0.7, 1.1, 1.0 and for inter-day precision 0.7, 0.7, 0.4, 0.7 respectively. The low values of standard deviation and coefficient of variation indicate high precision of the method. The results from recovery analysis are given in **Table 3.0**. The mean recovery is within acceptable limits, indicating the method is accurate.Conclusion

The proposed method is highly specific, accurate, selective, precise and reproducible and can therefore be used for a routine quality-control analysis and quantitative simultaneous determination of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in pharmaceutical preparations.

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