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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF PHENYLEPHRINE HYDROCHLORIDE, CAFFEINE, PARACETAMOL, CHLORPHENIRAMINE MALEATE IN PHARMACEUTICAL DOSAGE FORM USING METHOD OF LEAST SQUARES BY USING ULTRA VIOLET SPECTROPHOTOMETER

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ABSTRACT

A simple UV-Visible spectrophotometric method was developed for the determination of phenylephrine hydrochloride, caffeine, paracetamol, chlorpheniramine maelate in pure and its pharmaceutical formulation. They exhibited maximum absorption at 220nm for phenylephrine hydrochloride, 215nm for caffeine, 250nm for paracetamol and 205 nm for chlorpheniramine maelate. The following drugs obeyed linearity in the range of 1-25µg/ml for phenylephrine hydrochloride, 1-50µg/ml for caffeine, 1-5µg/ml for paracetamol and 10-60µg/ml for chlorpheniramine maleate. The proposed method was statiscally evaluated. All the proposed methods are simple, selective, reproducible, sensitive, and accurate with good precision. The selected solvent was water for the dosage form. The proposed methods can be used as an alternative methods to the reported ones for the routine determination of selected drugs under the study in bulk and pharmaceutical dosage forms.

KEYWORDS: Phenylephrine hydrochloride, Caffeine, Paracetamol, Chlorpheniramine maelate, UV-Visible spectrophotometer, Least squares.

INTRODUCTION

The UV-Visible spectrophotometric methods which fall in the wavelength region of 200-400nm and flourimetric methods (may fall in the UV and visible regions) are very simple, cheap and easy to carry out estimations of drugs in bulk form and their formulations.[1]

Phenylephrine hydrochloride is used as an nasal decongestant, used to block the running nose.[2] Caffeine is a CNS stimulating agent used to restore mental alertness or wakefulness during fatigue or drowsiness, it is also found in some headache and migraine medications and in many popular energy drinks. Paracetamol is an analgesic, anti-pyretic agent, used as pain reliever and a fever reducer.[3] Chlorpheniramine maleate is an antibiotic useful for the treatment of a number of bacterial infections, Its use is only recommended when safer antibiotics cannot be used.[4]

MATERIALS AND METHODS

DRUG SAMPLE

Phenylephrine hydrochloride, Paracetamol, Caffeine, Chlorpheniramine maelate was obtained from Pharma Deep remedies Hyderabad.

CHEMICALS AND REAGENTS

Methanol, HCl, NaOH, Ethanol are obtained from New Himalaya science and co.., Nellore. Distilled water was prepared in the house.

APPARATUS

A Schimadzu 1800 version 1.12 - Double Beam UV-Visible spectrophotometer. UV spectra of standard and sample solutions were recorded in 1 cm quartz cells at the wave length ranges of 200-400 nm.

METHOD DEVELOPMENT

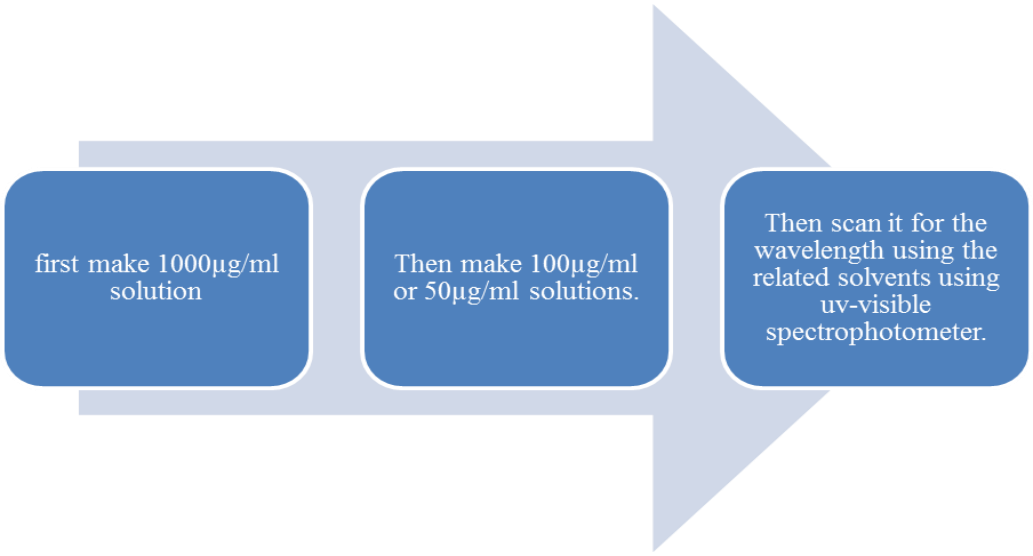
1. Solubility studies

The following samples of drugs like phenylephrine hydrochloride, paracetamol, caffeine, chlorpheniramine maelate was tested in various solvents like water, 0.1 N HCl, Methanol and Ethanol.

2. λ Max determination

We can prepare working concentrations from stock solution by using water, methanol, 0.1 N HCl for different drug samples. The stock solutions are prepared and are scanned in the UV

range from 200 to 400 nm and the absorbance values are noted. Based on these absorbance values the ^ max was determined. The wavelength at which maximum concentration takes place was noted.



3. Preparation of stock solution

- 1 mg/ml phenylephrine hydrochloride solution was prepared by dissolving 100 mg of PPH in 100 ml of water. The working standard solutions are prepared by taking 5 ml from the prepared solution and made up to the final volume of 50 ml with the respective solvent (water). Then it becomes 50 µg/ml.
- The following remaining drug samples like paracetamol, caffeine and chlorpheniramine maelate also follows the same procedure. but the solvent in which it dissolves varies.
- Caffeine and Chlorpheniramine maelate was dissolved in 0.1 N HCl.
- Paracetamol was dissolved in methanol.

4. Dosage form preparation

Weigh equivalent weight of the dosage form and dissolve it in 100 ml of water. From the above, 5 ml solution was taken and made up to the final volume with water and it is so 50 µg/ml.

As it is a combination of multiple drugs we have to take the highest amount of chemical to be weighed. In the dosage form paracetamol is highest. So the equivalent weight to be weighed is equal to the weight of the paracetamol in the dosage form.

METHOD VALIDATION

The proposed method was validated for the following parameters as per ICH.

- Accuracy
- Precision
- Linearity
- Limit of detection
- Limit of quantification
- Specificity
- Robustness
- Assay

ACCURACY

Accuracy is performed by diluting the stock solution in three different concentrations at 50%, 100%, 150%. 6 samples were prepared from 50% concentration, 3 samples was prepared from 100% concentration and another 6 samples from 150% concentration. All the samples were scanned at respective λ max in photometric mode and the absorbance of each sample was noted. Then percentage recovery and mean percentage recovery are calculated.

PRECISION

Precision is performed in which the stock solution is diluted in optimum concentration of six samples. The six samples were scanned at their respective λ max and their absorbance was noted.

According to ICH guidelines, precision should be performed at two different levels. They are repeatability and intermediate precision. Repeatability is the variation arising when all efforts are made to keep conditions constant by using the same instrument and operator and repeated during a short period of time.

Intermediate precision (also called with-in laboratory or within device) is a measure of precision under a defined set of conditions: same measurement procedure, same measuring system, same location, and replicate measurements on the same or similiar objects over an extended period of time.

In this method, precision was performed by determining the absorbance of six samples. The resulting data are tabulated by the absorbance of inter day, intraday, mean, standard deviation and percentage reletive standard deviation are calculated.

LINEARITY

Method

Linearity was determined by preparing 5 samples of different concentrations of the different samples of bulk (pure drugs). The concentrations are different for each drug. These concentrations are taken from the stock solution and diluted to final volume with respective solvent.

These concentrations are scanned at respective λ max in photometric mode and the absorbance was noted for each concentration.

The calibration curve is plotted by taking concentration on x-axis and absorbance on y-axis. The correlation coefficient and slope are calculated.

LIMIT OF DETECTION (LOD)

Limit of detection is processed by preparing very dilute concentrations like 0.1, 0.2, 0.5..... up to 10 µg/ml and scanned at respective λ max, where the concentration shows the detectable absorbance.

LIMIT OF QUANTIFICATION (LOQ)

Limit of quantification is processed by preparing very dilute concentrations like 0.1, 0.2, 0.5..... up to 10 µg/ml and scanned at respective λ max, where the concentration shows the quantified absorbance.

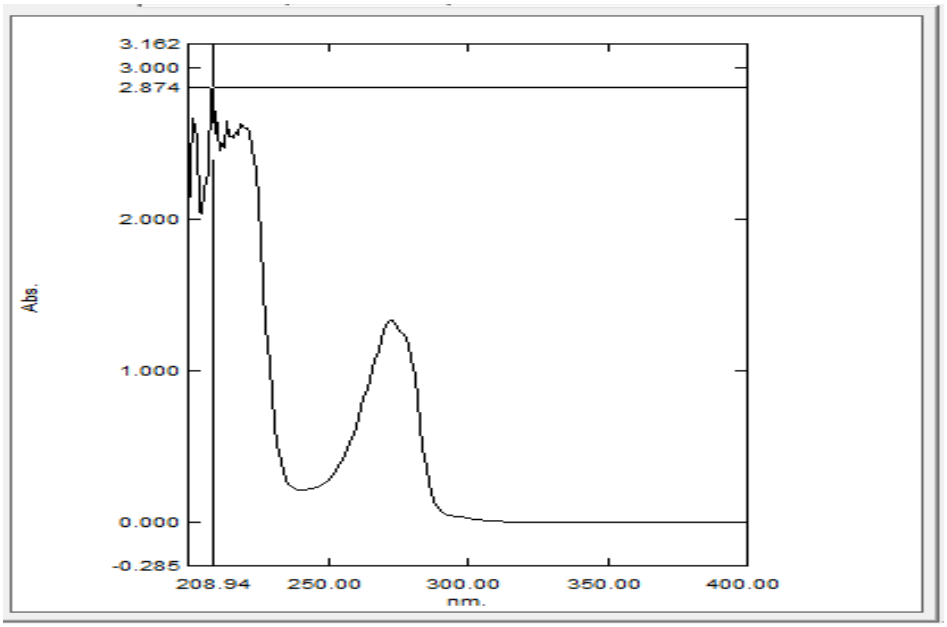
ROBUSTNESS

Robustness is performed by changing λ max, solvent and analyst. Sinoset tablets was prepared for 100% concentration (50µg/ml) and the above solution was scanned in a photometric mode by changing the λ max at 214nm, 216nm, 218nm and by changing the concentration of the solvent. The absorbance of each sample was noted and percentage purity was calculated.[5]

RESULTS AND DISCUSSION

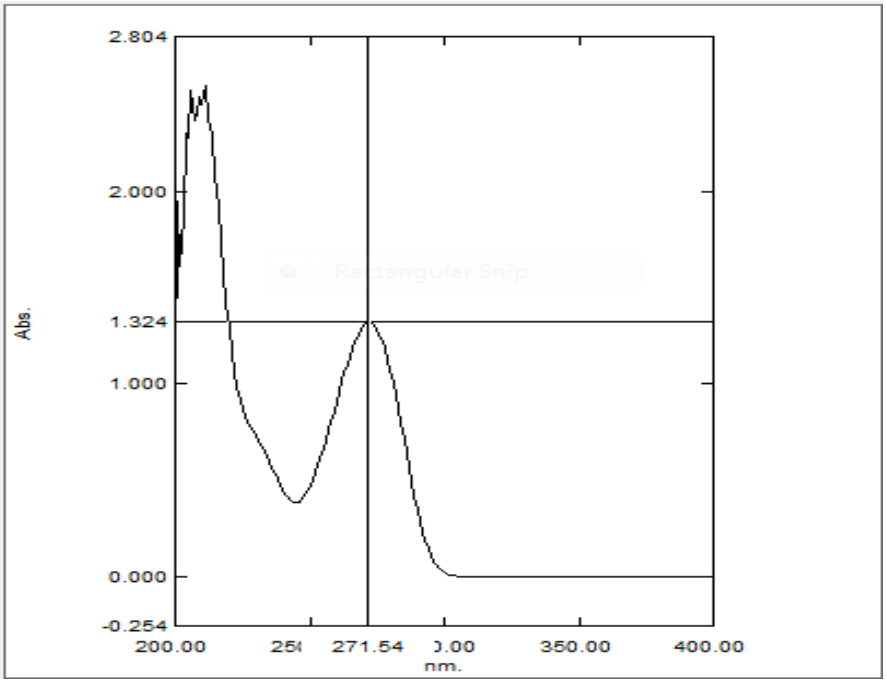
Determination of λ max

Determination of λ max of PPH



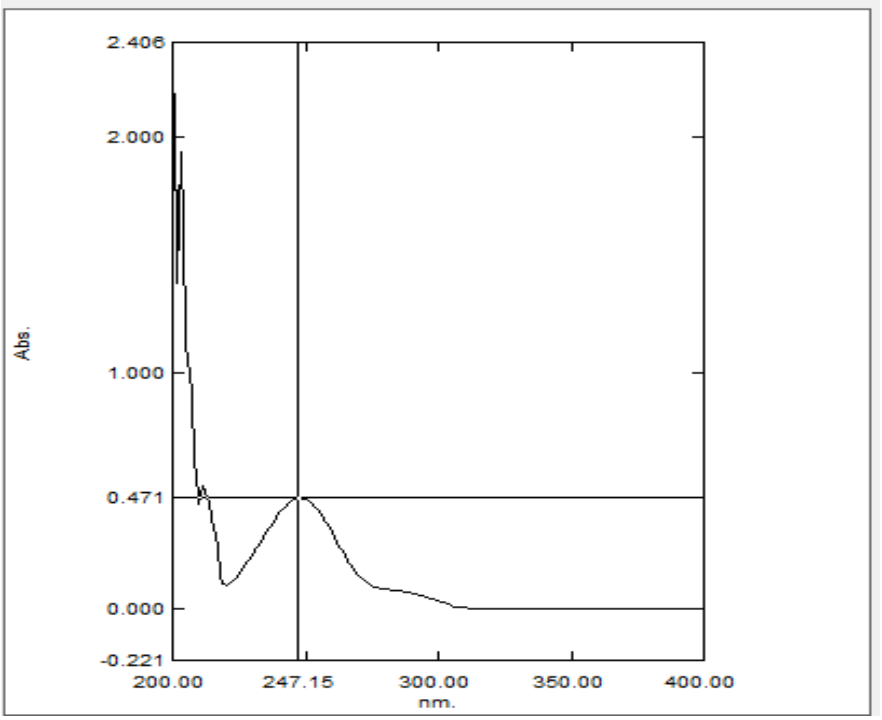
The Absorption maximum of PPH in water was found to be 220 nm.

Determination of λ max of Caffeine

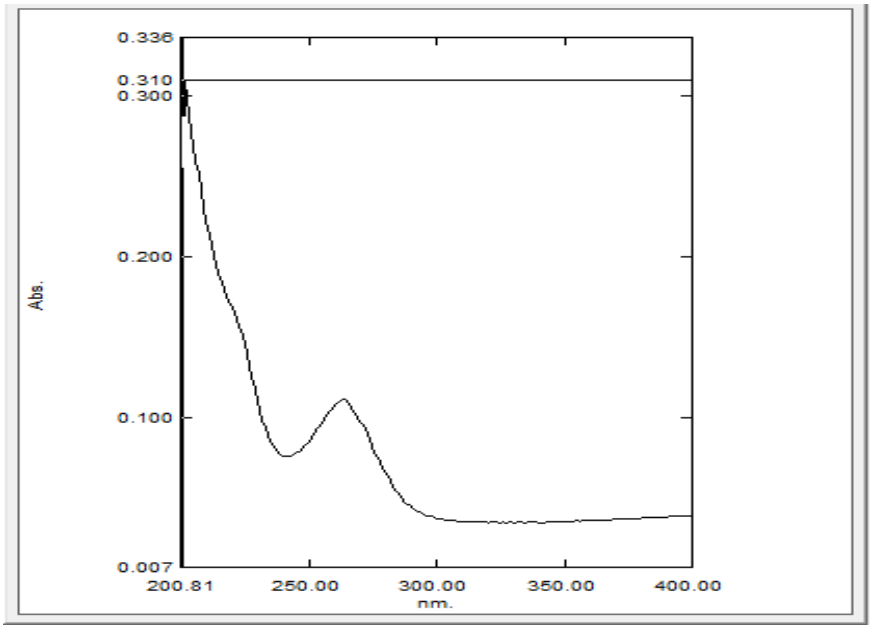


The Absorption maximum of caffeine in 0.1 N HCL was found to be 215nm.

Determination of λ max of Paracetamol

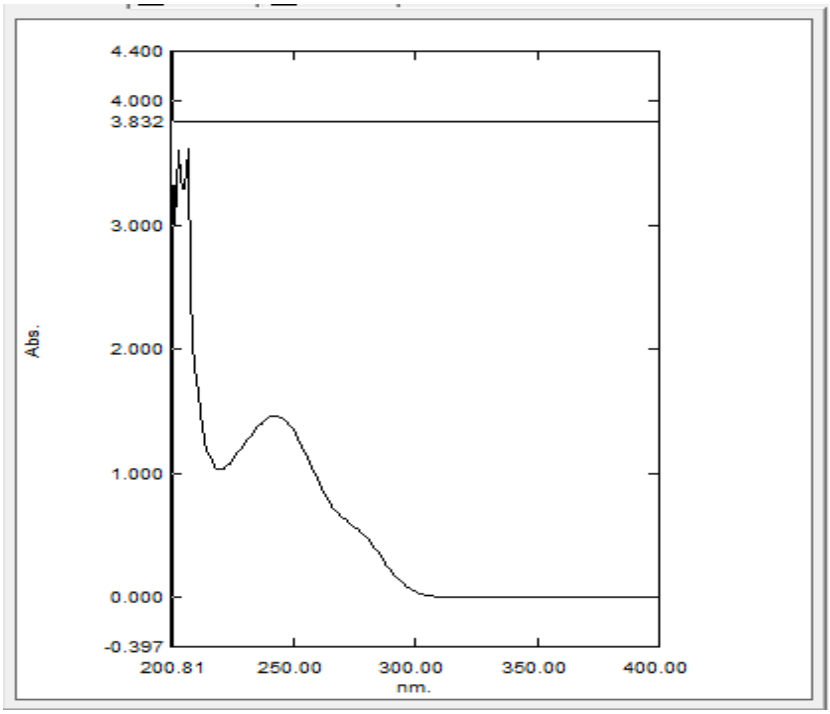


The Absorption maximum of Paracetamol was found to be 250 nm.



The absorption maximum of CPM was found to be 205 nm.

Determination of λ max of Dosage form



METHOD DEVELOPMENT AND VALIDATION BY ULTRA VIOLET SPECTROMETRY

Solubility Profile

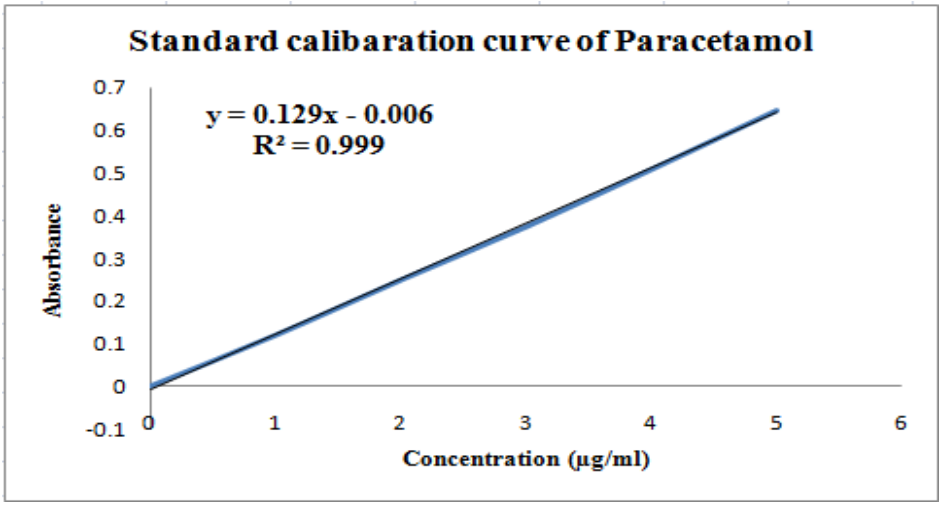
DRUGS	WATER	0.1N HCL	ETHANOL	METHANOL
PHENYLEPHRINE HYDROCHLORIDE	Very soluble	Very soluble	Soluble	Soluble
PARACETAMOL	Insoluble	Slightly soluble	Very soluble	Very soluble
CAFFEINE	Insoluble	Soluble	Insoluble	Insoluble
CHLORPHENIR-AMINE MAELATE	Soluble	Soluble	Insoluble	Insoluble
DOSAGE FORM	Soluble on heating or ultra-sonication. After that filter through whatmannfilterpa per no.40.	Insoluble	Slightly soluble	Soluble

LINEARITY

The linearity was evaluated in the concentration ranges as shown below. The calibration curve was constructed by plotting concentration verses absorbance. Good linearity was obtained with a straight line and thus regression coefficient was found to be $R^2 = 0.999$.

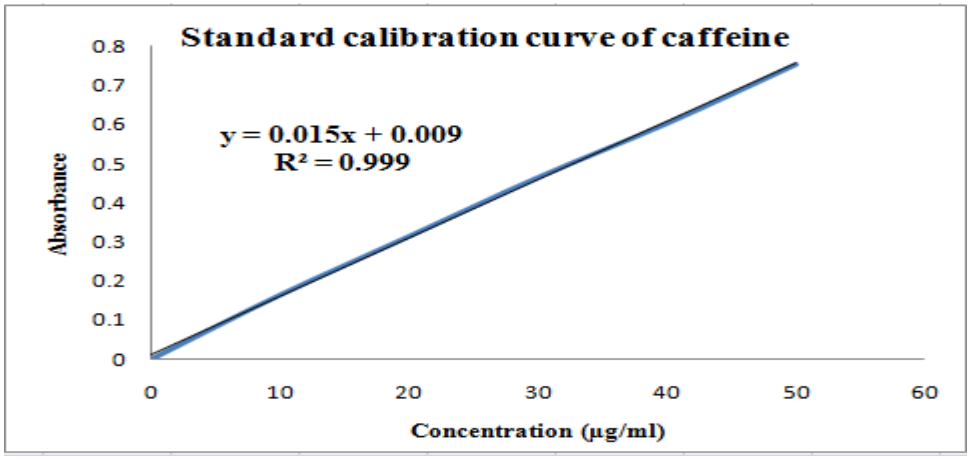
Linearity Profile of Paracetamol

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	1	0.12
3	2	0.25
4	3	0.375
5	4	0.51
6	5	0.648



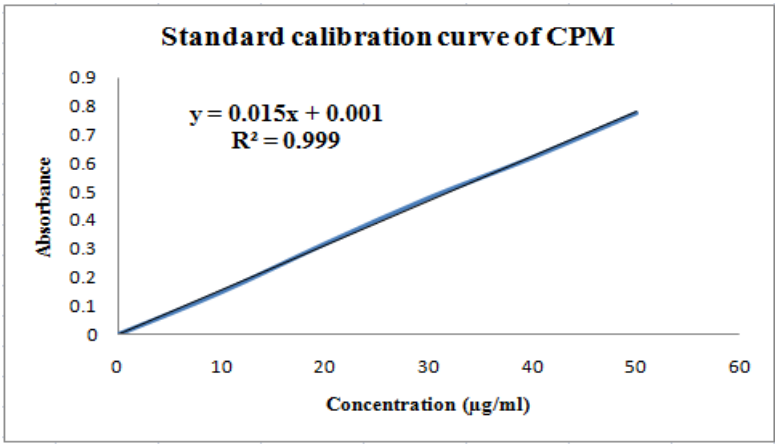
LINEARITY PROFILE OF CAFFEINE

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.165
3	20	0.315
4	30	0.466
5	40	0.604
6	50	0.755



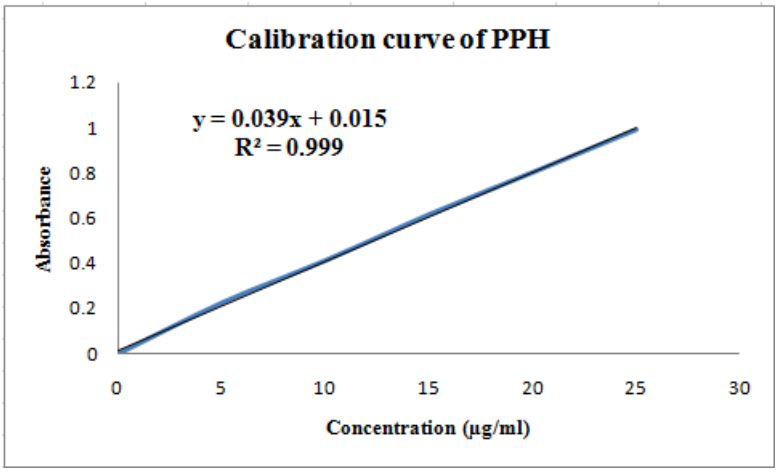
LINEARITY PROFILE OF CPM

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.15
3	20	0.32
4	30	0.481
5	40	0.623
6	50	0.779



LINEARITY PROFILE OF PPH

S.No	Concentration (µg/ml)	Absorbance
1	0	0
2	5	0.225
3	10	0.412
4	15	0.615
5	20	0.801
6	25	0.990



LIMIT OF DETECTION

The limit of detection is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit (generally 1%).

LOD = 3 × Standard deviation / Slope The LOD was found to be 1.380µg/ml.

LIMIT OF QUANTIFICATION

The quantification limit is the term used to describe the smallest concentration of a substance measured that can be reliably measured by an individual.

LOQ = 10 × Standard deviation / Slope. The

LOQ was found to be 5.101µg/ml.

✓ The obtained results were satisfactory.

PRECISION

Intraday precision (Repeatability) for paracetamol

Intraday precision day-1.

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
1	0.121	0.121	0.120	0.120	0.0005	0.48
3	0.375	0.374	0.375	0.374	0.0005	0.15
5	0.648	0.647	0.648	0.647	0.0005	0.09

Intraday precision day-2

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
1	0.163	0.163	0.162	0.162	0.0005	0.35
3	0.415	0.416	0.415	0.415	0.0005	0.14
5	0.695	0.693	0.690	0.692	0.0025	0.36

Intraday precision day-3

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
1	0.163	0.163	0.162	0.162	0.0005	0.35
3	0.416	0.416	0.412	0.414	0.0023	0.56
5	0.645	0.637	0.640	0.640	0.0040	0.63

Intermediate precision

Conc (µg/ml)	Absorbance						Average	SD[a]	RSD[b]
	Set1	Set2	Set3	Set4	Set5	Set6			
1	0.121	0.121	0.192	0.181	0.121	0.121	0.1416	0.034	0.85
3	0.375	0.374	0.385	0.384	0.374	0.374	0.377	0.005	1.4
5	0.641	0.641	0.677	0.677	0.641	0.641	0.653	0.018	1.85

Intraday precision (Repeatability) for Caffeine

Intraday precision Day-1

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
10	0.165	0.165	0.164	0.164	0.0005	0.35
30	0.465	0.464	0.465	0.646	0.0005	0.12
50	0.755	0.754	0.755	0.464	0.0005	0.12

Intraday precision Day-2

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
10	0.172	0.173	0.173	0.173	0.0005	0.33
20	0.481	0.481	0.480	0.480	0.0005	0.12
30	0.761	0.761	0.760	0.760	0.0005	0.08

Intraday precision day-3

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
10	0.165	0.171	0.169	0.168	0.0030	1.81
30	0.480	0.475	0.479	0.478	0.0026	0.55
50	0.760	0.756	0.758	0.758	0.0026	0.26

Intermediate Precision

Conc (µg/ml)	Absorbance						Average	SD[a]	RSD[b]
	Set1	Set2	Set3	Set4	Set5	Set6			
10	0.165	0.164	0.163	0.164	0.165	0.165	0.164	0.0008	0.50
30	0.465	0.463	0.465	0.467	0.465	0.464	0.464	0.0013	0.29
50	0.755	0.754	0.753	0.755	0.752	0.755	0.754	0.0012	0.17

Intraday precision (Repeatability) for CPM

Intraday precision Day-1

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
10	0.171	0.171	0.162	0.166	0.005	0.93
30	0.481	0.480	0.479	0.48	0.001	0.21
50	0.779	0.777	0.778	0.778	0.001	0.13

Intraday precision Day-2

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
10	**0.211**	**0.209**	**0.210**	**0.21**	**0.001**	**0.48**
30	0.499	0.498	0.497	0.498	0.001	0.20
50	0.801	0.799	0.801	0.800	0.001	0.14

Intraday precision day-3

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
10	**0.171**	**0.170**	**0.171**	**0.170**	**0.0005**	**0.34**
30	0.481	0.481	0.480	0.480	0.0005	0.12
50	0.779	0.775	0.779	0.777	0.002	0.30

Intermediate precision

Conc (µg/ml)	Absorbance						Average	SD[a]	RSD[b]
	Set1	Set2	Set3	Set4	Set5	Set6			
10	**0.171**	**0.169**	**0.168**	**0.171**	**0.170**	**0.172**	**0.170**	**0.0014**	**0.87**
30	0.480	0.481	0.483	0.482	0.483	0.480	0.4815	0.0013	0.29
50	0.774	0.773	0.776	0.775	0.773	0.775	0.774	0.0012	0.16

Intraday precision (Repeatability) for PPH

Intraday precision day-1

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
5	0.225	0.224	0.225	0.224	0.0005	0.26
15	0.615	0.615	0.616	0.615	0.0005	0.09
25	0.990	0.991	0.993	0.991	0.0015	0.15

Intraday precision Day-2

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
5	0.231	0.230	0.231	0.230	0.005	0.25
15	0.635	0.633	0.634	0.634	0.001	0.16
25	1.105	1.105	1.104	1.104	0.0005	0.05

Intraday precision Day-3

Conc (µg/ml)	Absorbance			Average	SD[a]	RSD[b]
	Set 1	Set 2	Set 3			
5	0.223	0.225	0.225	0.224	0.0011	0.51
15	0.615	0.616	0.613	0.614	0.0015	0.25
25	0.990	0.992	0.993	0.991	0.0015	0.15

Intermediate precision

Conc (µg/ml)	Absorbance						Average	SD[a]	RSD[b]
	Set1	Set2	Set3	Set4	Set5	Set6			
5	0.225	0.223	0.222	0.225	0.224	0.223	0.223	0.001	0.54
15	0.615	0.614	0.613	0.615	0.616	0.615	0.615	0.001	0.17
25	0.990	0.990	0.991	0.992	0.991	0.992	0.991	0.0008	0.09

ACCURACY

Limit: % recovery must be more than 98 and less than 102%.

ACCURACY OF PARACETAMOL

S.No	Accuray level	Wt. of the sample	Sample Absorbance	Amount added	Amount found	% recovery	Mean % recovery	% RSD
1	50	325	0.014	25	25.10	100.4	98.97	1.31
2		325	0.015	25	24.45	97.88		
3		325	0.015	25	24.66	98.64		
4	100	650	0.211	50	49.98	99.96	100.41	0.39
5		650	0.212	50	50.31	100.62		
6		650	0.211	50	50.33	100.66		
7	150	975	0.457	75	74.99	99.98	99.97	0.13
8		975	0.459	75	74.88	99.84		
9		975	0.458	75	75.11	100.1		

ACCURACY OF CAFFEINE

S.No	Accuracy level	Wt. of the sample	Sample Absorbance	Amount added	Amount found	% recovery	Mean % recovery	% RSD
1	50	12.5	0.001	25	24.88	99.52	99.77	0.85
2		12.5	0.001	25	25.18	100.72		
3		12.5	0.002	25	24.77	99.08		
4	100	25	0.303	50	50.48	100.96	100.63	1.53
5		25	0.304	50	50.99	101.98		
6		25	0.303	50	49.48	98.96		
7	150	37.5	0.602	75	74.99	99.9	100.14	0.86
8		37.5	0.604	75	75.88	101.1		
9		37.5	0.603	75	74.57	99.42		

ACCURACY OF PPH

S.No	Accuracy level	Wt. of the sample	Sample Absorbance	Amount added	Amount found	% recovery	Mean % recovery	% RSD
1	50	2.5	0.005	25	24.77	99.08	99.81	0.69
2		2.5	0.004	25	25.11	100.44		
3		2.5	0.004	25	24.98	99.92		
4	100	5	0.244	50	50.47	100.94	100.29	1.13
5		5	0.245	50	50.48	100.96		
6		5	0.242	50	49.49	98.98		
7	150	7.5	0.455	75	75.33	100.44	100.53	0.59
8		7.5	0.456	75	74.99	99.99		
9		7.5	0.460	75	75.88	101.17		

ACCURACY OF CPM

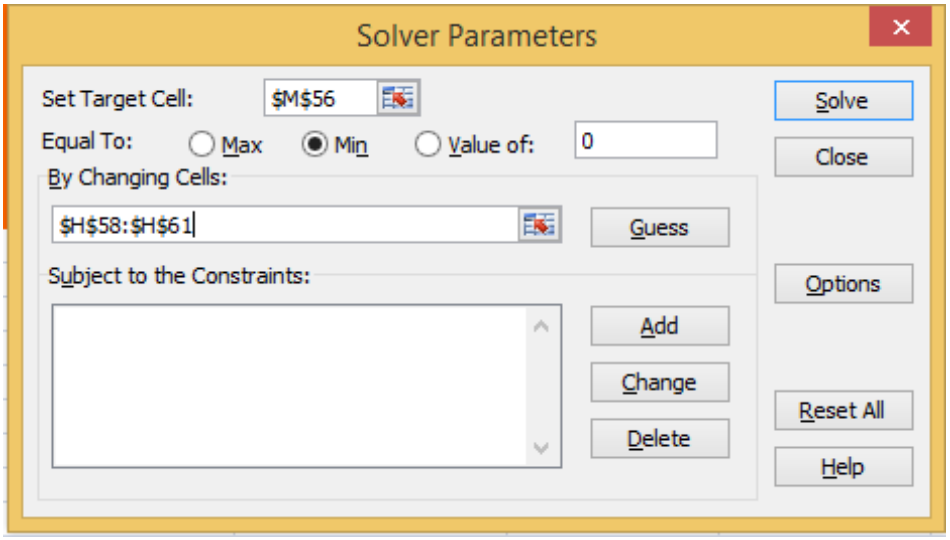
S.No	Accuracy level	Wt. of the sample	Sample Absorbance	Amount added	Amount found	% recovery	Mean % recovery	% RSD
1	50	1	0.001	25	25.11	100.44	99.32	1.24
2		1	0.001	25	24.88	99.52		
3		1	0.001	25	24.50	98		
4	100	2	0.211	50	50.32	100.64	99.50	0.99
5		2	0.233	50	49.48	98.96		
6		2	0.234	50	49.46	98.92		
7	150	3	0.433	75	75.33	100.44	100.49	0.77
8		3	0.435	75	74.81	99.75		
9		3	0.437	75	75.97	101.29		

ANALYSIS BY METHOD OF SIMPLE LEAST SQUARES:

Method of simple least squares

			standard								
Wave Length	paracetamol	caffeine	PPh	CPM	Am (FORML)	std absorpt paracetamol	std abso caffe	std absorp PPh	std abs CPM	Acalc	(Acalc - Am)^2
205	0.12	0.165	0.225	1.829	1.895	0.04	0.0055	0.015	0.060966667	2.36823621	0.223952510
210	0.201	0.315	0.412	1.993	1.932	0.067	0.0105	0.027466667	0.066433333	2.940359865	1.016789618
220	0.257	0.466	0.615	0.882	1.652	0.085666667	0.015533333	0.041	0.0294	2.197366305	0.297424407
230	0.375	0.604	0.801	0.527	1.998	0.125	0.020133333	0.0534	0.017566667	2.259948	0.068616755
240	0.512	0.755	0.999	0.32	1.548	0.170666667	0.025166667	0.0666	0.010666667	2.51672662	0.938431265
250	0.614	0.8	1.012	0.324	1.485	0.204666667	0.026666667	0.067466667	0.0108	2.674801683	1.415628044
255	0.648	0.95	1.512	0.459	1.387	0.216	0.031666667	0.1008	0.0153	3.473816395	4.354802667
										Sum =	8.315645265

paracetamol	3	paracet FOUND	2.86958569
caffeine	30	caffeine found	29.2538762
PPh	15	PPh found	14.4531611
CPM	30	CPM found	30.76696085



ASSAY

Assay Data by method of simple least squares

Formulation	Label Claim	Amount found	% Assay ± SD*
Sinoset	Paracetamol - 650mg	2.86958569	95.33 ± 0.0005
	Caffeine - 25 mg	29.2538762	97.5 ± 0.0026
	Phenylephrine HCL - 5 mg	14.4531611	96.33 ± 0.0015
	Chlorpheniramine Maleate - 2 mg	30.76696085	102.53 ± 0.0001

The obtained results were found to be accurate owing to analysis at multiple wavelengths with satisfactory assay results.

CONCLUSION

From the results it was found that the developed UV method was found to be simple, accurate, sensitive, precise, specific and rapid. The method is developed and validated in tablet dosage form in conjunction with robust chemometric statistical approach studies by UV spectrophotometry and method of simple least squares. Method of simple least squares multivariate analysis using solver - add in lead to appreciable results. Further statistical evaluation revealed the equal preciseness of the assay of the results of the developed methods. The developed method can be utilized for routine analysis in quality control laboratories.

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REFERENCES

1. Kirtimaya Mishra, B. Kiran kumar, M. Muthu Kumari, B. B. S. Subrahmanyam. New analytical method development and validation of chlorpheniramine maleate by using UV-Visible spectrophotometry. Indo American journal of pharmaceutical sciences, 2016; 3(7): 762-772.
2. P. Ptacek, J. Klima, J. Macek. Development and validation of liquid chromatography - tandem mass spectrometry method for the determination of the phenylephrine in human plasma and its application to a pharmacokinetic study. Journal of chromatography B, 2007; 858: 263-268.
3. Hemaraj Sharma, M. Anil Kumar Reddy, C. Naresh Babu, Hari Prasad Bhatta, Nabin Wagle, Hari Prasad Sapkota, Nim Bahadur Dangi. Method development and validation of Dual wavelength spectrophotometric method for simultaneous estimation of Paracetamol and Caffeine in combined dosage form by internal standard method. Asian journal of chemistry, 2015; 27(12): 4666-4668.
4. Kirtimaya Mishra, B.Kiran kumar, M. Muthu Kumari, B. B. S. Subrahmanyam. New analytical method development and validation of chlorpheniramine maleate by using UV-Visible spectrophotometry. Indo American journal of pharmaceutical sciences, 2016; 3(7): 762-772.
5. Validation of Analytical procedures: Text and Methodology Q_2 (R_1). ICH Harmonized Tripartite guidelines.