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Validated RP-HPLC method for the simultaneous quantification of Paracetamol, Codeine phosphate and Caffeine in Effervescent tablet formulation

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ABSTRACT: Background: A simple, rapid and precise method for the quantitative simultaneous determination to helps in reducing analysis time and maintaining good efficiency of multidrug therapy. **Aim:** The research was aimed to develop a simple, rapid and precise method for the quantitative simultaneous determination of three active moieties that are Paracetamol, Codeine phosphate and Caffeine, for development of an effervescent dosage form since no analytical method is available for effervescent triple component formulation in any of the Pharmacopeias. **Methods:** The analysis was performed on a Waters HPLC system with a Phenomenex Kinetex C_{18} column (4.6 × 100 mm I.D., 2.6µm) and an isocratic elution consisting of 15 mM Potassium dihydrogen orthophosphate as the buffer and acetonitrile and methanol as the organic solvents in the ratio 90.5: 4.5: 5 respectively as the mobile phase. The detection wavelength was 210 nm (Codeine and Caffeine) and 243 nm (Paracetamol) with an acquisition time of 7 min in which all the 3 moieties were well separated. **Results:** The method was validated and shown to be linear for Paracetamol, Codeine phosphate and Caffeine. The relative standard deviations for six replicate measurements in two sets of each drug in the tablets is always less than 2 % and mean % error of active recovery not more than ± 2.0 %. The method was validated for precision and accuracy. **Conclusion:** The proposed method was successfully applied to the pharmaceutical dosage forms containing the above-mentioned drug combination without any interference by the excipients.

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Keywords: Paracetamol, Codeine phosphate and Caffeine, Multi component, Effervescent tablets.

INTRODUCTIONS:

Drug combinations are single preparations containing two or more active pharmaceutical ingredients (APIs) for concurrent administration as a fixed dose 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. 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.

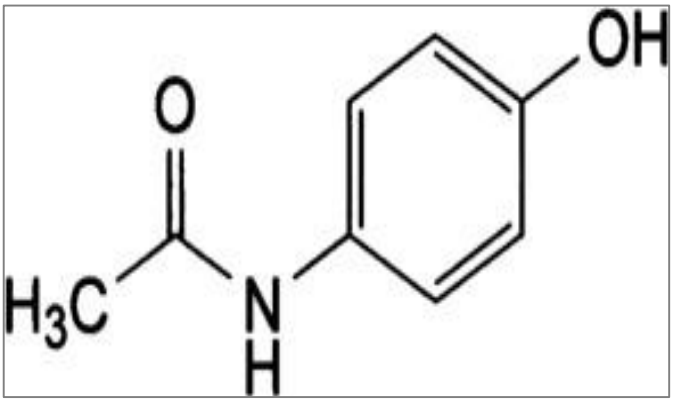

Fig 1. Structure of Paracetamol.

However, monographs in most official pharmacopoeia are for single component drugs, hence 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 reverse phase HPLC methods for the simultaneous estimation of various active components in multi-component drugs

The main objective of this work is to develop and validate a new, simple, accurate, linear, precise, specific, robust, sensitive and cost effective reverse phase HPLC method for simultaneous estimation of paracetamol, codeine phosphate and caffeine in multi-component effervescent tablet dosage form.

Paracetamol (acetaminophen), N-(4-Hydroxyphenyl)-acetamide (Fig 1) is a widely used analgesic and antipyretic agent for the relief of fever, headaches, minor pains, etc. It is a major ingredient in numerous cold and flu remedies. In combination with non-steroidal anti-inflammatory drugs and Opioid analgesics, Paracetamol is used also in the management of severe pain (such as post operative pain). Paracetamol alone or in combination with other drugs is reported to be estimated by titrimetry, Spectrophotometric method, HPLC, TLC, HPTLC, UHPLC, LC-MS, FT-IR, Amperometric determination and Fluorimetry.

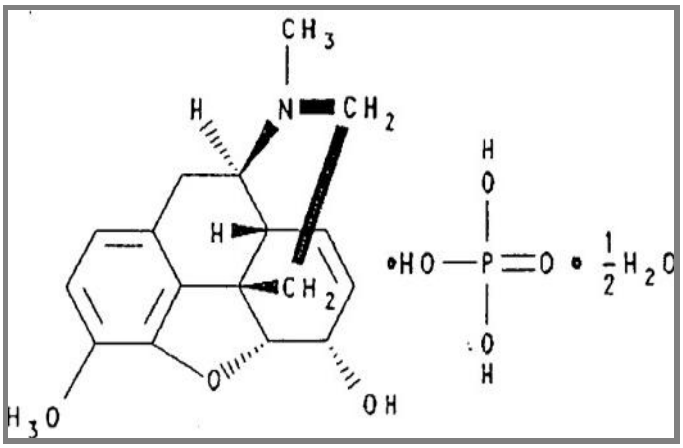

Fig 2. Structure of Codeine phosphate.

Codeine phosphate (7,8-Didehydro-4,5a-epoxy-3-methoxy-17- methylmorphinan-6a-ol) phosphate is predominant alkaloid in opium. It is considered as a pro-drug, metabolized to active compounds of morphine and codeine-6-glucoronide. Codeine (Fig 2) is the traditional choice for the treatment of moderate opioid- sensitive pains. Codeine phosphate in combination with other compounds has been determined in different pharmaceutical preparations by GLC, TLC, UV and HPLC. Combinations of Codeine with Paracetamol produce a significant increase in analgesia compared with Paracetamol alone. These pharmaceutical formulations accounted for 20 % of total non-opiate analgesics during the last decade. Their quality control is thus of paramount importance, especially the determination of Paracetamol in pharmaceuticals has been critically reviewed since its overdose can cause fulminating hepatic necrosis and other toxic effects.

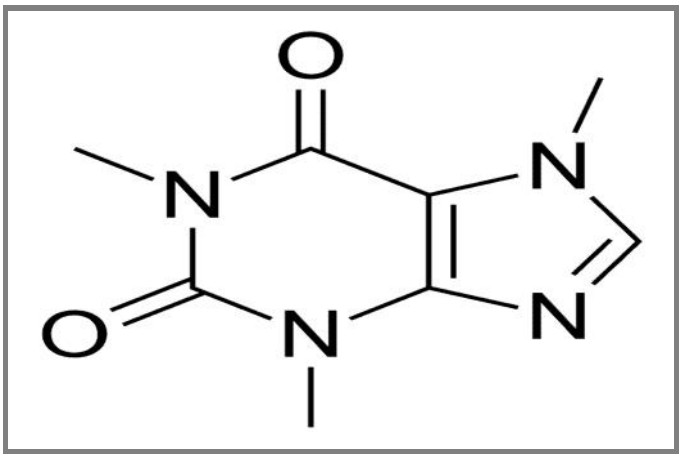

Fig 3. Structure of Caffeine.

Caffeine is a central nervous system (CNS) stimulant of the methylxanthine class (Fig 3). It is the world's most widely consumed psychoactive drug. Unlike many other psychoactive substances, it is legal and unregulated in nearly all parts of the world. There are several known mechanisms of action to explain the effects of caffeine. The most prominent is that it reversibly blocks the action of adenosine on its receptor and consequently prevents the onset of drowsiness induced by adenosine. Caffeine also stimulates certain portions of the autonomic nervous system.

Caffeine is a bitter, white crystalline purine, a methylxanthine alkaloid, and is chemically related to the adenine and guanine bases of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). It is found in the seeds, nuts, or leaves of a number of plants native to Africa, East Asia and South America, and helps to protect them against predator insects and to prevent germination of nearby seeds. The most well-known source of caffeine is the coffee bean, a misnomer for the seed of Coffee plants.

Validation of the current method is performed according to the requirements of International Conference on Harmonization (ICH) guideline.

MATERIALS AND METHODS:

Materials and Chemicals:

Paracetamol, Caffeine and Codeine phosphate working standards were used available in Oman Pharmaceutical Products L.L.C. Tablet formulation containing Codeine phosphate hemihydrate 8 mg, Caffeine 30 mg and Paracetamol 500 mg were taken from the R&D pilot batch performed at Oman Pharmaceutical Products L.L.C. HPLC grade Methanol and Acetonitrile was procured from Merck Ltd. All other chemical reagents were of analytical grade.

Chromatographic conditions:

The Chromatographic conditions for simultaneous determination and quantification of Paracetamol, Caffeine and Codeine phosphate, being present in effervescent triple component formulation, is given in Table 1.

Buffer preparation:

About 2 g of Potassium dihydrogen phosphate was dissolved in 1000 ml of water. The solution was sonicated and dissolved. The solution was mixed and filtered through 0.2 µ nylon membrane filter.

Table 1. Chromatographic conditions for quantitative analysis of Paracetamol, Caffeine and Codeine phosphate.

Parameter	Specification
Column	Kinetex C_{18} 100Å, (100 × 4.6 mm; 2.6µm)
Flow rate	1.3 mL/min
Injection volume	5 µl
Wavelength	Codeine and caffeine: 210 nm
	Paracetamol: 243 nm
Column	35 °C
Sampler temperature	10 °C
Retention time	For Paracetamol**:** about 2 min.
	For Caffeine**:** about 6 min.
	For Codeine**:** about 4 min.
Solvents	Methanol: Water (50:50 %v/v)
Run time	7 min

HPLC Column manufactured by Phenomenex.

Mobile phase:

The mobile phase for chromatographic study was prepared by mixing 1810 ml of buffer, 100 ml of Methanol and 90 ml of Acetonitrile.

Standard preparation:

About 65 mg of Paracetamol, 60 mg of Caffeine and mg of Codeine phosphate WS were weighed accurately and were taken in a 100 ml volumetric flask. To the flask, about 50 to 60 ml of solvent was mixed and sonicated, until unless the components were completely dissolved. The solution was diluted with same solvent system up to the mark, mixed and filtered. Further, the solution was diluted with 10 ml to 100 ml with solvent, in which the concentration of Paracetamol, Caffeine and Codeine were 65, 60 and 16 µg/ml respectively.

Sample preparation:

About 20 tablets were crushed and weighed. About 3 g (equivalent to about 325 mg of Paracetamol) of sample was weighed and transferred into a 500 ml volumetric flask. About 250 to 300 ml of solvent slowly (to ensure solution does not overflow due to effervescence). The mixture was sonicated for 20 min. The the solution was cooled amd diluted up to the mark with solvent. The solution was filtered through a 0.45µm PVDF filter. About 5 ml of filtrate was discarded. This solution was used for codeine phosphate and caffeine. Further, the 5

to 50 ml solution was diluted with same solvent and this solution was used for Paracetamol.

RESULTS AND DISCUSSION:

The developed method for determination of Paracetamol, Caffeine and Codeine phosphate was validated by using the following parameters:

Selectivity:

Selectivity of the current method was demonstrated by good separation of the three active ingredients (Paracetamol, Codeine phosphate and caffeine). Furthermore, matrix components, e.g. excipients, do not interfere at the retention time of the three actives.

Specificity:

Peak purity angle obtained was less than peak purity threshold were obtained for all three APIs in the chromatograms of sample solutions depicting that the method was very specific to the three APIs under consideration. There were no interfering peaks on the retention times of the APIs in the presence of excipients.

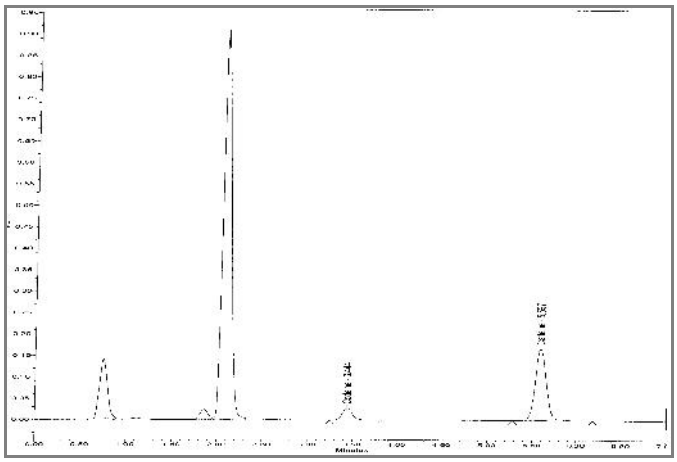

Fig 4. Blank injection overlaid with standard – Codeine and Caffeine.

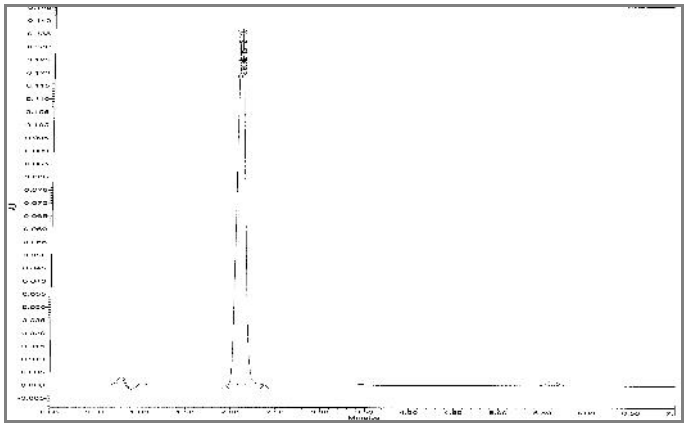

Fig 5. Blank injection overlaid with standard – Paracetamol.

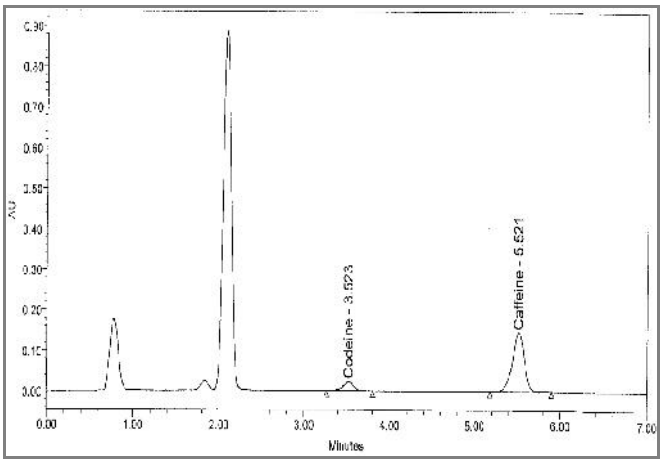

Fig 6. Codeine and caffeine – Oxidation degradation.

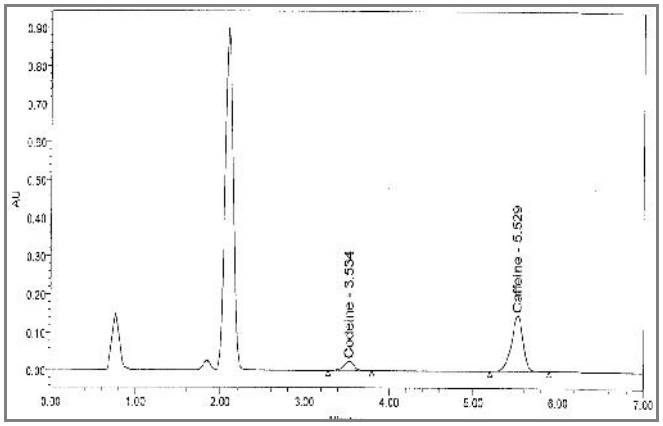

Fig 7. Codeine and caffeine – Basw degradation.

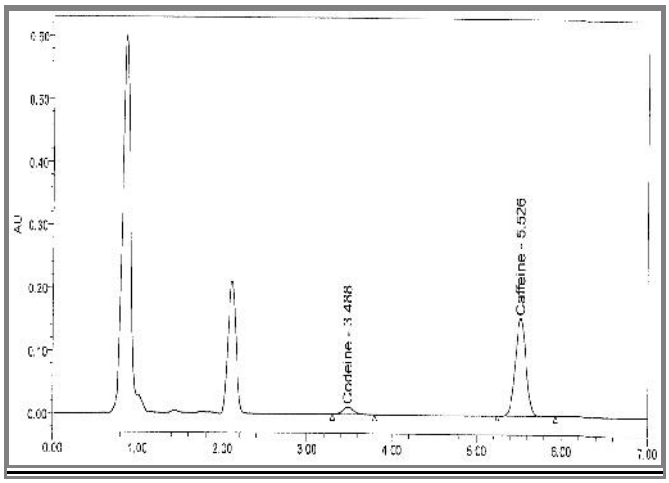

Fig 8. Codeine and Caffeine – Acid degradation.

Further, to demonstrate the specificity of the method, the sample had been subjected to acid, base, oxidation, thermal and photolytic degradation. This was evaluated by comparing the purity angle with purity threshold.

Linearity and Range:

Standard solutions containing Paracetamol (16.3 to 98.1 µg/ml), Codeine phosphate (4.1 to 24.4 µg/ml) and Caffeine (15.1 to 90.9 µg/ml) were prepared. Linearity was determined by duplicate injections of

six different concentrations (25, 50, 80, 100, 120 and 150 % of the target concentration). The average peak areas were plotted against concentrations. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r^2) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

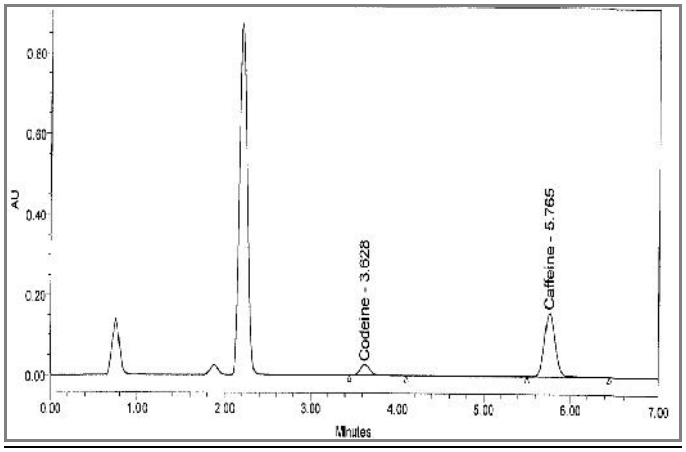

Fig 9. Codeine and caffeine – Thermal degradation.

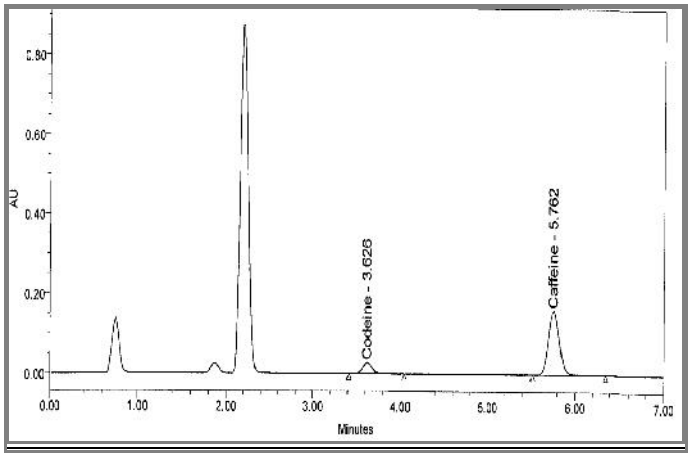

Fig 10. Codeine and Caffeine – Photolytic degradation.

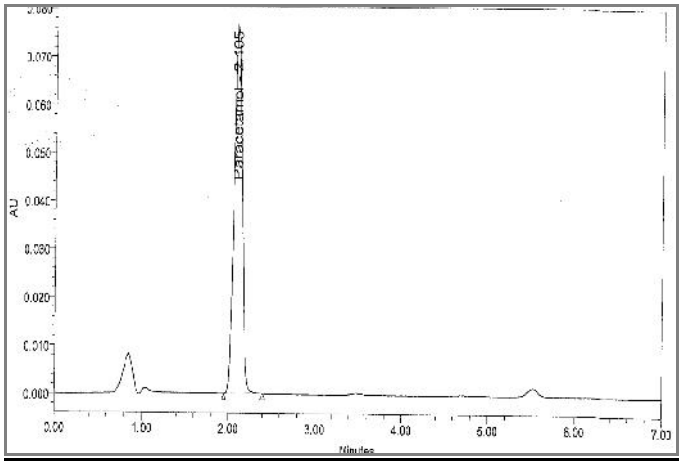

Fig 11. Paracetamol – Acid degradation.

The results obtained are shown in the Tables 1-3 and show that the current method was linear for the three analytes in the range specified above with a correlation coefficients better than 0.999.

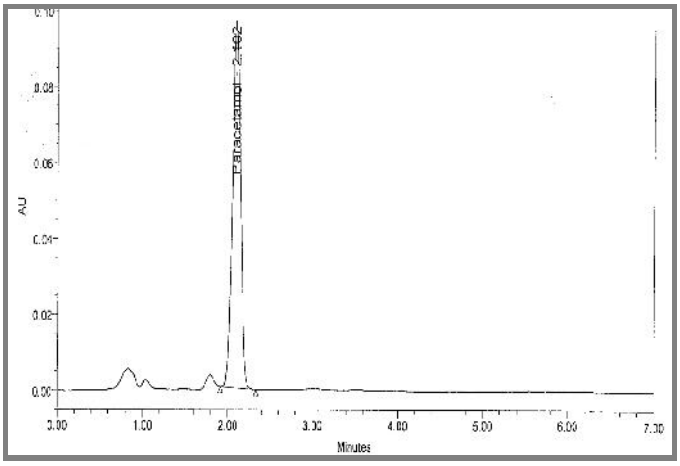

Fig 12. Paracetamol – Base degradation.

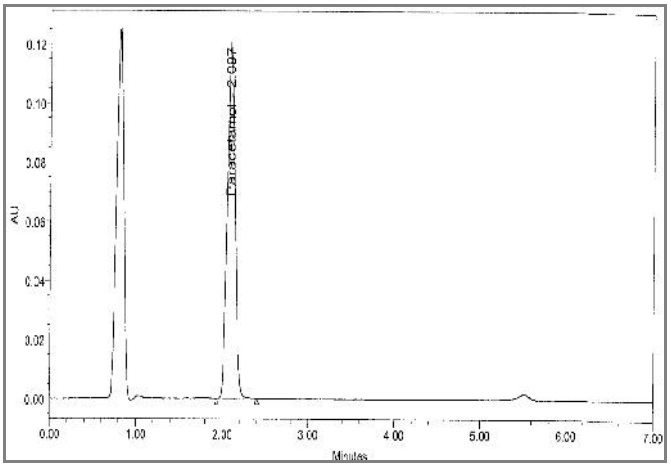

Fig 13. Paracetamol – Oxidation degradation.

Linearity and range:

Standard solutions containing Paracetamol (16.3 to 98.1 µg/ml), Codeine phosphate (4.1 to 24.4 µg/ml) and Caffeine (15.1 to 90.9 µg/ml) were prepared. Linearity was determined by duplicate injections of six different concentrations (25, 50, 80, 100, 120 and 150 % of the target concentration). The average peak areas were plotted against concentrations.

Then linearity was evaluated using the calibration curve to calculate coefficient of correlation, slope and intercept. In general, a value of correlation coefficient (r^2) > 0.999 is considered as the evidence of an acceptable fit for the data to the regression line.

The results obtained are shown in the Tables 1-3 and show that the current method was linear for the three analytes in the range specified above with a correlation coefficients better than 0.999.

Table 2. The purity data of Paracetamol, Codeine and Caffeine.

Stress Condition	Codeine		Caffeine		Paracetamol	
	PA	PT	PA	PT	PA	PT
Acid	1.94	90.0	0.132	14.87	0.225	3.98
Base	11.7	90.0	0.093	8.045	0.153	3.85
Oxidation	1.8	90.0	0.095	11.45	0.266	41.21
Thermal	1.5	90.0	0.066	3.303	0.058	1.25
Photolytic	1.18	90.0	0.051	3.004	0.059	0.93

PA and PT are purity angle and threshold.

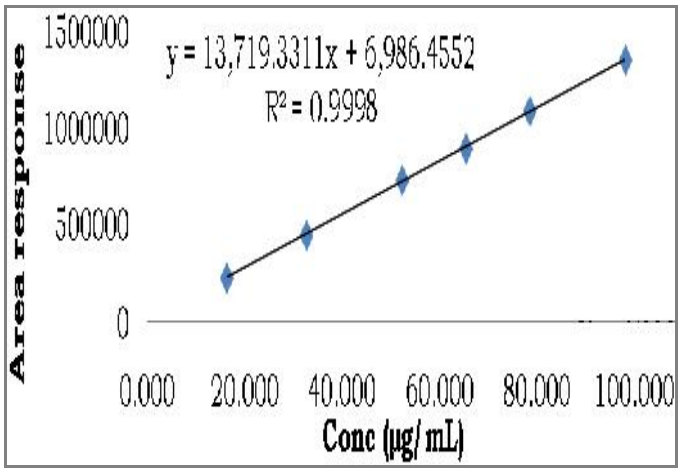

Fig 14. Linearity plot of Paracetamol.

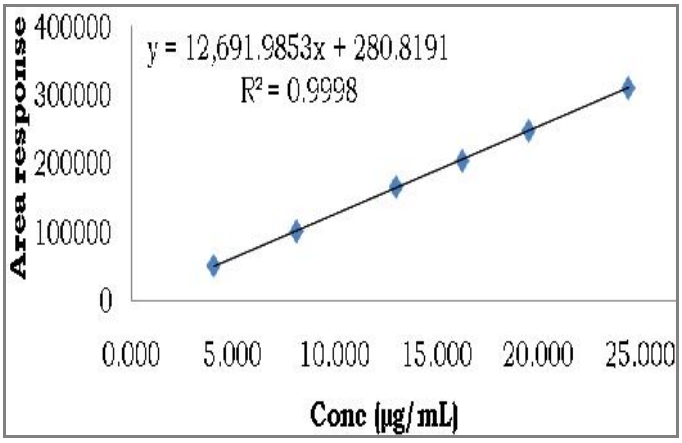

Fig 15. Linearity plot of Codeine Phosphate.

Precision:

The precision of an analytical procedure is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical procedure is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

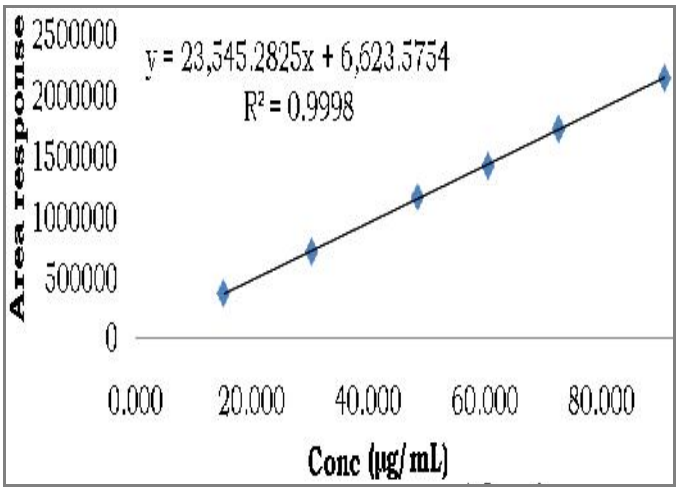

Fig 16. Linearity plot of Caffeine.

Table 3. Linearity data of Paracetamol, Codeine and Caffeine at different concentrations.

L %	Paracetamol		Codeine		Caffeine	
	Conc.	Mean Area	Conc.	Mean Area	Conc.	Mean Area
25	16.349	232059	4.066	52520	15.14	365831
50	32.699	450939	8.132	102502	30.28	711656
80	52.318	733716	13.010	166868	48.46	1161385
10	65.397	896234	16.263	204747	60.57	1419181
12	78.477	1088277	19.516	248460	72.69	1725074
15	98.096	1351034	24.395	310255	90.86	2144650
Slope		13719.3		12691.9		23545.3
Intercept		6986.45		280.82		6623.6
Correlation coefficient		0.9999		0.999		0.999
% bias		0.8		0.1		0.5
R^2		0.9998		0.999		0.999

L – Level. Concentration is expressed in µg/ml.

Table 4. Inter day precision – Standard.

Injec-tion#	Paracetamol area	Caffeine area	Codeine area
1	893960	1487395	204122
2	895337	1489005	204255
3	895813	1489677	204054
4	894153	1487172	203252
5	893407	1488175	203396
6	894183	1487945	203790
Mean	894476	1488228	203812
SD	908.635	958.671	409.402
%RSD	0.1	0.1	0.2

Table 5. Inter day precision – Sample.

Injection #	Assay Paracetamol	Assay Caffeine	Assay Codeine
1	101.5	98.6	100.0
2	101.7	98.6	99.9
3	102.1	98.6	99.9
4	101.7	98.3	100.1
5	102.1	98.7	100.1
6	101.9	98.6	99.9
Mean	101.8	98.6	100.0
SD	0.242	0.137	0.098
%RSD	0.2	0.1	0.1

Table 6. Intraday precision – Standard (Ruggedness).

Injection#	Paracetamol area	Caffeine area	Codeine area
1	166808	260363	37748
2	166943	260848	37913
3	170206	265690	38274
4	169650	264722	38380
5	168225	262554	38092
6	168897	263881	38279
Mean	168455	263010	38114
SD	1395.782	2133.578	244.304
%RSD	0.8	0.8	0.6

Data are expressed as %.

Table 7. Intraday precision – Sample (Ruggedness).

Injection #	Assay Paracetamol	Assay Caffeine	Assay Codeine
1	100.1	98.6	97.9
2	97.6	99.0	98.5
3	99.3	99.3	98.7
4	99.0	97.6	97.0
5	99.7	97.7	97.3
6	100.6	100.7	100.1
Mean	99.4	98.8	98.3
SD	1.042	1.148	1.120
%RSD	1.0	1.2	1.1

Data are expressed as %.

Precision of the method was determined based on the inter and intraday data generated. The results are presented as below. From the data, it can be concluded that the new developed is precise.

Accuracy:

The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range.

Table 8. The percentage recovery data of Paracetamol.

Recovered (mg/ml)	Added (mg/ml)	Recovery	Parameter	Data
0.0320	0.0320	100.0	Avg	99.1
0.0315	0.0320	98.4	Stdv	0.833
0.0316	0.0320	98.8	%RSD	0.8
0.0631	0.0639	98.7	Avg	98.5
0.0628	0.0639	98.3	Stdv	0.208
0.0630	0.0639	98.6	%RSD	0.2
0.0945	0.0959	98.5	Avg	98.7
0.0945	0.0959	98.5	Stdv	0.289
0.0949	0.0959	99.0	%RSD	0.3

Table 9. The percentage recovery data of Codeine Phosphate.

Recovered (mg/ml)	Added (mg/ml)	Recovery		
0.0197	0.0195	101.0	Avg	101.2
0.0197	0.0195	101.0	Stdv	0.289
0.0198	0.0195	101.5	%RSD	0.3
0.0391	0.0390	100.3	Avg	100.3
0.0391	0.0390	100.3	Stdv	0.000
0.0391	0.0390	100.3	%RSD	0.0
0.0578	0.0585	98.8	Avg	98.7
0.0577	0.0585	98.6	Stdv	0.115
0.0578	0.0585	98.8	%RSD	0.1

Table 10. The percentage recovery data of Caffeine.

Recovered (mg/ml)	Added (mg/ml)	Recovery		
0.0307	0.0303	101.3	Avg	101.1
0.0305	0.0303	100.7	Stdv	0.346
0.0307	0.0303	101.3	%RSD	0.3
0.0604	0.0606	99.7	Avg	99.5
0.0602	0.0606	99.3	Stdv	0.200
0.0603	0.0606	99.5	%RSD	0.2
0.0907	0.0909	99.8	Avg	100.0
0.0911	0.0909	100.2	Stdv	0.208
0.0910	0.0909	100.1	%RSD	0.2

Table 11. The precision data of analytical study of Paracetamol.

Sample	Method precision	1.1 ml/min	1.5 ml/min	COT (33°C)	COT (37°C)	89.5:5:6.5 Buffer:MeOH:ACN	92.5:5:2.5 Buffer:MeOH:ACN
1	101.5	101.6	101.9	101.6	101.1	103.7	101.0
2	101.7	101.3	101.4	101.5	100.8	103.0	100.2
3	102.1	101.1	101.6	100.9	100.7	101.5	99.7
Mean	101.8	101.3	101.6	101.3	100.9	102.7	100.3
SD	0.242	0.252	0.252	0.379	0.208	1.124	0.656
%RSD	0.2	0.2	0.2	0.4	0.2	1.1	0.7
OM		101.7	101.8	101.7	101.5	102.1	101.3
OSD		0.339	0.25	0.367	0.53	0.745	0.856
O %RSD		0.3	0.2	0.4	0.5	0.7	0.8

MeOH – Methyl alcohol. CAN – Acetonitrile. Om – Overall mean, O –Overall, COT – Column Oven Temperature.

Table 12. The precision data of analytical study of Codeine Phosphate.

Sample	Method precision	1.1 ml/min	1.5 ml/min	COT (33°C)	COT (37°C)	89.5:5:6.5 Buffer:MeOH:ACN	92.5:5:2.5 Buffer:MeOH:ACN
1	98.6	102.8	102.9	102.8	102.1	101.6	101.2
2	98.6	101.8	101.9	101.3	101.2	101.5	99.9
3	98.6	102.3	102.0	101.3	101.1	101.1	100.0
Mean	98.6	102.3	102.3	101.8	101.5	101.4	100.4
SD	0.137	0.500	0.551	0.866	0.551	0.265	0.723
%RSD	0.1	0.5	0.5	0.9	0.5	0.3	0.7
OM		99.8	99.8	99.6	99.5	99.5	99.2
OSD		1.886	1.873	1.677	1.48	1.427	0.976
O %RSD		1.9	1.9	1.7	1.5	1.4	1.0

MeOH – Methyl alcohol. CAN – Acetonitrile. Om – Overall mean, O –Overall, COT – Column Oven Temperature.

Table 13. The precision data of analytical study of Caffeine.

Sample	Method precision	1.1 ml/min	1.5 ml/min	COT (33°C)	COT (37°C)	89.5:5:6.5 Buffer:MeOH:ACN	92.5:5:2.5 Buffer:MeOH:ACN
1	100.0	100.8	101.2	101.3	100.2	99.2	98.6
2	99.9	99.8	100.2	99.6	99.1	99.3	97.3
3	99.9	100.7	100.3	99.7	99.1	98.9	97.3
Mean	100.0	100.4	100.6	100.2	99.5	99.1	97.7
SD	0.098	0.551	0.551	0.954	0.635	0.208	0.751
%RSD	0.1	0.5	0.5	1.0	0.6	0.2	0.8
OM		100.1	100.2	100.1	99.8	99.7	99.2
OSD		0.364	0.409	0.495	0.417	0.444	1.188
O %RSD		0.4	0.4	0.5	0.4	0.4	1.2

MeOH – Methyl alcohol. CAN – Acetonitrile. Om – Overall mean, O –Overall, COT – Column Oven Temperature.

CONCLUSION:

This intended study can be concluded as: the proposed method is economical, simple, ultra fast, sensitive and reliable and is found to be more accurate, precise, specific, stability indicating, rugged and robust hence it can be employed for routine estimation of for four actives Paracetamol, Codeine and Caffeine

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