Research Article

UPLC Analytical Method Development and Validation for the Simultaneous Estimation of Paracetamol and Caffeine Capsules Dosages Form

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Abstract

In the present study, a rapid, an accurate and precise Ultra Performance Liquid Chromatography (UPLC) method was developed and validated for simultaneous estimation of paracetamol and caffeine in its capsule dosage form (325 mg and 30 mg) by selecting chromatographic parameters. The UPLC method was developed using 2.1 × 50 mm, reverse phase C_{18} column (Acquity UPLC ethylene bridge hybrid (BEH) C_{18} 1.7 µm) with mobile phases containing 0.1% w/v H_3PO_4 and 100% v/v buffer as mobile phase A and methanol: Acetonitrile (50:50) as mobile phase B and water: acetonitrile: H_3PO_4 (80:20:0.1) as diluent and the run considered as an isocratic elution. Flow rate was 0.5 ml/min with PDA detection at (λ_{max}) 275 nm for paracetamol and caffeine and the injection volume was set at 2 µL with run time 7 min. The method was validated by using various validation parameters like accuracy, precision, linearity and specificity. These results show the method could find practical application as a quality control tool for analysis of the drug in its capsule dosage forms in pharmaceutical industries. The developed validated method and stability testing of new dosage forms as per ICH-Q2 (R1) and ICH-Q1C guidelines applicable for the analysis of bulk drug and in its capsules dosage form.

Keywords: UPLC; Validation; Precision; System suitability

Introduction

Ultra Performance Liquid Chromatography (UPLC) is a relatively new modern technique which gives a new direction for liquid chromatography and it is applicable for particle having less than 2 µm in diameter to acquire better resolution, speed and sensitivity as compared with High-Performance Liquid Chromatography (HPLC). It uses fine particles and saves time and reduces solvent consumption. The UPLC system reduces analysis time up to nine times comparing to the conventional system using 5 µm particle packed analytical columns. In UPLC the separation is performed under tremendous pressures (up to 100 MPa is possible), but it has no negative impact on analytical column as well as other components of chromatographic system. Separation efficiency remains maintained and also it is even improved more [1-4]. Paracetamol or acetaminophen which is chemically N-(4-hydroxyphenyl) acetamide or an "aniline analgesics", non-steroidal anti-inflammatory drug by some sources, and not as an NSAID by others sources while most sources implicitly distinguish them in today's scenario as shown in Figure 1 [5-10]. Paracetamol has few anti-inflammatory effects in comparison to other NSAIDs. The antipyretic, analgesic and anti-inflammatory effects of paracetamol are due to inhibiting prostaglandin synthesis such as cyclooxygenase-1(COX-1) and cyclooxygenase-2 (COX-2) [11-13]. The major therapeutic uses of paracetamol are headache, migraine, neuralgia, muscular aches, rheumatic pain, backache, joint pain, toothache and reduction of fever in bacterial or viral origin [14,15]. Caffeine (1,3,7-trimethyl xanthine) is the world's most widely consumed psychoactive drug as depicted in Figure 1 [16]. Apart from that it is also used as diuretic, CNS stimulant and CVS stimulant [17]. The merits of combination of paracetamol along with caffeine as an analgesic and antipyretic are properly established in pharmaceutical formulations [18]. From the various literature findings, it has been seen that paracetamol in combination with caffeine were analytically validated by different spectroscopic and chromatographic techniques [19-23]. The major objectives of this research is to develop a new, convenient UPLC method for determination of paracetamol and caffeine in capsule dosage forms, in accordance with international conference harmonized guidelines [24].

Figure 1: Chemical structure of paracetamol (a) and caffeine (b).
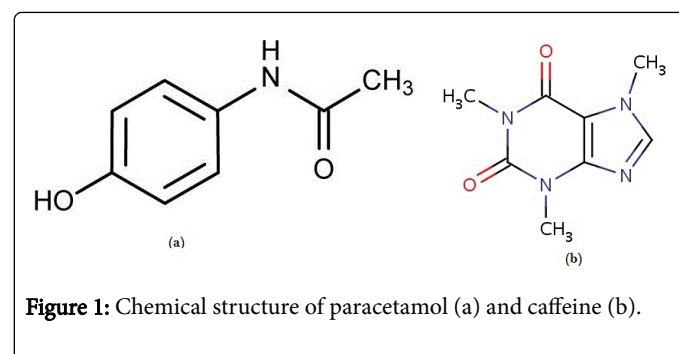
Experimental

Materials and reagents

The paracetamol and caffeine capsule samples were contain 99.71% (w/w) and 99.62% (w/w) respectively, on dried basis. The samples of

paracetamol and caffeine capsules [325 mg, 30 mg] were obtained from local market for analysis. Methanol, acetonitrile, ortho-phosphoric acid, milli-Q water is used as a solvent of UPLC grade. Acquity UPLC (WATERS) liquid chromatographic system consists of following components: A gradient pump, variable wavelength programmable PDA detector, a flow rate 0.5 ml/min. It's injection facility having 2 μl. Chromatographic analysis was performed using Acquity UPLC ethylene bridge hybrid (BEH) C_{18}, column of dimensions of particle size 1.7 μm, and standard diameter (2.1 × 50 mm). In addition, UV Spectrophotometer (Shimadzu 1800) an electronic balance (Meter Toledo), pH meter (Metter Toledo), Sonicator (Power sonic), Hot air oven (Serve well), Centrifuge (REMI), Vacuum oven (Centex), Humidity cum photo stability chamber (Thermo lab), and Shaking water bath (JEIO Tech) were used in this study.

Instrumentation and chromatographic condition

The analytical method development was performed with a chromatographic system (WATERS), Acquity UPLC consisting of binary solvent manager along with PDA detector. It is having acquity UPLC ethylene Bridge Hybrid (BEH) C_{18} column of particle size 1.7 μm and internal diameter 2.1 × 50 mm. The injection facility with 2 μl and flow rate of 0.5 ml/min is maintained. Samples were injected with rheodyne injector system with PDA detection at (λ_{max}) 275 nm. The system data analysis was performed with the Empower 2 software and peak areas were integrated with 7 min run time.

Preparation of solutions

Standard preparation: Weigh accurately about 325 mg of paracetamol to a 250 ml volumetric flask. Add 50 ml of acetonitrile and dissolved it (Solution-A) to get 1300 μg/ml.

Caffeine stock solution: Weigh about 151 mg of caffeine and transfer in to 100 ml volumetric flask. Dissolve it properly and make up to the mark with water. Pipetted out 20 ml of caffeine stock in volumetric flask (solution-A) to get 120 μg/ml and make up the volume with the diluent. For preparation of buffer, dilute 0.5 ml H_3PO_4 to 500 ml with water, i.e., (0.1% H_3PO_4) and for mobile phase mix buffer and methanol in the ratio of 550:450 (v/v). The diluent contains acetonitrile and water in the ratio 50:50 (v/v). Filtration is performed by using 0.22 μm membrane filter.

Test preparation

Weigh accurately about 10 capsules and take the average weight, remove the capsule cell and then transfer the capsules content in to the powder form and accurately take weight equivalent to 380 mg of capsule and transfer it in to the 250 ml volumetric flask. After that add 50 ml acetonitrile and dissolved it properly. Then add the diluent and sonicate it for 5 min until it dissolves the material and make up to the volume with the diluent. Filter the above solution through 0.22 μm PVDF Filter and then inject the clear solution in to the UPLC system.

Procedure

Separately inject equal volumes of about 2 μl of diluent as blank, six replicate injections of standard preparation and one injection of two test preparations into the to the UPLC system and record the chromatograms.

System suitability

Tailing factor for the paracetamol and caffeine capsules is not more than 2.0 for the first chromatogram of the standard injection. The relative standard deviation of sum of the peak areas of paracetamol and caffeine from six replicate injections of standard preparation should not be more than 2.0%.

Calculation

The paracetamol and caffeine present in the capsule as % of labelled amount by the formula:

Paracetamol:

$$T_1 = \frac{AT_1}{AS_1} \times \frac{WS_1}{250} \times \frac{250}{WT} \times \frac{AW}{LC} \times \frac{P}{100} \times 100 \ (1)$$

Where, AT_1 is the sum of the paracetamol peak areas obtained from the test preparation of paracetamol and caffeine capsules and AS_1 is the sum of the Paracetamol peak areas obtained from the standard preparation of Paracetamol, caffeine. WS_1 is the weight of paracetamol working standard taken in mg for standard preparation. WT is the weight of test sample taken in mg for test preparation, AW is the average weight of paracetamol, caffeine capsules, P is the % purity of paracetamol, caffeine capsules working standard calculated as on as such basis and LC is the label claim of paracetamol, caffeine capsules in mg (of respective strength). For second assay test preparation (T_2) is calculated by applying same formula, the average assay results as given below (% & mg);

$$Average \ assay \ results \ in \ \% = \frac{T1 + T2}{2} \tag{2}$$

$$Average \ assay \ value \times \frac{mg}{capsules} = \frac{(\% \ of \ average \ Assay \ value \times Label \ Claim)}{(100)} \tag{3}$$

Caffeine:

$$T_2 = \frac{AT_2}{AS_2} \times \frac{WS_2}{100} \times \frac{20}{250} \times \frac{250}{WT} \times \frac{AW}{LC} \times \frac{P}{100} \times 100 \ (4)$$

Where, AT_2 is the sum of the caffeine peak areas obtained from the test preparation of paracetamol, caffeine capsules and AS_2 is the sum of the caffeine peak areas obtained from the standard preparation of paracetamol, caffeine. WS_2 is the weight of caffeine working standard taken in mg for standard preparation. WT is the weight of test sample taken in mg for test preparation. AW is the average weight of paracetamol, caffeine capsules, P is the % purity of paracetamol, caffeine capsules working standard calculated as on as such basis and LC is the label claim of paracetamol, caffeine capsules in mg (of respective strength). For second assay Test preparation (T_2) is calculated by applying same formula, the average assay results as given below (% & mg).

$$Average \ assay \ result \ in \ \% = \frac{T1 + T2}{2} \tag{5}$$

$$Average \ assay \ value \times \frac{mg}{capsules} = \frac{\% \ of \ average \ assay \ value \times Label \ Claim}{100} \tag{6}$$

Method Development

Determination of maximum wavelength for paracetamol using PDA detector

The maximum wavelength for the paracetamol and caffeine were observed at 275 nm using PDA detector in UPLC.

At 275 nm paracetamol and caffeine both were showing absorbance.

So for estimation of these combinations we have selected 275 nm for paracetamol and caffeine respectively (Figure 2).
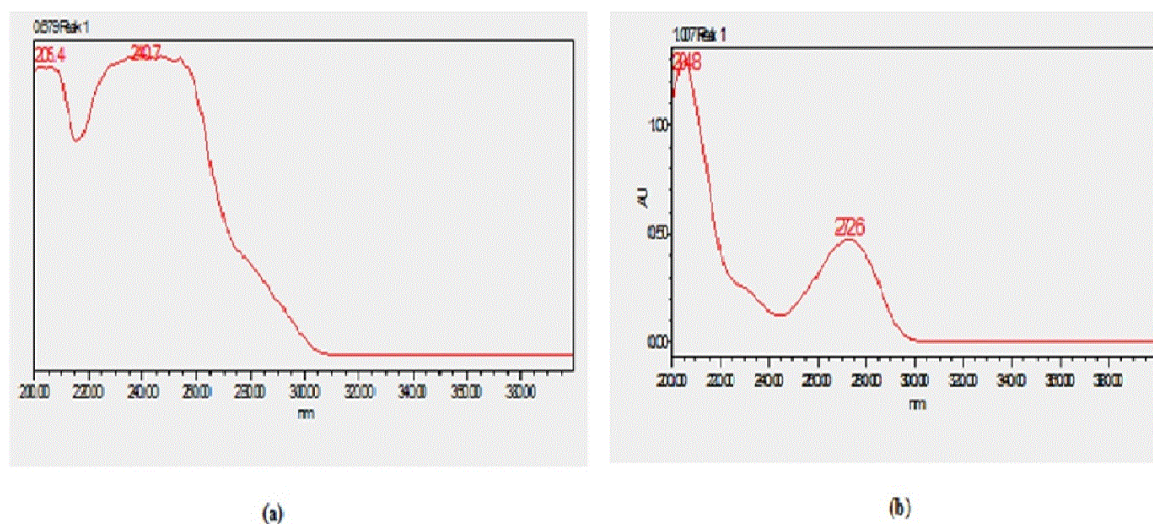

Figure 2: Spectrum for paracetamol (a) and caffeine (b) by PDA detector in UPLC.

Optimization method

Preparation of mobile phase: Mix 0.5 ml of Ortho Phosphoric Acid (OPA) and diluted to 500 ml with water (0.1% H_3PO_4), (mobile phase A) and it is filtered through 0.22 μ membrane filter. Similarly mix methanol and acetonitrile in the ratio of 50:50 (mobile phase B). Filtration is done through 0.22 μ membrane filter and both the mobile phases were sonicated for 5 min. The column temperature has to be maintained at 50°C with gradient composition of pressure 92:8.

Chromatographic condition: By Acquity UPLC (BEH C_{18} 1.7 μm, 2.1 × 50 mm) with isocratic elution and a flow rate of 0.5 ml/min, the detail analysis was performed. The column temperature was maintained at 40°C. All solvents were filtered through a 0.22 μ membrane filter before use and degassed in an ultrasonic bath. The prepared standard solutions were injected into the column. By using PDA detector quantification was measured at 275 nm. The chromatographic run time was observed in 7 min.

Observation and major findings: Resolution between paracetamol and caffeine was 10.3. In this trail paracetamol is having a good resolution from caffeine peaks. It's all passes the system suitability parameters caffeine peak shape is also good. This trail is considered as the optimized method trail (Figure 3). The observed data has been shown in Table 1.

S. No.	Name	R_t	Area	% Area	Height	USP Tailing	USP Plate count	USP Resolution
Chromatogram data for standard paracetamol and caffeine								
1	Paracetamol	0.68	2323336	74.53	1207177	1.4	2927	
2	Caffeine	1.78	785452	25.2	191242	1.4	4397.6	10.1
Chromatogram data for sample paracetamol and caffeine								
1	Paracetamol	0.67	4856755	78.55	1207177	1.1	841.1	
2	Caffeine	1.77	1326508	21.45	201785	1	1940	8.8

Table 1: Chromatogram data for standard, samples of paracetamol and caffeine.

Preparation of sample: Weigh accurately about 10 capsules and take an average weight, than transfer the capsules content in to the powder form and accurately take weight equivalent to 380 mg of capsule and transfer it in to the 250 ml volumetric flask. After that add 50 ml acetonitrile and dissolved it properly. Then add the diluent and sonicate it for 5 min until it dissolves the material and make up to the

volume with the diluent. Filter the above solution through 0.22 μm PVDF filter and then inject the clear solution in UPLC (Figure 4).

Observation and major findings: In case of sample also all the individual impurities are resolved from the main peak of paracetamol and caffeine (Figure 4). So there is a good resolution is observed between the main peaks of all the two drugs. No interferences are observed and the results are shown in Table 1. The final developed method is confirmed to be a new analytical method for the simultaneous estimation of paracetamol and caffeine capsules and this method is to be validated.
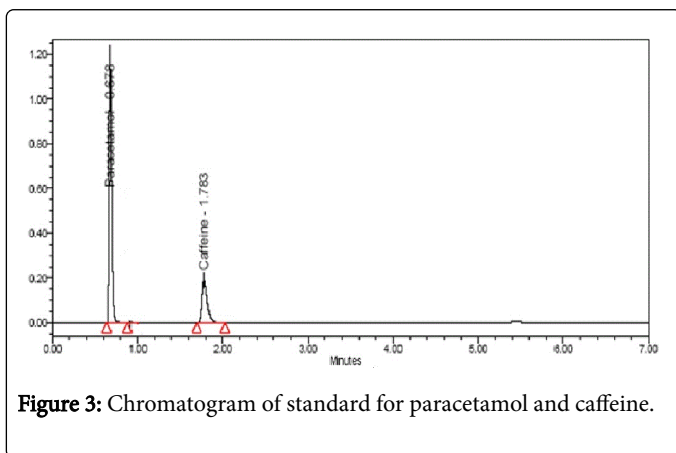

Figure 3: Chromatogram of standard for paracetamol and caffeine.

The samples in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Here at the Retention Time (Rt) of paracetamol and caffeine having no interference of the mixture of excipients peak. So no interference of mixture of excipients is observed with the retention time of paracetamol and caffeine (Figure 5).
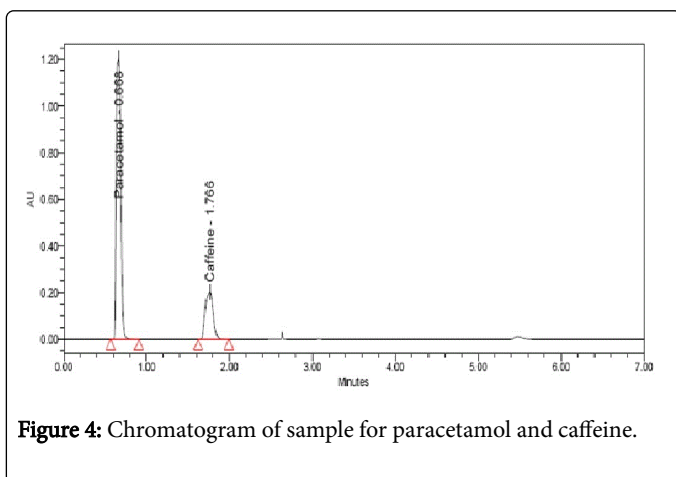

Figure 4: Chromatogram of sample for paracetamol and caffeine.

Method Validation

The method validation for the assay of paracetamol, caffeine in paracetamol and caffeine capsules (USP 325 mg, 30 mg) has been performed. This report shows and discusses the results obtained in the validation study for the parameters such as system suitability, precision, specificity, linearity, accuracy, robustness. The method of analysis was validated as per the recommendations of ICH and USP [25,26].
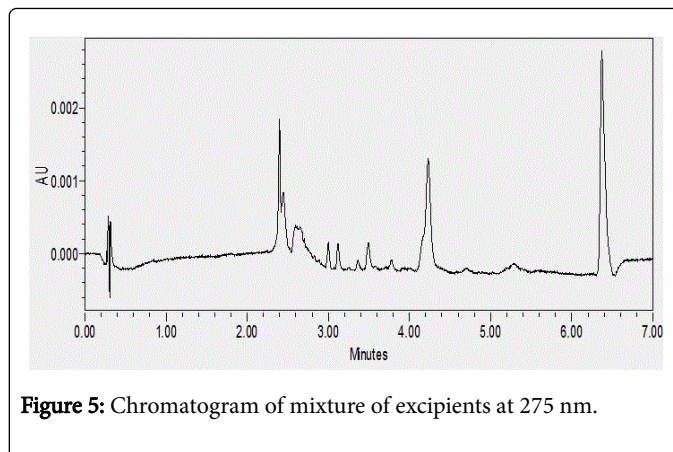

Figure 5: Chromatogram of mixture of excipients at 275 nm.

System suitability test

The sample reproducibility was observed of the chromatographic system to measurement of peak area and was carried out using three replicates of standard and sample of having same concentration.

Precision

System precision: As per the test method standard solution was prepared by using paracetamol and caffeine working standard and six replicate injections were injected into the UPLC system. The percentage relative standard deviation (% RSD) was estimated.

Method precision: As per the test procedures, the precision of test method was found out by performing six samples assay, prepared from paracetamol and caffeine capsules 325 mg and 30 mg. Relative standard deviation of assay results was calculated.

Intermediate precision: For the determination of intermediate precision of assay method, the analyst to analyst variation study was conducted. Here the same UPLC systems and same UPLC columns were used. The assay of six samples was calculated as per the test procedures. The percentage relative standard deviation (% RSD) of assay results was found out and results are reported.

Specificity

A study to establish the interference of mixture of excipients was conducted. It was determined with excipients of formulated capsules dosage form. Assay was performed on paracetamol and caffeine capsules (32 5 mg and 30 mg) mixture of excipients in duplicate, equivalent to the weight of mixture of excipient present in portion of test preparation as per the test method and the results are found out. It is concluded that the method was specific.

Linearity

Linearity was conducted by plotting a graph between concentration on X-axis and peak area on Y-axis and the correlation coefficient (R^2) value was determined. Six different concentrations of paracetamol and caffeine working standard ranging from 25%, 50%, 75%, 100%, 150% and 200% (325-2600 ppm for paracetamol and 30-240 ppm for caffeine) to were prepared and analyzed as per test method. The calibration curves were obtained by plotting peak areas versus known concentrations in μg/mL.

Accuracy

The accuracy of assay method was found out by quantifying paracetamol and caffeine content at various concentrations ranging from 650 ppm, 1300 ppm, 1950 ppm for paracetamol and 60 ppm, 120 ppm, 180 ppm for caffeine of spike level 50%, 100%, 150% of the final test concentration (i.e., paracetamol and caffeine capsules 325 mg and 30 mg respectively) by mixing of mixture of excipients and drug substance. Assay was performed in triplicate for all levels and the results are reported and percentage of recovery was calculated.

Robustness

Robustness of the method was determined to ensure its capacity to remain unaffected by small deliberate variation in the method parameters such as mobile phase ratio, column oven temperature, flow rate of mobile phase, and pH. Robustness of the method was conducted by making slight changes in the chromatographic conditions, such as change in composition of mobile phase and flow rate.

Results and Discussion

System suitability

Standard solution of paracetamol and caffeine were prepared by using paracetamol, caffeine working standard as per the test method and made six replicate injections into the UPLC system (Table 2). Various parameters such as theoretical plates, tailing factor, retention time and resolution factor, were computed as reported by ICH and USP [25,26]. The relative standard deviation (RSD) for area response obtained from six replicate injections was found to be not more than 2.0. The tailing factor was found to be 1.8, 1.6 which are well within the acceptance criteria of NMT 2.0% respectively.
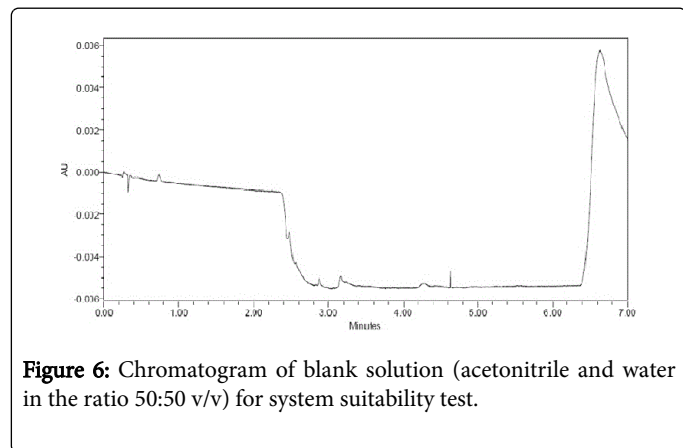

Figure 6: Chromatogram of blank solution (acetonitrile and water in the ratio 50:50 v/v) for system suitability test.

The number of theoretical plates is a measure of column efficiency which shows the high separation efficiency of the column used and was found to be 1824 and 4575.1 for paracetamol and caffeine capsules respectively which is in the acceptance criteria of NLT 1500. The typical chromatograms of blank, standard solution for system suitability test are shown in Figures 6 and 7.
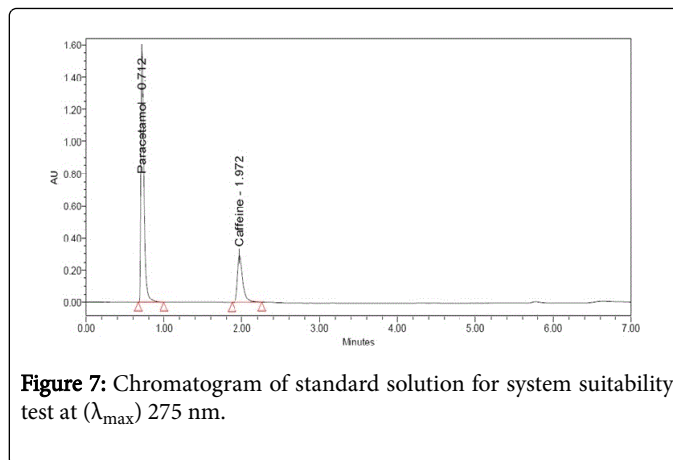

Figure 7: Chromatogram of standard solution for system suitability test at (λ_{max}) 275 nm.

It was observed from the results that the system suitability parameters meet the requirement of method validation. The results of all system suitability parameters were shown in Table 3.

S. No	Name	Injection No	R_t of paracetamol	R_t of caffeine	Paracetamol area	Caffeine area
1	Standard	1	0.703	1.942	4318243	1399718
2	Standard	2	0.704	1.94	4323621	1402161
3	Standard	3	0.705	1.941	4315551	1399509
4	Standard	4	0.706	1.94	4310359	1398194
5	Standard	5	0.706	1.941	4325271	1402422
6	Standard	6	0.705	1.939	4326283	1403116
Mean			0.705	1.941	4319888	1400853
Standard deviation			0.001	0.001	6262.996	1972.63
%RSD			0.13	0.05	0.11	0.2

Table 2: Standard injections responses for system suitability test.

Precision

System precision was carried out by using preparation of standard to ensure that the analytical solution was working properly and giving optimum, precise results. The RSD obtained from six replicate injections were well within the acceptance limit of NMT 2.0%. Method precision was carried out by the same analyst on the same day with same instrument repeatedly for six times to ensure that the method gives consistent results. The RSD was calculated for standard and sample on six determinations and was found to be in the acceptance limit of NMT 2.0%. Intermediate precision was carried out on different days in different instruments to ensure that analytical results remain unaffected with change in instrument and day. The RSD was calculated for standard and sample on six determinations and was found to be less than 2.0%. The results of system, method and intermediate precision date are reported in the Table 4.

System suitability parameters	Observed value	Acceptance criteria	R_t	Area	%Area
Tailing factor for the paracetamol and caffeine capsules is not more than 2.0 for first injection of the standard solution	1.8, 1.6	Not more than 2.0	0.712	4229520	75.62
The % Relative standard deviation of sum of peak areas of paracetamol and caffeine from six replicate injections should not be more than 2.0	0.1, 0.1	Not more than 2.0	5.768	2880560	100
The USP plate count for paracetamol and caffeine	18244575	Not less than 1500	1.972	1363933	24.38

Table 3: System suitability test parameters for paracetamol and caffeine.

Injection No	Peak response of system precision		Peak response of method precision		Peak response of intermediate precision			
					Analyst 1		Analyst 2	
	Paracetamol	Caffeine	Paracetamol	Caffeine	Paracetamol	Caffeine	Paracetamol	Caffeine
1	4318243	1399718	4197528	1389898	101.7	98.4	99.7	99.2
2	4323621	1402161	4126330	1385496	99.9	98.4	101.3	99.3
3	4315551	1399509	4194117	1392549	101.6	98.8	99.9	98.3
4	4310359	1398194	4192119	1375869	101.5	98.3	99.1	98.8
5	4325271	1402422	4130056	1375269	100	99.1	101.5	100.3
6	4326283	1403116	4118578	1398965	99.8	100	99.4	100.2
Mean	4319888	1400853	4159788	1386341	100.8	98.8	100.2	99.4
Standard deviation	0.001	1972.63	38339.99	9418.311	0.9394	0.647	1.0075	0.7817
% Relative standard deviation	0.13	0.1	0.9	0.67	0.9	0.7	1	0.8

Table 4: Standard injection of paracetamol and caffeine peak response by system, method and intermediate precision test.
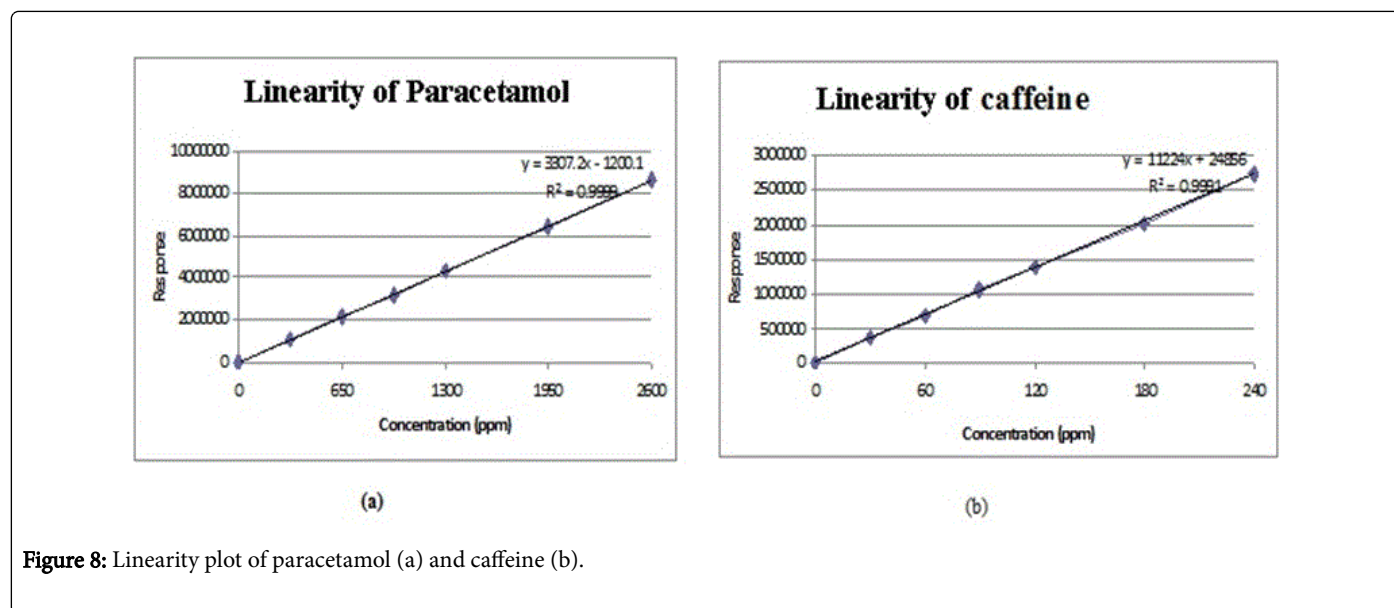
Linearity

Figure 8: Linearity plot of paracetamol (a) and caffeine (b).

This was performed to determine whether test results were proportional to the concentration of analyte in samples in a given range. It was determined using standard solutions of paracetamol and caffeine. The response was found to be linear over a concentration range of (25-200%) 325-2600 ppm for paracetamol and 30-240 ppm for caffeine. The correlation coefficient was found to be 0.999942 within the acceptance criteria limit of NLT 0.999. The results are mentioned in Table 5 and Figure 8.

Level	Stock weight taken (mg)		Area response	
	Paracetamol 325 mg Concentration (ppm)	Caffeine 30 mg Concentration (ppm)	Paracetamol	Caffeine
25%	325	30	1087658	358436
50%	650	60	2154992	690650
75%	975	90	3186906	1081396
100%	1300	120	4318243	1399798
150%	1950	180	6425282	2006893
200%	2600	240	8614658	2718438
Slope			Y = 03307 2x-1200.1	Y=1124x+24856
Correlation Coefficient (R^2)			0.9999	0.9991

Table 5: Response of paracetamol and caffeine at various linearity levels.

Specificity

Interference from mixture of excipients: Specificity of the method was carried out and the peaks of diluent, excipients of capsules, mobile phase did not interfere with the peaks of two drugs. A study to establish the interference from mixture of excipients was conducted. The specificity of the method was assessed by comparing chromatograms obtained from drug standard with that obtained from capsule solutions. The R_t of drug standard and the drug from sample solution were same, so the method was specific. The method was also specific and selective because there was no interference of impurities with paracetamol and caffeine peaks. Thus, the method indicated a high degree of specificity (Figure 5).

Accuracy

Accuracy of the method was performed to ensure closeness of agreement between true value and reference value in three levels each. The mean % recoveries at concentrations ranging from (spike level) 50%, 100%, 150% were found to be 99.2 to 101.7 which were in the acceptance limit of 98.0 to 102.0% and the RSD was within the limit of NMT 2.0%. The results are shown in Table 6.

Sample No.	Spike Level (%)	Area	"Mg added" paracetamol	"Mg found" paracetamol	%Recovery paracetamol	Mean % Recovery paracetamol	Area	"Mg added" caffeine	"Mg found" caffeine	% Recovery caffeine	Mean % Recovery caffeine
1	50	2137864	162.7	161.9	99.51		698893	15.1	14.9	100.08	
2	50	2118864	162.7	161.2	99.3	99.4	696984	14.8	14.9	99.8	99.65
3	50	2139562	162.8	161.7	99.5		692092	15.3	15.2	99.07	
4	100	4279243	325.3	325.8	100.2		1378789	30.2	30.1	100.52	
5	100	4329481	325.1	324.9	99.4	99.8	1369843	30.4	30.2	99.85	99.8
6	100	4293364	324.9	323.1	99.8		1366962	29.9	30	99.64	
7	150	6468797	487.3	486.5	99.7		2048762	45.2	45.1	100.17	
8	150	6387653	487.2	485.9	100.1	99.9	2027764	45.4	45.2	99.13	99.9
9	150	6401765	487.6	488	99.29		2036587	44.8	44.9	99.57	

Table 6: Percentage recovery of paracetamol and caffeine at different spike level.

Robustness

The system suitability parameters obtained from normal experimental conditions and that obtained from small and deliberately changed conditions for each of the system suitability parameters like RSD, tailing factor and theoretical plates passes for all the conditions. The results are reported in the Table 7.

Parameter	Flow Rate (+10%)	Flow Rate (-10%)	Column Temp. (+50°C)	Column Temp. (-50°C)	Wave length (+5 nm)	Wave length (-5 nm)	Flow Rate (+10%)	Flow Rate (-10%)	Column Temp. (+50°C)	Column Temp. (-50°C)	Wave length (+5nm)	Wave length (-5nm)
Peak Area (Inj.1)	4135056	4081671	4181519	4138536	4228698	4167561	1361581	1308261	1371621	1368267	1264894	1218894
Peak Area (Inj.2)	4160862	4060719	4172961	4151795	4209276	4149417	1359492	1311772	1374192	1366193	1270454	1213738
Peak Area (Inj.3)	4092617	4032781	4190178	4092578	4212851	4157158	1357235	1318784	1370784	1360173	1266282	1215932
Peak Area (Inj.4)	4151680	4050917	4157287	4117953	4201968	4098171	1369836	1309213	1366245	1358226	1273939	1213498
Peak Area (Inj.5)	4168076	4020761	4196151	4141395	4231317	4128643	1358737	1313287	1367279	1359239	1268384	1208584
Avg. Area	4141658	4049370	4179419	4128451	4216822	4140190	1361376	1312263	1370024	1362419	1268790	1214129
SD	30065.6	23812.9	15375.1	23512.1	12693.5	27482.5	4981.84	4157.27	3252.54	4505	3567.73	3783.32
% RSD	0.725	0.588	0.367	0.569	0.301	0.663	0.365	0.316	0.237	0.33	0.281	0.311

Table 7: Robustness data of paracetamol and caffeine.

Stability of analytical solution

This parameter was determined by injecting sample and standard solutions at regular intervals. The standard and sample solutions were found to be stable up to 48 h. The proposed method was validated and met the requirements as per the ICH guidelines. Hence, the method can be conveniently adopted for the routine analysis in quality control laboratories.

Conclusion

The present investigation represents a rapid, convenient, accurate and precise UPLC method was developed for the simultaneous estimation of paracetamol and caffeine in pharmaceutical formulation. The assay provides a linear response across a wide range of concentrations. This method can be said to be more economical as compared to other methods. The method is suitable for the determination of these drugs in capsules, and hence can be used for routine quality control of paracetamol and caffeine in this dosage form. From the results of linearity it was found that these drugs obey linearity within the level of 25-200 % for paracetamol and caffeine. From the results of precision it was found that % RSD is less than 2%; which indicates that the proposed methods have good reproducibility. From the results of accuracy, it was found that the percentage recovery values of pure drug from the pre-analyzed solutions of formulations were in between 99.65-102%, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the formulations were not interfering in the proposed method. The method was validated by using various validation parameters like system suitability, precision, specificity, linearity, accuracy, robustness, stability in analytical solution. All the validation parameters were found to be well within the acceptance criteria. It is shown that the method was accurate, reproducible, repeatable, linear, precise, and selective, proving the reliability of the method. The run time is 7 min which enables proper separation and resolution between the peaks and quality control analysis of capsule formulations. The same solvent used throughout the experimental work and no interference from any excipient was observed. The proposed method is simple, economical, accurate, and precise and could be successfully employed in routine quality control for the simultaneous analysis of paracetamol and caffeine in pharmaceutical formulations.

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