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Method Development and Validation for Simultaneous Determination a Multiple Drug Dosage Form of Paracetamol, Phenylepherine and Caffeine by RP-HPLC

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A B S T R A C T

This method describes a procedure to quantify the assay of Paracetamol, Phenylepherine and caffeine tablet using a mobile phase containing containing mixture of 880 mL of sodium perchlorate buffer, 120 mL of acetonitrile and degassed for about 5 minutes in a sonicator. Paracetamol, Phenylepherine and caffeine is subsequently analyzed by reverse phased HPLC using Phenomenex, Kinetex XB- C18 (100x4.6 mm i.d. 2.6 µm), column. The retention times of Paracetamol, Phenylepherine and caffeine are about 1.2, 1.5, and 2.3 minutes respectively. Quantitative linearity was observed over the concentration range of 81.25 to 487.50µg/mL for Paracetamol 1.25 to 7.5 µg/mL for Phenylepherine and 16.5 to 97.5 µg/mL for Caffeine. The regression equations of concentration of Paracetamol, Phenylepherine and Caffeine are found to be y=7840.x + 32320, y=12582x + 401.8, y=18502x+ 22155 respectively, where y is the peak area and x is the concentrations of drugs (µg/mL). The numbers of theoretical plates obtained were 10123, 5695 and 14288 for Paracetamol, Phenylepherine and Caffeine respectively, which indicates the efficiency of the column.

Keywords: Paracetamol, Phenylepherine and Caffeine, RP-HPLC

A R T I C L E I N F O

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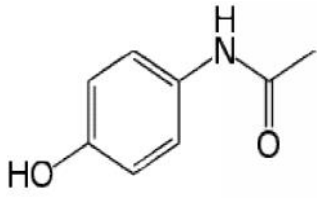
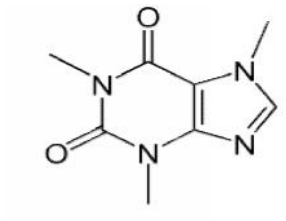
1. Introduction

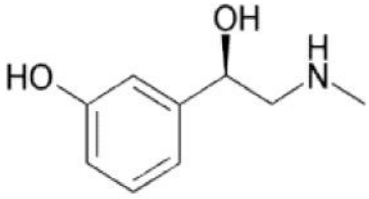
This combination of drugs was found to be more effective in relieving mild to moderate pain from certain muscle problems. It may also be used for other conditions as determined by your doctor. Paracetamol, Phenylepherine and caffeineis a muscle relaxant, salicylate and stimulant combination. It works by decreasing pain and inflammation, which helps muscles to relax. This HPLC method determines assay of Paracetamol, Phenylepherine and caffeine tablet formulation. This method describes a procedure to quantify the assay of Paracetamol, Phenylepherine and caffeine tablet using a mobile phase containing mixture of 880 mL of sodium perchlorate buffer, 120 mL of acetonitrile and degassed for about 5 minutes in a sonicator. Paracetamol, Phenylepherine and caffeine is subsequently analyzed by reverse phased HPLC using Phenomenex, Kinetex XB- C18 (100x4.6 mm i.d. 2.6 µm), column. Present literature survey shows that there are no methods published exclusively for the combination of these drugs in bulk or formulation. Extensive literature search was done for the methods on UV- Visible spectroscopy, HPLC, LCMS/MS, TLC, & GC. Based on the methods available in single or in combination with other drugs, the chromatographic conditions are optimized and method was developed and validated. The structures of the drugs chosen for the research.



Paracetamol



Caffeine



Phenylephrine

2. Materials and Methods

Reagents and chemicals:

The active pharmaceutical ingredient of Paracetamol, Caffeine and Phenylephrine, were obtained as gift sample from AIZANT Drug Research pvt.Ltd, all solvent and reagent used were of HPLC and spectroscopic grade. HPLC grade, Acetonitrile, Millipore water obtained from (Milli Q) was used in all experiments, Hydrochloric acid used is of AR Grade, sodium perchlorate monohydrate and perchloric acid used is of GR grade

Instrumentation parameters:

The chromatographic separation performed using Agilent HPLC system with PDA detector, Model:1200 series. Software was used for monitored and integrate the outputsingle at wavelength 280 nm. Sample injection was done with a Rheodye 7725 injection valve viaa 20µL loop.

Analytical balance used is electronic semi microbalance, (accuracy: ± 0.01mg) Sartorious make (model ME235P). Drug separation achieved at room temperature with Phenomenex, Kinetex Xb-C18 (100x4.6mm), i.d 2.6µm. was used for method development.

Preparation of Sodium Perchlorate Buffer:

Dissolved 1.4 g of sodium perchlorate was dissolved in 1000 mL of water and adjusted the resulting pH of the solution to 3.0 with perchloric acid. Filtered through 0.45µ nylon membrane filter.

Preparation of Mobile Phase:

880 mL of sodium perchlorate buffer, 120 mL of acetonitrile was mixed and degassed for about 5 minutes in a sonicator.

Preparation of Diluents / Blank (0.1 N HCl):

Pipetted out and transferred 8.5 mL of concentrated HCl and transferred into 1000 mL volumetric flask and diluted with water, mixed thoroughly.

Preparation Standard Solutions:

Weighed accurately and transferred 325mg of Paracetamol, 65 mg of Caffeine and 5mg of Phenylepherine working standards into 100mL volumetric flask. Added about 30mL of diluents and sonicated to dissolve. Diluted upto the mark with the diluent. Pipette out 5 mL of above solution, into 50 mL of volumetric flask and madeup the volume with diluent and mixed well. The standard preparation is performed in duplicate, one standard is designated as the calibration standard and the other is designated as the check standard. The final concentration for Phenylepherine HCl, Paracetamol and Caffeine is approximately 0.005 mg/mL, 0.325 mg/mL and 0.065 mg/mL respectively.

Sample preparation:

Weighed 5 tablets and noted the reading, Placed 5 tablets into1000 mL of volumetric flask and added about 700mL of diluents and sonicated for about 30 minutes with intermittent shaking. Taken out the flask and madeup the volume with diluents and mixed well. Pipetted out 5mL of the supernatant solution into 25 mL of volumetric flask and madeup the volume with diluents and mixed well. Filtered a portion of solution through 0.45 µm Nylon membrane filter and injected into a HPLC.

Optimization of the chromatographic conditions:

The initial literature search indicated that many HPLC methods are available forindividual drugs. Based on literature search, attempts were made to develop a simple methodwhich has less retention time and higher

selectivity, top priority was given for completeseparation ofparacetamol, caffeine and phenylepherine. These are hydrophilic, almost soluble in aqueous solution and freely soluble in methanol. Severalmobile phases were tested until good resolution was obtained between three drugs. In preliminary experiments, all the three drugs paracetamol, caffeine and phenylepherine, were subjected to separation by reversephase HPLC equipped with Phenomenex, Kinetex XB- C18 (100x4.6 mm i.d. 2.6 µm), column with a flowrate of 1.2 mL/min, and sample detection was done at a wavelength of 280 nm. Column temperature was maintained at 30°C. Injection volume was 10 µL, and run time was for 8 min.Mobile phase containing sodium perchlorate buffer pH 3 buffer (dissolve about 1.4g of sodium perchlorate in 1000 mL of water and adjust the resulting pH of the solution to 3.0 with perchloric acid. Filter through 0.45µ Nylon membrane filter)and acetonitrile in the ratio of (88:12 v/v) were passed through 0.45µm nylon membrane filter and degassed.The retention times of Phenylephrine, Paracetamol and Caffeine peaks are about 1.2, 1.5, and 2.3 minutes respectively.

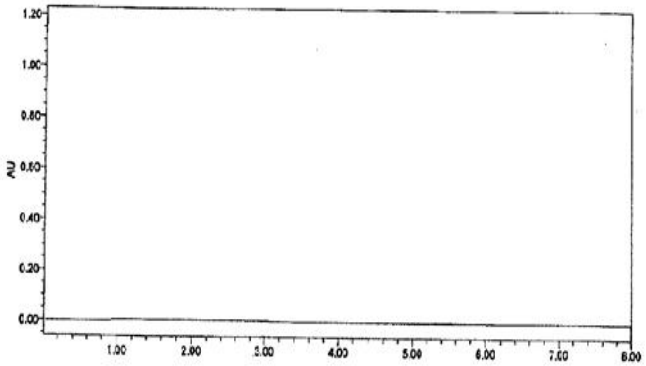


Figure 1: Blank chromatogram of Phenylephrine, Paracetmol and caffeine

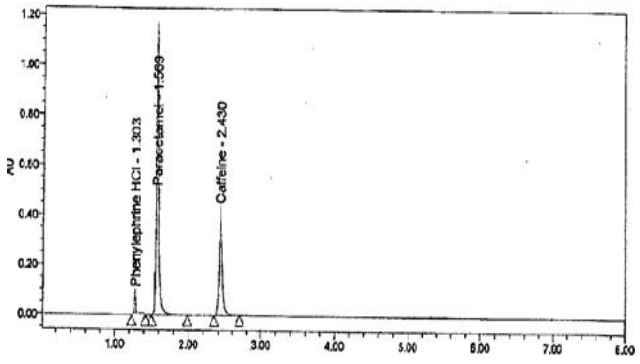


Figure 2: Standard chromatogram of Phenylephrine, Paracetmol and Caffeine

Validation:

The method was successfully validated as per ICH guideline Q2 (R1): validation of analyticalprocedures: text and methodology, international conference on harmonization, Food and DrugAdministration, USA, November 2005. The method was validated and parameters were linearity,range, accuracy, precision, LOQ, LOD.

Linearity and Range:

The Linearity of detector response to different concentration of all the three drugs was studied with a series of working standard solutions prepared by diluting the stock solution with mobile phase. Thestandard plots were constructed between concentration vs. peak area a linear response of peak area was observed over the concentration range of 81.25 to 487.50µg/mL for Paracetamol 1.25 to 7.5 µg/mL for Phenylepherine and 16.5 to 97.5 µg/mL for Caffeine. 10 microlitre of each sample was injected under above chromatographic conditions and peak area was measured. Keeping the values to the straight line equation of calibration curve, quantification was carried, the data of linearity curve was summarized in thetable:1 and figure 3,4 and 5and it was found that correlation coefficient (R^2) and regression analysis were within the limits.

Table 1: Correlation coefficient values

Drug	Conc.Range(µg/mL)	Equation	R^2
Phenylephrine	1.25 - 7.5	Y=12582x+ 401.8	0.999
Paracetamol	81.25-487.50	Y=7840.x+ 32320	0.999
Caffeine	16.5 -97.5	Y=18502x+ 2155	0.999

Table 2: Linearity data of Paracetamol

S.No.	Concentration(µg/ml)	Peak area
1	81.25	649891
2	162.50	1319586
3	243.75	1946261
4	325.00	2608569
5	406.25	3198560
6	487.50	3849541

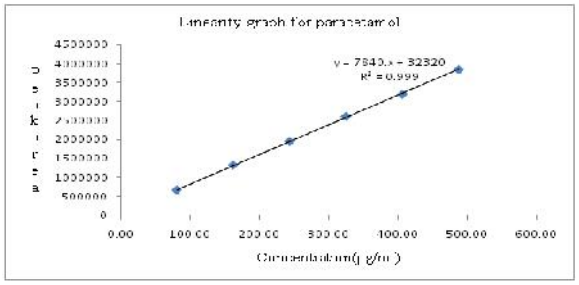


Figure 3: Linearity graph of Paracetamol

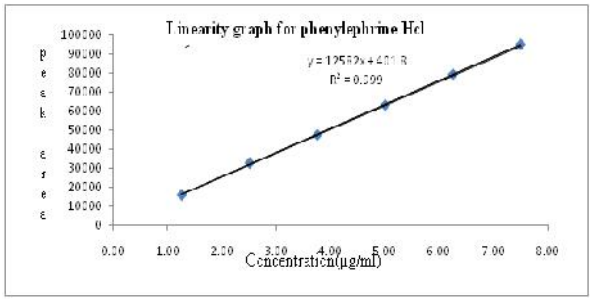


Figure 4: Linearity graph of phenylephrine

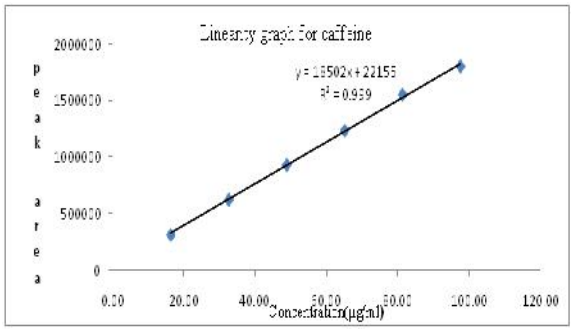


Figure 5: Linearity graph of caffeine

Table 5: LOQ, LOD Values

Drugs	LOD µg/ml	LOQ µg/ml
Paracetamol	0.66	2.2
Phenyephrine	0.8	2.6
Caffiene	1	3.3

Precision

According to ICH guidelines repeatability should be assessed by using a minimum of nine determinations covering the specified range for the procedures (i.e., three concentrations and three replicates of each concentration) precision was studied to find out intra and interday variations of the proposed method at three different levels (50, 100 and 150% or 80, 100,120%) 162.5, 325 and 487.5 µg/mL for Paracetamol, 2.5, 5.0 and 7.5 µg/mL for Phenylephrine and 32.5, 65 and 97.5 µg/mL for Caffeine. On the same and on three different days respectively. The results were interpreted by statistical analysis by calculating % RSD values and all the results were within the acceptance criteria of not more than 2 % and the results are tabulated in the tables:6,7,8,9,10 and 11.The % RSD values for intraday and interday were <2%, indicating that the method was sufficiently precise.

Table 3: Linearity data of Phenylephrine

S.No	Concentration(µg/ml)	Peak area
1	1.25	15821
2	2.50	32599
3	3.75	47319
4	5.00	62941
5	6.25	79151
6	7.50	94858

Table 4: Linearity data of caffeine

S.No.	Concentration(µg/ml)	Peak area
1	16.25	311632
2	32.50	623241
3	48.75	929652
4	65.00	1232562
5	81.25	1549862
6	97.50	1799626

Limit of detection and limit of quantification:

A study to establish the limit of detection and limit of quantification was conducted. Limit of detection and limit and quantification were established based on signal to noise ratio. A series of dilutions of the test solution were injected. Limit of detection was established by identifying the concentration which gives signal to noise ratio of about 3. Limit of quantification was established by identifying the concentration which gives signal to noise ratio of about 10.The results of the LOQ and LOD are given in the table no.5

Table 6: Intraday precision of Paracetamol

S.No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	162.16	99.3	Mean=99.633
2	163.28	99.5	SD=0.416
3	162.65	100.1	%RSD=0.418
4	325.21	99.6	Mean=99.733
5	326.63	99.7	SD=0.153
6	326.69	99.9	%RSD=0.153
7	487.42	100.3	Mean=100.300
8	488.76	100.8	SD=0.500
9	487.55	99.8	%RSD=0.499

Table 7: Interday precision of paracetamol

S.No	Concentration(µg/ml)	% Assay	Statistical parameters
1	162.32	100.6	Mean=100.033
2	162.71	99.9	SD=0.495
3	162.56	99.6	%RSD=0.495
4	325.25	99.6	Mean=100.200
5	325.86	100.2	SD=0.346
6	325.31	100.8	%RSD=0.346
7	487.22	100.4	Mean=100.200
8	487.52	99.6	SD=0.611
9	487.21	100.6	%RSD=0.610

Table 8: Intraday precision of Phenylephrine

S.No.	Concentration(µg/ml)	% Assay	Statistical parameters
1	2.49	100.6	Mean=100.333
2	2.47	100.9	SD=0.737
3	2.52	99.5	%RSD=0.735
4	5.32	99.8	Mean=100.300
5	5.15	100.3	SD=0.500
6	5.32	100.8	%RSD=0.499
7	7.49	100.6	Mean=99.833
8	7.56	99.6	SD=0.681
9	7.63	99.3	%RSD=0.682

Table 9: Interday precision of Phenylephrine

S.No.	Concentration(µg/ml)	% Assay	Statistical parameters
1	2.52	99.5	Mean=99.433
2	2.56	99.6	SD=0.071
3	2.48	99.2	%RSD=0.071
4	5.26	100.6	Mean=100.233
5	5.23	100.2	SD=0.721
6	5.19	99.9	%RSD=0.719
7	7.52	99.6	Mean=100.000
8	7.49	100.6	SD=0.513
9	7.61	99.8	%RSD=0.513

Table 10: Intraday precision of caffeine

S.No	Concentration(µg/ml)	% Assay	Statistical parameters
1	31.92	99.8	Mean=99.933
2	32.62	100.6	SD=0.611
3	33.31	99.4	%RSD=0.611
4	64.96	99.4	Mean=100.067
5	65.15	100.6	SD=0.611
6	66.01	100.2	%RSD=0.611
7	96.93	99.7	Mean=100.033
8	97.15	99.6	SD=0.666
9	96.48	100.8	%RSD=0.666

Table 11: Interday precision of caffeine

S.No	Concentration(µg/ml)	% Assay	Statistical parameters
1	32.78	99.2	Mean=99.733
2	31.93	99.8	SD=0.424
3	33.02	100.2	%RSD=0.425
4	64.56	100.8	Mean=100.300
5	65.35	100.5	SD=0.300
6	65.49	99.6	%RSD=0.299
7	96.58	99.6	Mean=99.667
8	97.21	99.3	SD=0.173
9	96.36	100.1	%RSD=0.174

Accuracy:

Accuracy for Paracetamol, Phenylephrine and Caffeine was conducted by spiking these three drugs to the placebo powder at three different levels of the target concentration (i.e. 50%, 100%, and 150%) and each level three times. The mean %Recovery and %RSD values were calculated. The %Recovery values for all the three drugs were found to be in between 98.0% to 102.0% and %RSD values were found to be less than 2.0%. The accuracy results were tabulated in the tables12,13 and 14

Table 12: Recovery studies of Paracetamol

S.No	%Spike level	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	50	162.21	161.13	99.334	Mean=100.220
2	50	163.32	164.32	100.612	SD=0.768
3	50	162.82	163.98	100.712	%RSD=0.767
4	100	326.26	326.59	100.101	Mean=99.920
5	100	325.92	324.29	99.500	SD=0.365
6	100	326.69	327.21	100.159	%RSD=0.365
7	150	488.36	487.98	99.922	Mean=100.090
8	150	486.32	486.98	100.136	SD=0.150
9	150	486.29	487.32	100.212	%RSD=0.150

Table 13: Recovery studies of Phenylephrine HCL

S.No	Amount added(µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	2.56	2.57	100.391	Mean=100.140
2	2.58	2.56	99.225	SD=0.819
3	2.49	2.51	100.803	%RSD=0.817
4	5.21	5.18	99.424	Mean=100.131
5	5.18	5.21	100.579	SD-0.619
6	5.14	5.16	100.389	%RSD=0.618
7	7.56	7.52	99.471	Mean=99.335
8	7.53	7.49	99.469	SD=0.233
9	7.49	7.42	99.065	%RSD=0.235

Table 14: Recovery studies of Caffeine

S.No	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	32.56	32.49	99.785	Mean=99.926
2	32.19	32.43	100.746	SD=0.759
3	33.21	32.96	99.247	%RSD=0.760
4	65.21	64.32	98.635	Mean=99.065
5	65.35	65.39	100.061	SD=0.866
6	65.23	64.25	98.498	%RSD=0.874
7	96.65	95.65	98.965	Mean=99.227
8	97.41	96.56	99.127	SD=0.324
9	97.36	96.96	99.589	%RSD=0.326

System suitability parameters

According to USP system suitability tests are an integral part of chromatographic method validation. The tests were used to verify that the reproducibility of the chromatographic system is adequate for analysis. To ascertain its effectiveness system suitability tests were carried out on freshly prepared standard stock solution containing 325 µg/mL for paracetamol, 5.0 µg/mL for Phenylephrine and 65.0 µg/mL for caffeine. 10µL of solution was injected into the optimized chromatographic system. For system suitability 6 replicates of working standard samples were injected and the parameters like retention time (RT), plate number(N), peak area and peak asymmetry of sample were calculated these results are presented in the tables15,16 and 17.

Table 15: System suitability of Paracetamol

S.No.	Retention time	Peak area	Tailing Factor	USP plate count
1	1.569	2609703	1.3	10520
2	1.568	2598636	1.3	10490
3	1.567	2589635	1.29	9456
4	1.567	2608695	1.29	10123
5	1.566	2612365	1.29	10856
6	1.568	2613659	1.3	10230
Mean	**1.568**	**2605449**	**-**	–
SD	**0.001**	**9384.751**	**-**	-
%RSD	**0.067**	**0.360**	**-**	-

Table 16: System suitability of Phenylephrine

S.No	Retention time	Peak area	Tailing Factor	USP plate count
1	1.289	63148	1.37	5896
2	1.276	62980	1.37	5695
3	1.28	62715	1.37	5689
4	1.279	62521	1.36	5598
5	1.268	62256	1.37	5579
6	1.289	63823	1.37	5586
Mean	**1.280**	**62907**	**-**	–
SD	**0.008**	**549.932**	**-**	-
%RSD	**0.628**	**0.874**	**-**	-

Table 17: System suitability of caffeine

S.No	Retention time	Peak area	Tailing Factor	USP plate count
1	2.433	1243814	1.22	14288
2	2.432	1229856	1.23	14208
3	2.429	1223695	1.22	13695
4	2.412	1229865	1.22	14236
5	2.413	1214569	1.22	13989
6	2.428	1239369	1.22	12369
Mean	**2.425**	**1230195**	**-**	–
SD	**0.009**	**10547.845**	**-**	-
%RSD	**0.391**	**0.857**	**-**	-

3. Results and Discussion

To optimize the mobile phase, various proportions of buffers with methanol were tested. Mobile phase composition was changed and the method development was started withreversephase HPLC equipped with Phenomenex, Kinetex XB- C18 (100x4.6 mm i.d. 2.6 µm), column with a flow rate of 1.2 mL/min, and sample detection was done at a wavelength of 280 nm. Column temperature was maintained at 30^{o}C. Injection volume was 10 µL, and run time was for 8 min.Mobile phase containing sodium perchlorate buffer pH 3 bufferand acetonitrile in the ratio of (88:12 v/v). The retention times of Phenylepherine, Paracetamol and Caffeine peaks are about 1.2, 1.5, and 2.3 minutes respectively. Quantitative linearity was observed over the concentration range of 81.25 to 487.50µg/mL for Paracetamol 1.25 to 7.5 µg/mL for Phenylepherine and 16.5 to 97.5 µg/mL for Caffeine The regression equations of concentration of Paracetamol, Phenylepherine and Caffeine are found to be y=7840.x + 32320, y=12582x + 401.8, y=18502x+ 22155respectively, where y is the peak area and x is the concentrations of drugs (µg/mL). The numbers of theoretical plates obtained were 10123, 5695 and 14288 for Paracetamol, Phenylepherine and Caffeine respectively, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 0.66, 2.2 and 0.8, 2.6 and 1, 3.3 µg/mL for Paracetamol, Phenylepherine and Caffeine respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

4. Conclusion

A simple, specific, accurate, precise, stability indicating reverse phase high performance liquid chromatography (RP-HPLC) method has been developed which can be used for accuratequantitative estimation of Paracetamol, Caffeine and Phenylepherine for routine analysis of individual andcombination of drugs. Method was validated as per ICH Q2 (R2) so it can be used by analytical department

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