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METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS DETERMINATION A MULTIPLE DRUG DOSAGE FORM OF PARACETAMOL, ORPHENEDRINE, IBUPROFEN BY RP-HPLC

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ABSTRACT

This method describes a procedure to quantify the assay of Paracetmol, Orphenedrine Citrate and Ibuprofen tablet using a mobile phase containing mixture of orthophosphoric acid, water and acetonitrile in gradient mode. Paracetamol, Orphenedrine citrate and ibuprofen is subsequently analyzed by reverse phased HPLC using Zorbax SB-C18 (150x4.6 mm, 3.5 µm) column. The retention times of Paracetamol, Orphenadrine and Ibuprofen peaks are about 3.29, 11.29 and 21.46 minutes respectively. Quantitative linearity was observed over the concentration range of112.65 to 675.90 µg/mL for Paracetamol 8.788 to 52.725µg/mL for Orphenadrine and 100.15 to 600.90 µg/mL for Ibuprofen The regression equations of concentration of Paracetamol, Orphenadrine and Ibuprofen are found to be y = 7302.x - 10898, y = 3446.x + 782.2, y = 6249.x - 45282 respectively, where y is the peak area and x is the concentrations of drugs (µg/mL). The numbers of theoretical plates obtained were 8656, 70036 and 64826 for Paracetamol, Orphenadrine and Ibuprofen respectively

KEY WORDS: Multiple drug dosage form, RP-HPLC

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. Orphenadrine/aspirin/caffeine is 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, Orphenedrine citrate and Ibuprofen tablet formulation. This method describes a procedure to quantify the assay of Paracetmol, Orphenedrine Citrate and Ibuprofen tablet using a mobile phase containing mixture of orthophosphoric acid, water and acetonitrile in gradient mode. Paracetamol, Orphenedrine citrate and ibuprofen is subsequently analyzed by reverse phased HPLC using Zorbax SB-C18 (150x4.6 mm, 3.5 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.

MATERIALS AND METHODS

Reagents & Chemical:

The Active pharmaceutical ingredient of Paracetamol, Orphenadrine and Ibuprofen, 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, orthophosphoric acid (88%) of GR grade are used. Paracetamol, Ibuprofen, Orphenadrine citrate are used as working or reference standard.

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Instrument parameters:

The chromatographic separation performed using Agilent HPLC system with DAD detector, Model, 1200 series. Software was used to monitor and integrate the output single at wavelength 280 nm. Sample injection was done with a Rheodyne 7725 injection valve via a 20 µL loop. Analytical balance used is electronic semi microbalance, (accuracy: ± 0.01mg) Sartorious make (model ME235P). Drug separation achieved at room temperature, Zorbax SB-C187 (150x4.6 mm, 3.5µm) column was used for method development. The method was conducted using an isocratic reverse phase technique. Eppendorf; model no: 5810 centrifuge was used.

Preparation of mobile phases:

Mobile phase –A: 1 mL of Orthophosphoric acid (88%) was transferred into 1000mL of water. Mixed well and filtered and degassed through 0.45 µm membrane filter.

Mobile phase-B: Acetonitrile- 100%

Diluents preparation:

Mobile phase- A was mixed with mobile phase –B in (50:50) % V/V and sonicated for about 5minutes.

Preparation of standard stock solution – I:

About 90 mg of paracetamol working standard or reference standard was weighed and transferred into a 20 mL volumetric flask, 15 mL of diluent was added and sonicated for 3 minutes to dissolve the material completely and made up the volume with diluents and mixed well.

Preparation of standard stock solution- II:

35mg of Orphenadrine working standard or reference standard was weighed and transferred into a 100 mL volumetric flask, 70 mL of diluent was added, and sonicated for 3 minutes to dissolve the material completely and made up the volume with diluents and mixed well.

Preparation of standard stock solution –III

80mg of ibuprofen working standard or reference standard was weighed and transferred into a 20 mL volumetric flask, 15 mL of diluent was added and sonicated for 3 minutes to dissolve the material completely and made up the volume with diluents and mixed well.

Preparation of standard solution for 450/35/200mg strength: 2 mL of standard stock solution –I, II & III were transferred in to 20 mL volumetric flask and diluted up to the volume with diluent.

Test preparation of 450/35/200 mg strength:

Weighed and crushed 10 tablets to a fine powder in a mortar with pestle. Weighed and transferred tablet powder about 5050 mg(equivalent to 2250 mg of Paracetamol, and 175 mg of Orphenadrine citrate and 1000 mg of Ibuprofen) into a 1000 mL individual volumetric flask, add about 800 mL of diluents and sonicated for 30 min with intermittent shaking and dilute with diluents to volume and mix well. Take a portion of sample from the above solution and filter through 0.45µ nylon membrane filter. Take 5 mL of filtrate into 25 mL volumetric flask and dilute to volume with diluent.

Optimization of the chromatographic conditions:

The initial literature search indicated that many HPLC methods are available for individual drugs and in combination with different drugs. Based on literature search, attempts were made to develop a simple method which has less retention time and higher selectivity, top priority was given for complete separation of Paracetamol, Orphenadrine and ibuprofen. Paracetamol, Orphenadrine and Ibuprofen are hydrophilic almost soluble in aqueous solution and freely soluble in methanol. Several mobile phases were tested until good resolution obtained between two drugs.

In preliminary experiments, all the three Paracetamol, Orphenadrine and Ibuprofen were subjected to separation by reverse phase HPLC equipped with the, Zorbax SB-C187(150x4.6 mm, 3.5 µm) column and with a flow rate of 1.5 mL/min, and detection wavelength of 225 nm. Column temperature was maintained at 40^{o}C. Injection volume is 5 µL, and run time is for 30 min.

The mobile phase consists of Orthophosphoric acid 20mM buffer and methanol as Organic base modifiers. These drugs were able to be separated on the chromatogram but peak shape was not good for Paracetamol and Ibuprofen, tailing was seen more than 2.

The effect of pH (6, 5.5 4 and 3.0) and mobile phase composition was also checked. It improved peak shape at pH 3.0 slight extent but the number of theoretical plates is very less. Acetonitrile was found to be better than methanol in terms of resolution and peak shapes. Initially trials with mixture of methanol acetonitrile shown good peak shape with theoretical plates but Paracetamol got merged with solvent peak. Resolution was also not good. Then only acetonitrile was used. The chromatogram obtained was improved. For reducing tailing triethylamine was used at the conc 0.1% but no improvement in tailing factor then 0.2% was used. Finally a method was developed with orthophosphoric acid buffer and acetonitrile by gradient program given in the table 1. The chromatogram obtained was better than the previous one in all aspects with good peak shape, tailing factor, resolution and theoretical plate as per USP requirement. The retention times of Paracetamol, Orphenadrine and Ibuprofen peaks are about 3.29, 11.29 and 21.46 minutes respectively. The chromatograms were shown in the figures 1 & 2.

Table 1: Gradient program:

Time (minutes)	Mobile phase- A (% v/v)	Mobile phase –B (% v/v)
0	95	5
10	66	34
15	55	45
20	55	45
25	30	70
30	30	70
31	95	5
35	95	5

Figure 1: A Typical blank chromatogram of Paracetamol, Orphenadrine and ibuprofen:

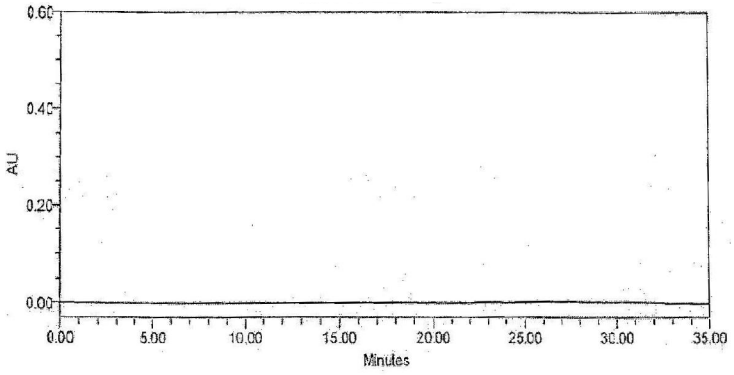
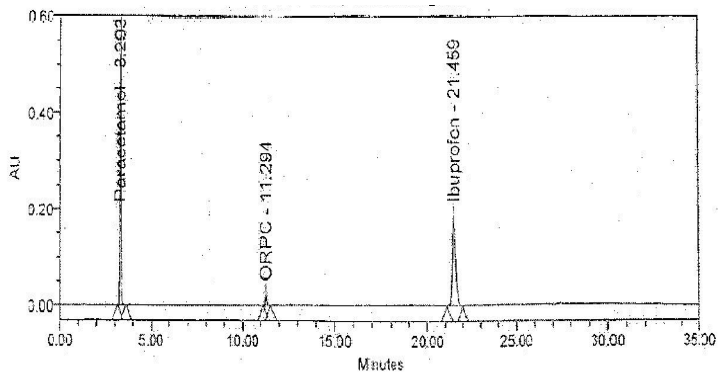


Figure 2: Standard chromatogram of paracetamol, Orphenadrine and Ibuprofen



Validation:

The method was successfully validated as per ICH guideline kQ2 (R1): validation of analytical procedures: text and methodology, international conference on harmonization, Food and Drug Administration, 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. The Standard plots were constructed between concentration vs. peak area a linear response of peak area was observed over the concentration range of 112.65 to 675.90µg/mL for paracetamol, 100.15 to 600.90 µg/mL for Ibuprofen and 8.788 to 52.725 µg/mL for Orphenadrine. Five micro-liter of each sample was injected under above chromatographic conditions and peak area was measured. The data of linearity curve was summarized in the tables 2 to 5 and figures 3 to 5 and it was found that correlation coefficient (R^2) and regression analysis were within the limits.

Table 2: Linearity data showing equation of regression line and coefficient of determination

Drug	Conc. Range (µg/mL)	Equation	R^2
Paracetamol	112.65 - 675.90	Y = 7302.x - 10908	0.999
Ibuprofen	100.15 - 600.90	Y = 6249.x - 45282	0.999
Orphenadrine	8.788 - 52.725	Y = 3446.x + 782.2	0.999

Table 3: Linearity data of paracetamol

S. No.	Concentration (µg/mL)	Peak area
1	112.65	816297
2	225.30	1649895
3	337.95	2396872
4	450.60	3305163
5	563.25	4146460
6	675.90	4895086

Figure 3: Linearity graph for paracetamol

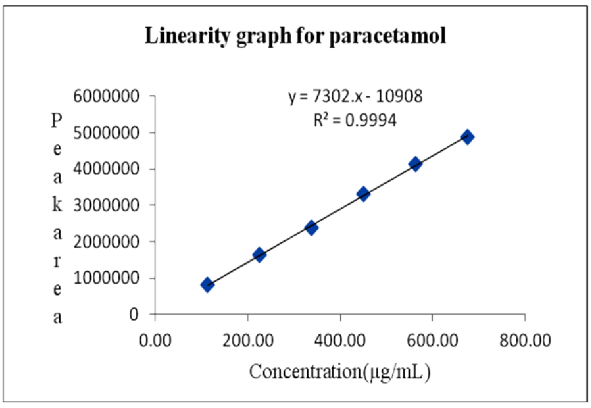


Table 4: Linearity data of Orphenadrine

S. No.	Concentration(µg/mL)	Peak area
1	8.788	30589
2	17.575	61208
3	26.363	91968
4	35.150	123108
5	43.938	151896
6	52.725	181956

Figure 4: Linearity graph of Orphenadrine

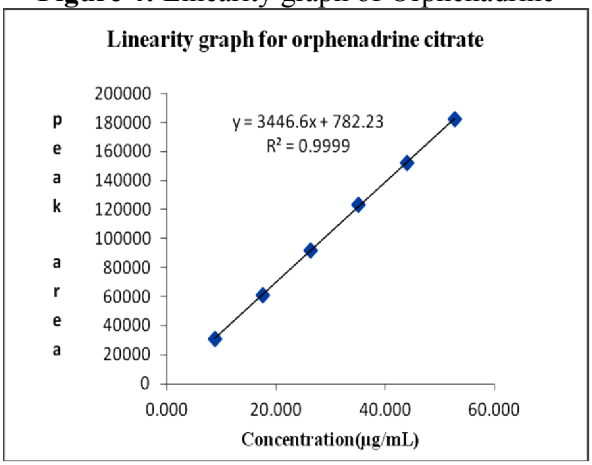
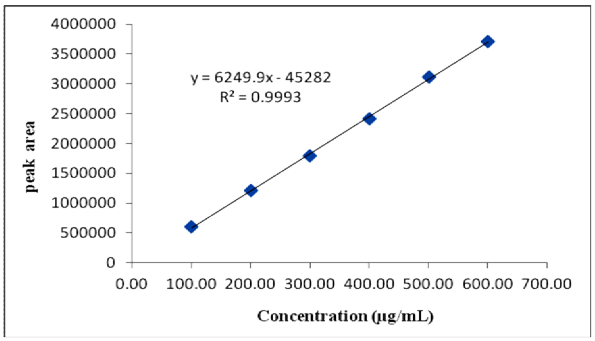


Table 5: Linearity data of Ibuprofen

S. No.	Concentration(µg/mL)	Peak area
1	100.15	603689
2	200.30	1212978
3	300.45	1798742
4	400.60	2419892
5	500.75	3121864
6	600.90	3715621

Figure 5: Linearity graph of Ibuprofen



Limit of Detection (LOD) and Limit of Quantification (LOQ):

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 6

Table 6: LOQ, LOD Values

Drugs	LOD µg/mL	LOQ µg/mL
Paracetamol	1.8	6
Orphenadrine	2.1	7
Ibuprofen	2.4	8

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 inter day variations of the proposed method at three different levels (50, 100 and 150% or 80, 100,120%) i.e. 225, 450 and 675 µg/mL for Paracetamol, 17.5, 35 and 52.5µg/mL for Orphenadrine and 200, 400 and600 µg/mL for Ibuprofen.

On the same and on three different days respectively. The %RSD values less than 2.0% indicate that the method was precise .The precision data was given in the tables 7 to 12

Table 7: Intraday precision of Paracetamol

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	225.23	100.2	Mean=99.867
2	224.34	99.8	SD=0.305
3	225.82	99.6	%RSD=0.306
4	450.63	99.8	Mean=99.567
5	451.92	99.6	SD=0.252
6	451.61	99.3	%RSD=0.253
7	675.83	100.4	Mean=100.067
8	676.16	99.6	SD=0.416
9	675.22	100.2	%RSD=0.416

Table 8: Interday precision of Paracetamol

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	224.81	99.2	Mean=99.667
2	225.93	99.6	SD=0.503
3	225.26	100.2	%RSD=0.55
4	451.23	99.3	Mean=99.333
5	450.82	99.2	SD=0.153
6	451.63	99.5	%RSD=0.154
7	676.26	100.1	Mean=100.233
8	675.82	100.8	SD=0.513
9	675.27	99.8	%RSD=0.512

Table 9: Intraday precision of Orphenadrine

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	17.562	100.2	Mean=99.633
2	17.421	99.2	SD=0.513
3	17.491	99.5	%RSD=0.515
4	35.621	99.8	Mean=100.200
5	35.482	100.6	SD=0.400
6	35.731	100.2	%RSD=0.399
7	52.582	99.2	Mean=99.700
8	52.721	99.6	SD=0.557
9	52.491	100.3	%RSD=0.558

Table 10: Interday precision of Orphenadrine

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	17.581	99.2	Mean=99.333
2	17.492	99.6	SD=0.231
3	17.610	99.2	%RSD=0.232
4	35.812	100.8	Mean=100.200
5	35.460	100.2	SD=0.600
6	35.821	99.6	%RSD=0.599
7	52.442	99.2	Mean=99.633
8	52.622	99.6	SD=0.451
9	52.563	100.1	%RSD=0.453

TabIe 11: Intraday precision of Ibuprofen

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	201.31	100.2	Mean=99.633
2	201.14	99.2	SD=0.513
3	200.96	99.5	%RSD=0.515
4	401.23	99.8	Mean=100.200
5	400.92	100.6	SD=0.400
6	400.56	100.2	%RSD=0.399
7	601.36	99.2	Mean=99.700
8	601.48	99.6	SD=0.557
9	601.23	100.3	%RSD=0.558

Table12: Interday precision of Ibuprofen

S. No.	Concentration (µg/mL)	% Assay	Statistical parameters
1	201.21	99.2	Mean=99.333
2	201.31	99.6	SD=0.231
3	200.86	99.2	%RSD=0.232
4	401.22	100.8	Mean=100.200
5	401.63	100.2	SD=0.600
6	401.55	99.6	%RSD=0.599
7	601.23	99.2	Mean=99.633
8	601.86	99.6	SD=0.451
9	601.65	100.1	%RSD=0.458

Accuracy:

Accuracy for Paracetamol, Orphenadrine and Ibuprofen 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 table No.'s 13 to 15.

Table 13: Recovery studies of paracetamol

S. No.	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	225.81	225.21	99.734	Mean=99.670
2	224.91	223.92	99.560	SD=0.095
3	225.26	224.62	99.715	%RSD=0.096
4	451.23	450.80	99.905	Mean=99.732
5	450.91	449.12	99.603	SD=0.156
6	450.62	449.22	99.689	%RSD=0.156
7	675.22	674.12	99.837	Mean=99.788
8	674.91	672.91	99.704	SD=0.073
9	676.12	674.92	99.823	%RSD=0.073

Table 14: Recovery studies of Orphenadrine

S. No.	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	17.58	17.51	99.602	Mean=99.870
2	17.22	17.32	100.581	SD=0.622
3	17.41	17.31	99.426	%RSD=0.623
4	35.65	35.31	99.046	Mean=99.024
5	34.92	34.56	98.969	SD=0.048
6	34.96	34.63	99.056	%RSD=0.048
7	52.81	52.26	98.959	Mean=99.189
8	52.54	52.21	99.372	SD=0.210
9	52.36	51.96	99.236	%RSD=0.212

Table 15: Recovery studies of Ibuprofen

S. No.	Amount added (µg/mL)	Amount found (µg/mL)	%Recovery	Statistical parameters
1	200.81	199.56	99.378	Mean=99.502
2	201.22	200.12	99.453	SD=0.221
3	201.63	200.32	99.350	%RSD=0.223
4	402.31	401.62	99.828	Mean=99.801
5	400.91	401.32	100.102	SD=0.322
6	401.23	400.93	99.925	%RSD=0.322
7	600.82	601.91	100.181	Mean=99.916
8	602.51	601.31	99.801	SD=0.230
9	601.92	600.51	99.766	%RSD=0.230

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 450 µg/mL for Paracetamol, 35 µg/mL for Orphenadrine and 400 µg/mL for Ibuprofen. 5µL 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 table 16 to 18

Table 16: System suitability of Paracetamol

S. No.	Retention time	Peak area	Tailing Factor	USP plate count
1	3.281	3305290	1.13	8720
2	3.272	3316272	1.13	8528
3	3.268	3305896	1.12	8656
4	3.256	3298658	1.12	8562
5	3.269	3289789	1.12	8708
6	3.285	3316256	1.13	8893
Mean	**3.272**	**3305360**	**-**	-
SD	**0.010**	**10251**	**-**	-
%RSD	**0.315**	**0.310**	**-**	-

Table 17: System suitability of Orphenadrine

S. No.	Retention time	Peak area	Tailing Factor	USP plate count
1	11.289	122139	1.25	70058
2	11.265	121862	1.25	70027
3	11.269	121986	1.25	70045
4	11.259	121568	1.25	70036
5	11.267	120869	1.25	70050
6	11.288	123058	1.25	70089
Mean	**11.273**	**121914**	**-**	-
SD	**0.013**	**718**	**-**	-
%RSD	**0.112**	**0.589**	**-**	-

Table 18: System suitability of Ibuprofen

S. No.	Retention time	Peak area	Tailing Factor	USP plate count
1	21.457	2409989	1.33	64868
2	21.421	2401356	1.32	64856
3	21.523	2415638	1.32	64826
4	21.369	2398655	1.32	64529
5	21.562	2399872	1.32	64502
6	21.426	2405892	1.32	64499
Mean	**21.460**	**2405234**	**-**	**-**
SD	**0.071**	**6608**	**-**	**-**
%RSD	**0.332**	**0.275**	**-**	**-**

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 byZorbax SB C18 (150x4.6 mm, 3.5 µm) column and with a flow rate of 1.5 mL/min, and detection wavelength of 225 nm. Column temperature was maintained at 40^{o}C. Injection volume is 5 µL, and run time is for 30 min. The mobile phase consists of Orthophosphoric acid 20mM buffer and methanol as Organic base modifiers for reducing tailing triethylamine was used at the conc. 0.2% finally a method was developed with orthophosphoric acid buffer and acetonitrile by gradient program. The retention times of Paracetamol, Orphenadrine and Ibuprofen peaks are about 3.29, 11.29 and 21.46 minutes respectively.

Quantitative linearity was observed over the concentration range of112.65 to 675.90 µg/mL for Paracetamol 8.788 to 52.725µg/mL for Orphenadrine and 100.15 to 600.90 µg/mL for Ibuprofen The regression equations of concentration of Paracetamol, Orphenadrine and Ibuprofen are found to be y = 7302.x - 10898, y = 3446.x + 782.2, y = 6249.x - 45282 respectively, where y is the peak area and x is the concentrations of drugs (µg/mL).

The numbers of theoretical plates obtained were 8656, 70036 and 64826 for Paracetamol, Orphenadrine and Ibuprofen respectively, which indicates the efficiency of the column. The limit of detection and limit of quantitation were found to be 1.8, 6 and 2.1, 7 and 2.4, 8 µg/mL for of Paracetamol, Orphenadrine and Ibuprofen respectively, which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate.

CONCLUSION

A simple, specific, accurate, precise, stability indicating reverse phase high performance liquid chromatography method has been developed which can be used accurately for quantitative estimation of Paracetamol, Orphenadrine and Ibuprofen for routine analysis of individual and combination of drugs. Method was validated as per ICH Q2 (R2) so it can be used by analytical department.

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