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Original Research Article

Gradient High Performance Liquid Chromatography Method Development and Validation for Simultaneous Determination of Phenylephrine and Ibuprofen in Tablet Dosage Form

Venkata Raveendra Babu Vemula[1]* and Pankaj Kumar Sharma[2]
[1]Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur-313024, [2]School of Pharmaceutical Sciences, Jaipur National University, Jaipur-302025, Rajasthan, India

For correspondence: **Email: raveendra.vemula@gmail.com; **Tel:** 91+9705126244*

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Abstract

Purpose: *To develop a gradient high performance liquid chromatography (HPLC) method for the simultaneous determination of phenylephrine (PHE) and ibuprofen (IBU) in solid dosage form.*
Methods: *HPLC determination was carried out on an Agilent XDB C-18 column (4.6 x 150mm, 5 µ particle size) with a gradient mobile phase composed of 0.1 % orthophosphoric acid and acetonitrile at a ratio of: 0.01/95/5, 2.5/95/5, 6/10/90, 8/10/90, 8.1/95/5 and 13/95/5 for time (min)/0.1 % orthophosphoric acid (%)/acetonitrile (%) at a flow rate of 1.0 mL/min. Column temperature was maintained at 30 °C and detection was carried out using a photodiode array (PDA) detector at 210 nm. Validation parameters, including system suitability, linearity, precision, accuracy, specificity, limit of detection (LOD), limit of quantification (LOQ), stability of sample and standard stock solutions as well as robustness were obtained as per International Conference on Harmonization (ICH) guidelines. The proposed method was applied to the determination of phenylephrine and ibuprofen in commercial tablets.*
Results: *Retention time for phenylephrine and ibuprofen were 2.7 and 8.4 min, respectively while % recovery was 99.42 and 99.80 %, respectively. The relative standard deviation (% RSD) for assay of the tablets was < 2 %.*
Conclusion: *The method is fast, accurate, precise and sensitive, and hence it can be employed for routine quality control of tablets containing both drugs in quality control (QC) laboratories and pharmaceutical industry.*

Keywords: *Phenylephrine, Ibuprofen, Simultaneous determination, Validation, Gradient HPLC.*

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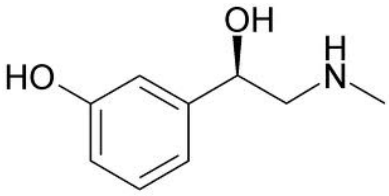
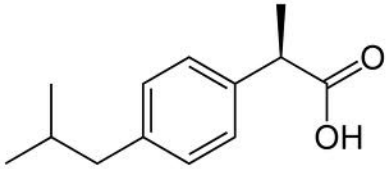
INTRODUCTION

Phenylephrine (PHE) is chemically named as (R)-3-[-1-hydroxy-2-(methylamino) ethyl] phenol (Figure 1A). It is a nasal decongestant which helps to relieve a blocked nose. It reduces the size of the blood vessels in the nose and sinuses thus enabling one to breathe more easily. It is also used as paroxysmal supraventricular tachycardia, mydriasis, and haemorrhoids [1]. Ibuprofen (IBU) is chemically named as (RS)-2-(4-(2-methylpropyl) phenyl) propanoic acid (Figure 1B). It is used to relieve symptoms of a wide range of illnesses such as headaches, backache, pains, migraine, cold and flu symptoms and arthritis. Its effects are due to the inhibitory actions on cyclo-oxygenases, which are involved in the synthesis of prostaglandins.

Prostaglandins have an important role in the production of pain, inflammation and fever [2].



(a)



(b)

Figure 1: Structure of (a) Phenylephrine and (b) Ibuprofen

Various ultra violet (UV) and HPLC assay methods have been reported in the literature for the determination of phenylephrine [3-6] and ibuprofen [7-11] individually and in-combination with other drugs. According to the literature, there is no official method for the simultaneous determination of both drugs by reverse phase HPLC in combined tablet dosage forms. Hence, an attempt has been made to develop new method for simultaneous determination [12-14] and validation of phenylephrine and ibuprofen in a tablet formulation in accordance with ICH guidelines [15-17].

EXPERIMENTAL

Instrumentation

Chromatography was performed with Water's 2695 HPLC systems provided with Hamilton syringe, auto sampler and 2996 photodiode array detector. All HPLC systems were equipped with a column compartment with temperature control and an on-line degasser. Data acquisition, analysis, and reporting were performed by Empower2 (Waters) chromatography software.

Reagents and chemicals

The reference samples of PHE and IBU were provided as gifts from Spectrum Pharma research solutions, Hyderabad. HPLC grade acetonitrile, HPLC grade methanol and all other chemicals were obtained from Merck chemical division, Mumbai. HPLC grade water obtained from Milli-Q water purification system was used throughout the study. Commercial tablets (ADVIL - dosage: PHE - 10 mg and IBU - 200 mg) were purchased from a local pharmacy.

Chromatographic conditions

The mobile phase consisted of 0.1 % Ortho phosphoric acid and acetonitrile was taken in gradient ratio of time (min.)/0.1 % orthophosphoric acid (%)/acetonitrile (%) as follows: 0.01/95/5, 2.5/95/5, 6/10/90, 8/10/90, 8.1/95/5 and 13/95/5, at a flow rate of 1.0 mL/min. Agilent XDB C-18 column (4.6 × 150 mm, 5 µ particle size) was used as the stationary phase. Although the PHE and IBU have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 210 nm was selected as the detection wavelength for PDA detector.

Preparation of standard stock solution

Standard stock solutions were prepared by transferring 10 mg of phenylephrine and 200 mg of ibuprofen into a clean and dry 100 mL volumetric flask, to which 70 mL of diluent was added, sonicated for 5 min and volume made up to 100 mL with diluent to get stock solution.

Preparation of working standard solutions

Aliquot of 0.5, 0.75, 1.0, 1.25, 1.5 & 2.5 mL pipette out from stock solution into 10 mL volumetric flask separately for both PHE and IBU and volume was made up to 10 mL with diluent. This gives the solutions of 5, 7.5, 10, 12.5, 15 and 25 µg/mL for phenylephrine and 100, 150, 200, 250, 300 and 500 µg/mL for ibuprofen, respectively.

Sample preparation

Twenty tablets were weighed and crushed into fine powder. An amount of the powder equivalent to the weight of five tablets was taken and dissolved in 1000 mL diluent, sonicated for 20 min and filtered through PVDF 0.45 µ filter. From the filtrate, 1 mL was pipetted into a 10 mL volumetric flask and the solution made up to the volume with the diluent.

Method validation

System suitability test

To ensure that the resolution and reproducibility of the HPLC system was adequate for the analysis, a system suitability test was established. Data from six injections of 10 µL of the working standard solutions of PHE and IBU were used for the evaluation of the system

suitability parameters like tailing factor, the number of theoretical plates, retention time and resolution factor.

Linearity

By appropriate aliquots of the standard PHE and IBU solutions with the mobile phase, six working solutions ranging between 5 - 25 µg/mL of PHE and 100 - 500 of IBU µg/mL were prepared. The linearity point of each experiment was performed in triplicate according to the optimized chromatographic conditions. The peak areas of the chromatograms were plotted against the concentration of PHE and IBU to obtain the calibration curve.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method. Previously analyzed samples of PHE and IBU to which known amounts of standard PHE and IBU corresponding to 50, 100 and 150 % of target concentration, were added. The accuracy was expressed as the percentage of analyte recovered by the proposed method.

Precision

Precision was determined as repeatability and intermediate precision (ruggedness), in accordance with ICH guidelines. The intra-day and inter-day precision were determined by analyzing the samples of PHE and IBU. Determinations were performed on the same day as well as well as on consequent days.

Limit of detection and the limit of quantification

Limit of detection (LOD) and limit of quantification (LOD) of PHE and IBU were determined by calibration curve method. Solutions of both PHE and IBU were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations: LOD = (3.3 × Syx)/b, LOQ = (10.0 × Syx)/b, where Syx is residual variance due to regression and b is slope.

Robustness

The robustness of the method was performed by deliberately changing the chromatographic conditions. Organic strength was varied by ± 5 %, column temperature by ± 5 ^{o}C and flow rate by ± 0.1 mL.

Stability

The sample and standard solutions were injected at 0 h (control) and after 24 h (stability sample) at ambient room temperature. Stability was determined by determining RSD for sample and standard solutions.

Statistical analysis

Where applicable, results were expressed as mean ± SD. % RSD and data were analyzed statistically by using t- test with aid of Microsoft Excel-2007 software and data were considered significantly different at $p \leq 0.05$.

RESULTS

Method development

Initially reverse phase liquid chromatography separation was tried using various ratios of methanol and water, acetonitrile and water as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs.

To improve the tailing factor, the pH of mobile phase became an important factor. At pH 3, both drugs eluted with better separation. Thereafter, buffer: acetonitrile were taken in gradient: T (min)/ %buffer / % acetonitrile: 0.01/95/5, 2.5/95/5, 6/10/90, 8/10/90, 8.1/95/5 and 13/95/5 using a flow rate of 1.0 mL/min. Agilent XDB C-18 column (4.6 × 150 mm, 5 µ particle size) was selected as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1.

To analyze both drugs, detection was tried at various wavelengths from 205 nm to 280 nm. Both PHE and IBU showed maximum absorption at a wavelength of 210 nm, which was selected as the detection wavelength for PDA detector. The retention times were found to about 2.7 min and 8.4 min for PHE and IBU, respectively. The chromatogram obtained was shown in the Figure 2.

Method validation

System suitability

System suitability parameters such as number of theoretical plates, peak tailing, retention time and resolution factor were determined. The total run time required for the method is only 13 min for

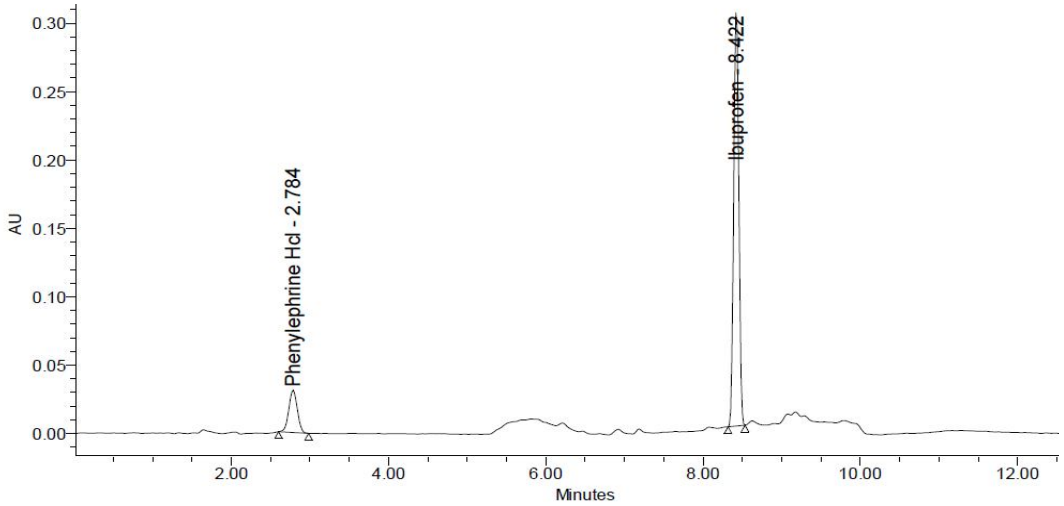


Figure 2: Representative chromatogram of phenylephrine and ibuprofen

Table 2: Accuracy for PHE and IBU

Preanalysed sample solution concentration (µg/mL)		Standard drug concentration (µg/mL)		Amount recovered (µg/mL)		Recovery (%)	
PHE	***IBU***	***PHE***	***IBU***	***PHE***	***IBU***	***PHE***	***IBU***
10	200	5	100	4.9	100.7	98.7	100.7
10	200	5	100	4.9	100.7	98.1	100.7
10	200	5	100	5.1	100.8	101.0	100.8
10	200	10	200	9.8	203.0	98.1	101.5
10	200	10	200	9.8	201.9	98.0	100.9
10	200	10	200	9.9	203.5	99.0	101.7
10	200	15	300	15.1	300.5	100.9	100.2
10	200	15	300	15.0	298.9	100.0	99.6
10	200	15	300	14.9	299.3	99.6	99.8
					Mean	99.27	100.66
					SD	1.197	0.705
					% RSD	1.2	0.7

eluting both PHE and IBU. The results obtained are shown in Table1.

Table 1: System suitability of PHE and IBU

Variable	PHE	IBU
No. of theoretical plates	3036	77131
Tailing factor	1.03	1.03
Resolution factor	36.4	
Retention time	2.7 min	8.4 min
Mean area	232598.0	1367853.7
RSD	0.9	0.1

Linearity

PHE showed a linearity of response between 5 - 25 µg/mL and IBU showed a linearity of response between 100 - 500 µg/mL. These were represented by a linear regression equations as follows: y(PHE) = 22901.x + 6949 (r^2 = 0.999); y(IBU) = 7079.x - 1586 (r^2 = 0.999) and regression line was established by least squares method; correlation coefficient (r^2) for PHE and IBU was > 0.98. Hence, the curves were linear.

Accuracy

To pre-analyzed sample solution, a definite concentration of standard drug (50, 100 and 150 % level) was added and recovery was studied. The percentage Mean recovery for PHE and IBU are 99.27 and 100.66 %, respectively and these results are within acceptable limit of 98-102%. The % RSD for PHE and IBU are 1.2 and 0.7, respectively and the percentage RSD for PHE and IBU is within limit of ≤ 2. Hence the proposed method is accurate and the results were summarized in Table 2.

Precision

Repeatability

Six replicates injections in same concentration were analyzed in the same day for repeatability

and the % RSD for PHE and IBU were found to be 1.1 and 1.0, respectively and which is for PHE and IBU found to be within the acceptable limit of ≤2 and hence, the method is reproducible as presented in Table 3.

Intermediate precision

Six replicates injections in same concentration were analyzed on two different days with different analyst and column for verifying the variation in the precision and the % RSD for PHE and IBU was 0.3 and 1.3, respectively, and is within the acceptable limit of ≤ 2. The overall % RSD for PHE and IBU was found to be 0.8 and 1.1, respectively, and it is within the acceptable limit of ≤ 2 and hence, the method is reproducible on different days with different analyst and column and the results are as shown in Table 3.

Statistical Analysis of Precision Result: Probability value (P) for PHE and IBU at 5% significance level was found to be 0.75 and 0.68, respectively, which are greater than 0.05 and hence no significant difference was observed in the precision results carried out for two consecutive days, and the results are shown in Table 4.

Robustness

The robustness was established by changing the flow rate, column temperature and composition of the mobile phase within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean RT and RSD is within limit of ≤ 2 .The tailing factor, resolution factor and number of theoretical plates were found to be within

Table 3: Precision data for PHE and IBU

Validation parameter	Sample no.	PHE	IBU
Repeatability (Day1, Analyst 1)	1	237093	1382263
	2	237444	1398591
	3	239179	1357440
	4	235016	1383691
	5	231882	1370765
	6	234674	1376931
	Mean	235881.33	1378280.2
	SD	2568.49	13786.08
	% RSD	1.1	1.0
Intermediate precision (Day 2, Analyst 2)	1	236742	1381213
	2	236010	1347947
	3	235070	1358811
	4	236706	1380356
	5	236243	1384521
	6	236592	1394036
	Mean	236227.2	1374480.7
	SD	634.65	17393.32
	% RSD	0.3	1.3
Global statistics (Inter day precision)	**Overall Mean**	236054.3	1376380.4
	SD	1792.88	15094.33
	Overall % RSD	0.8	1.1

SD = standard deviation RSD = relative standard deviation

Table 4: Students t-test data for precision of results for PHE and IBU

Validation parameter	PHE		IBU	
	Mean response	**Probability, *P* (≥ 0.05)**	**Mean response**	**Probability *P* (≥ 0.05)**
Repeatability -Day 1	235881.3	0.75	1378280.2	0.68
Intermediate precision - Day 2	236227.1		1374480.7	

Table 5: Robustness data for PHE

Analytical conditions	Flow rate (ml/min)		Column temperature (°C)		Mobile phase composition	
Evaluation parameters	***1.1***	***0.9***	***35***	***25***	***+5 %***	***-5 %***
Mean RT	2.29	2.69	2.17	2.31	2.0	2.8
Mean area	189917	213938	252511	2.2985	204678	234264
SD	3557	6294.984	4590	3583	3575	4168
RSD%	1.9	2.9	1.8	1.7	1.7	1.8
Tailing factor	1.11	1.08	0.97	1.03	1.02	1.0
No. of theoretical plates	3017	3154	2719	2731	5910	3050

Table 6: Results of Robustness for IBU

Analytical conditions	Flow rate (ml/min)		Column temperature (°C)		Mobile phase composition	
Evaluation parameters	***1.1***	***0.9***	***35***	***25***	***+5 %***	***-5 %***
Mean RT	8.17	8.50	8.31	8.31	8.31	8.43
Mean area	1288586	1463597	1368963	1344180	2662752	1367854
SD	6252.5	2999	22732	2499	32229	1333
RSD%	0.5	0.2	1.7	0.2	1.2	0.1
Tailing factor	1.01	1.01	1.01	1.00	1.01	1.02
No. of theoretical plates	71459	72979	78646	75429	74417	73614

acceptable limits for both PHE and IBU. Hence, the method is reliable with variations in the analytical conditions and the results for PHE are shown in Table 5 while the results for IBU are shown in Table 6.

Stability of sample solution

The sample and standard solutions were injected at 0 h (comparison sample) and after 24 h (stability sample) at ambient room temperature 30 °C. The RSD for 0 h and 24 h for sample and standard solutions of PHE are 1.1, 0.2 and 1.8, 0.3, respectively. The RSD of 0 and 24 h for sample and standard solutions of IBU are 1.0, 1.0 and 0.1, 1.3, respectively. RSD results for both PHE and IBU are within the acceptable limits of ≤ 2 and hence, the sample and standard stock are stable for 24 h in ambient room temperature and the results are shown in Table 7.

LOD and LOQ

LOD and LOQ for PHE were 0.03895 and 0.11803 µg/mL respectively, and LOD and LOQ for IBU were 0.338187 and 1.024809 µg/mL, respectively.

Results of method application to tablet

The content of PHE and IBU in the tablets was found by the proposed method and the results were shown in Table 8.

DISCUSSION

RP-HPLC method was developed and validated for the simultaneous determination of phenylephrine and ibuprofen in tablet dosage form. The resolution between two peaks is always more than 2. The system suitability tests revealed that numbers of theoretical plates were above 2000 and tailing factor is less than 2. PHE and IBU showed a linearity of response between5-25 µg/ml and 100-500 µg/ml. The mean peak area of the chromatograms was plotted against the concentration of PHE and IBU to obtain the calibration curve. Linearity was high as well as recovery of PHE and IBU, indicating high accuracy of the method. Repeatability and intermediate precision values were within the acceptable limits. This indicates that the method is precise. Specificity experiment shows that there is no interference or overlapping of the peaks of excipients or diluents with the main peaks of PHE and IBU. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The stability studies indicate that both

Table 7: Sample and standard stock solution stability data for PHE and IBU

Conc. (ug/ml)	Phenylephrine				Ibuprofen			
Injection no.	Sample stock solution area		Standard stock solution area		Sample stock solution area		Standard stock solution area	
	0 h day1	After 24 h	0 h-day1	After 24 h	0 h-Day1	After 24 h	0 h-Day1	After 24 h
1	237093	237052	230261	236742	1382263	1382261	1367303	1381213
2	237444	237146	231405	236010	1398591	1398572	1369168	1347947
3	239179	238212	232703	235070	1357440	1357462	1365533	1358811
4	235016	237457	241727	236706	1383691	1383635	1369027	1380356
5	231882	238013	236179	236243	1370765	1370732	1368168	1384521
6	234674	237126	233313	236592	1376931	1376645	1367923	1394036
Mean	235881.3	237501.0	234264.7	236227.2	1378280	1378217.8	1367854	1374481
SD	2568.493	497.56	4169.00	634.65	13786.08	13778.98	1333.90	17393.32
% RSD	1.1	0.2	1.8	0.3	1.0	1.0	0.1	1.3

Table 8: Results of HPLC Analysis of Tablet for PHE and IBU

No. of sample assayed	Label amount (mg)		Amount found (mg)		% Assay (mean ± SD)		RSD (%)	
6	PHE 10	IBU 200	PHE 10.09	IBU 200.5	PHE 100.90±1.10	IBU 100.25±1.00	PHE 1.1	IBU 1.0

standard and sample drugs were stable up to 24 h. Change in flow rate, temperature and mobile phase composition did not cause any significant change in the results, confirming the stability of the developed method. RSD for precision was < 2 % which confirms that method is sufficiently precise. The total run time required for the method was only 13 min for eluting both phenylephrine and ibuprofen.

CONCLUSION

A new gradient HPLC method has been developed and validated for the simultaneous determination of phenylephrine and ibuprofen in tablet dosage form. The method is fast, accurate, precise and sensitive, and hence, it can be employed for routine quality control of tablets containing both drugs in quality control laboratories and industry.

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