A Validated, Specific Stability Indicating Reverse Phase Liquid Chromatographic Method for the Simultaneous Estimation of Phenylephrine HCL, Betamethasone Valerate & Lignocaine HCL in Pharmaceutical Ointment

Safeena Sheikh, Suhail Asghar, Showkat Ahmad Patni

Unijules Life Sciences Ltd, Nagpur - 441501 (Maharashtra) India

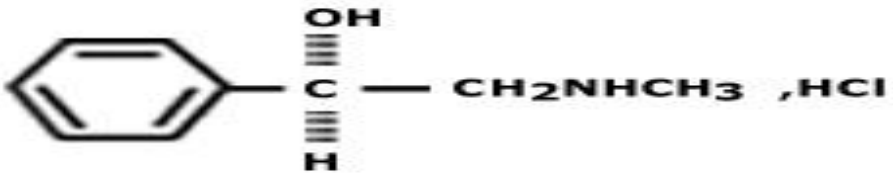
Abstract- A validated, specific, stability indicating reverse phase liquid chromatographic method has been developed for simultaneous quantitative analysis of Phenylephrine HCl, Lignocaine HCl and Betamethasone valerate in pharmaceutical ointment base products. The method was optimized by analysis of the samples and sample solutions spiked with each analyte for recovery study. Good resolution between the analytes was achieved in formulation and combined standards on Merck' C18 (250mm X 4.6mm, 5µ) column with mobile phase constituted of phosphate buffer (0.01M) and acetonitrile (46: 54% v/v) further the pH of the mobile phase was adjusted to pH=7.0(±0.05) with triethylamine. Detection was performed at 270nm.The method was validated in accordance with ICH guidelines and validation data showed that the assay is sensitive, specific and reproducible for the simultaneous estimation of Phenylephrine HCl, Lignocaine HCl and Betamethasone Valerate in the presence of other pharmaceutical excipient.

Index Terms- Estimation, Phenylephrine HCl, Lignocaine HCl, Betamethasone Valerate, HPLC-UV, Assay, Method Validation.

I. INTRODUCTION

The product is an anti-hemorrhoid ointment. It is a topical preparation that is applied directly to external hemorrhoids. The ointment is helpful in soothing the inflamed veins and as well as relieving some of itchiness commonly associated with hemorrhoids. It contains combinations of three main ingredients; Lignocaine hydrochloride, Phenylephrine Hydrochloride and Betamethasone Valerate. An extensive literature survey revealed HPTLC, HPLC and colorimetric determination for Lidocaine and Phenylephrine hydrochloride in combination with other drugs; an HPLC method utilized for the simultaneous determination of Miconazole nitrate and Lidocaine and HPTLC method for Lidocaine and Phenylephrine hydrochloride gel dosage form. But there is no method which describes the simultaneous determination of Phenylephrine hydrochloride and Lignocaine hydrochloride and Betamethasone Valerate from ointment dosage form meant for external application. The objective of this investigation was to develop simple, precise accurate and economical procedures for simultaneous estimation of Phenylephrine HCl, Lignocaine HCl and Betamethasone Valerate from an ointment dosage form.

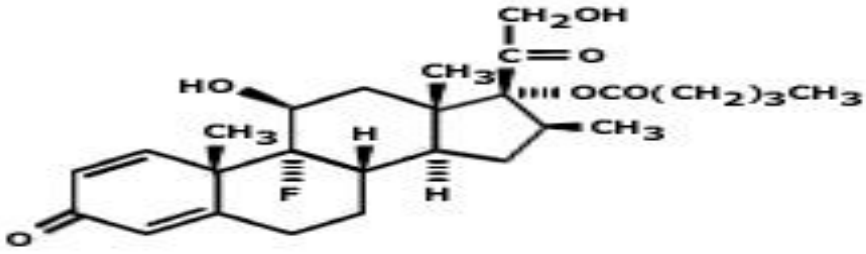
Phenylephrine Hydrochloride is a vasoconstrictor that reduces swelling and relief itching and discomfort by tightening blood vessels. Freely soluble in water and in ethanol (95%); practically insoluble in chloroform.



$C_9H_{13}NO_2$, HCl Mol. Wt. 203.67

Figure 1: Chemical Structure of Phenylephrine HCl

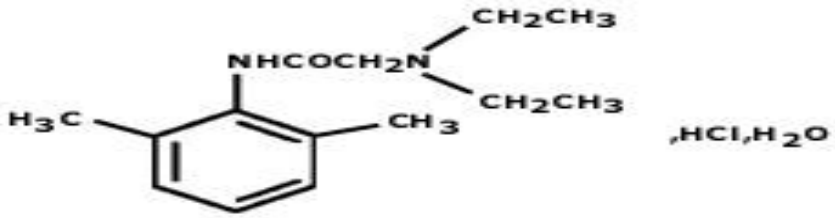
Betamethasone Valerate is an adrenocortical steroid that suppresses inflammation. It is freely soluble in chloroform; soluble in ethanol (95%); practically insoluble in water and in light petroleum.



$C_{27}H_{37}FO_6$ Mol. Wt. 476.58

Figure 2: Chemical Structure of Betamethasone Valerate

Lidocaine Hydrochloride is a local anesthetic to temporarily relieve pain. It is very soluble in water, freely soluble in ethanol (96 per cent). Melting point is 74 °C to 79 °C.



$C_{14}H_{22}N_2O$, HCl, H2O Mol. Wt. 288.82

Figure 3: Chemical Structure of Lignocaine Hydrochloride

II. Material and Methods

All the reagents were of analytical grade or HPLC grade unless stated otherwise. HPLC grade water was used throughout the experiment. Potassium dihydrogen phosphate, acetonitrile and triethylamine were of Merck". Betamethasone Valerate (B-Val), Phenylephrine HCl (PH-HCl), Lignocaine HCl (L-HCl) and the formulation were of Unijules Life Sciences Ltd.

III. Instrumentation

The HPLC system used was of Jasco LC-Net II/ADC (B211161095), pump, auto sampler, and an UV-2075 variable wavelength detector was controlled through Borwin software. Injection volume was 20µl was used. Analytical column used for this method is Merck" C_{18} (250mm X 4.6mm, 5µ).

IV. Mobile phase preparation

First the phosphate buffer (0.01M) was prepared by dissolving 1.36g of monobasic phosphate buffer in 1000ml of distilled water. Then the solution was mixed with Acetonitrile in the ratio 460:540 and then the pH of the mobile phase was adjusted to 7.0 (±0.05) with triethylamine.

V. Preparation of standard solution

Standard Stock solution for Phenylephrine HCl

Weigh accurately 10 mg Phenylephrine HCl and transfer it in to 100.0ml volumetric flask, add about 50.0ml of methanol and make up the volume to 100.0ml with methanol. (Solution A)

Standard Stock Solution for Betamethasone Valerate

Weigh accurately 10.0 mg Betamethasone Valerate and transfer to 10.0 ml volumetric flask, add 5.0 ml purified methanol and allow it to dissolved with sonication and make up the volume with methanol to 10.0ml. Take 5ml stock solution in 100.0ml volumetric flask and dilute with methanol up to the mark. (Solution B)

Standard Stock solution for Lignocaine HCl

Weigh accurately 250.0mg of Lignocaine HCl and transfer it in to 100.0ml volumetric flask, add about 50.0ml of methanol and make up the volume to 100.0ml with methanol. (Solution C).

Combine Standard

Take accurately 5.0ml of each of the above solutions (i.e. solution A, B and C) in 50.0ml volumetric flask and dilute up to the mark with mobile phase.

Sample Solution

Take an accurately 5.0g weighed amount of the sample in 100.0ml of beaker add 40.0ml of methanol and warm on water bath at 60ºC for about 10-15minutes; cool with stirring. Transfer the supernatant liquid to 100.0ml volumetric flask keeping the ointment in beaker. Repeat the procedure thrice with another fresh quantity of methanol. Allow to cool the volumetric in ice bath and make up the volume to 100.0ml with methanol. Dilute 5.0ml of the above solution to 50.0ml with mobile phase.

Chromatographic Conditions

The mobile phase was filtered through 0.45µm, Nylon membrane filter and degassed using vaccum before delivering it in to the HPLC system. For detection of analyte Phenylephrine HCl, Lignocaine HCl and Betamethasone Valerate UV-detector were used. The chromatographic conditions used for the estimation were given below.

Column	:	Merck' C_{18} (250mm X 4.6mm, 5µ)
Wavelength	:	270 nm
Injection Volume	:	20µl
Flow rate	:	1.5 ml/min
Column Temperature	:	Ambient

VI. Result and Discussion

Method development

The primary target in developing this stability indicating LC method was to achieve the resolution between Phenylephrine HCl, Betamethasone Valerate and Lignocaine HCl and the formulation excipient. To achieve the separation of related substances, stationary phase of C18 and a combination of mobile phase phosphate buffer with methanol and Acetonitrile were used. The separation of formulation excipient and all three active ingredients was achieved a Merck" C18 column and a mobile phase composed of [potassium dihydrogen phosphate buffer and Acetonitrile in the ratio 460**:**540 and Triethylamine was used to adjust the pH of the mobile phase to 7.0. Mobile phase flow rate was maintained at 1.5ml/min and eluent were monitored at 270nm. A 20µl of the sample was injected using a fixed loop. The developed method/chromatograms showed that the method was highly specific and all the actives were well resolved from each other. The Chromatograms of all the actives and sample (ointment) are given below (Figure 4a & 4b).

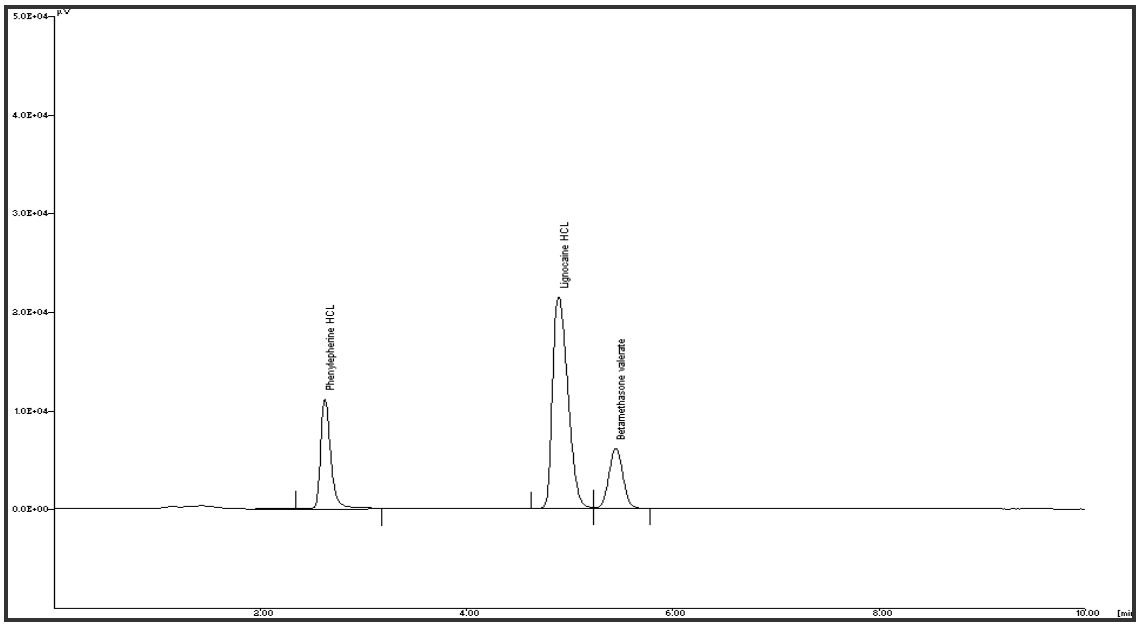


Figure 4 (a): Typical chromatograms of combined standards and product.

Info:
STD – 3-0028E1201
Group: UNIJULES
Control Method:

#	Name	RT	Area [µV.Sec]	Factor	Plates
1.	Phenylephrine HCl	2.652	110362.695	1.000	4952.03
2	Lignocaine HCl	4.815	384922.210	1.000	5962.41
3	Betamethasone Valerate	5.421	89510.674	1.000	9263.25

Total Area of Peak = 584795.579 [µV.Sec]

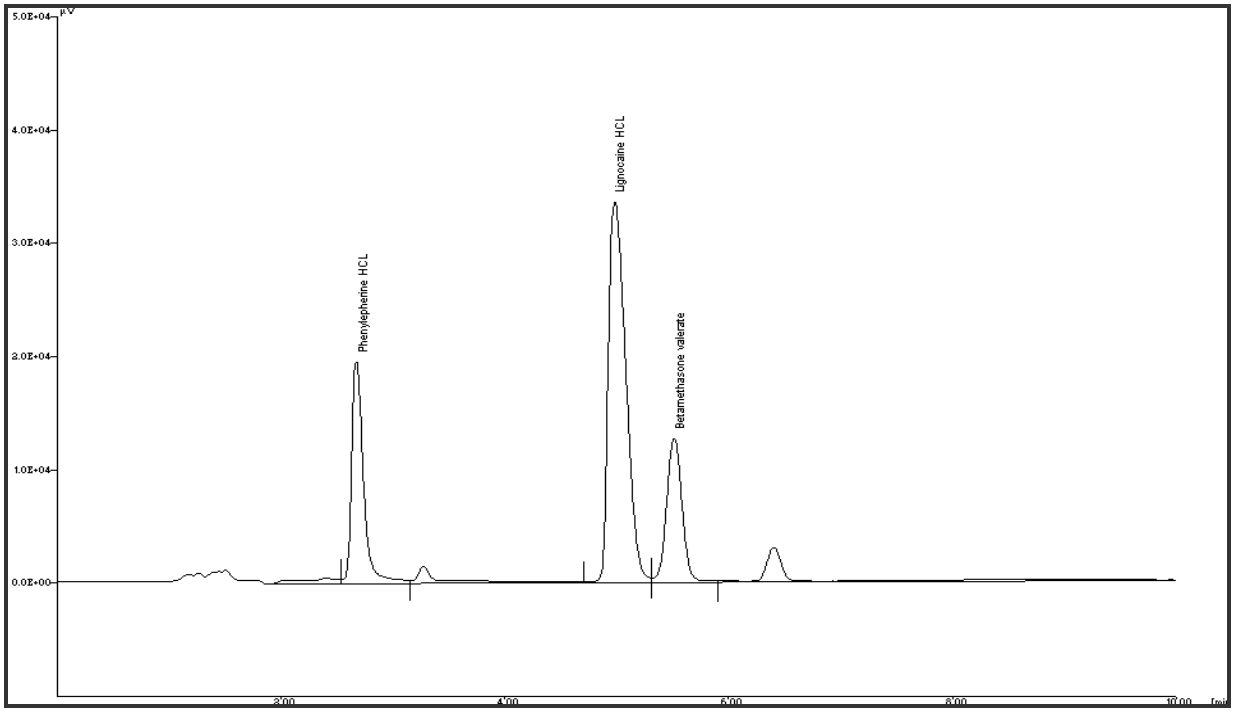


Figure 4 (b): Typical Chromatogram of Sample

Info:
OINTMENT
Group: UNIJULES
Control Method:

#	Name	RT	Area [µV.Sec]	Factor	Plates
1	Phenylephrine HCl	2.633	110212.253	1.000	4985.36
2	Lignocaine HCl	4.862	385422.104	1.000	5962.14
3	Betamethasone Valerate	5.421	89523.514	1.000	9245.17

Total Area of Peak = 585157.871 [µV.Sec]

VII. METHOD VALIDATION

Specificity

Specificity is the ability of the method to asses unequivocally the analyte in the presence of components, which may be expected to be present. Typically these might include impurities, degradation-products, matrix, etc. The specificity of the developed method for Phenylephrine hydrochloride, Betamethasone Valerate and Lignocaine hydrochloride. The placebo was not interfering with the actives.

Linearity and range

The linearity of detector response to different concentrations of actives was studied in the range of 1.0 to 12.0mcg/ml for Phenylephrine hydrochloride, 1.0 to 8.0mcg/ml for Betamethasone Valerate and 40.0 to 320.0mcg/ml for Lignocaine hydrochloride at different levels. The data were subjected to statistical analysis using a linear model; the calibration curves of **PH-HCl, L-HCl** and **B-Val** are shown in (figure 5(a), 5(b), 5(c)). Regression characteristics of the proposed HPLC method are given in (Table 1).

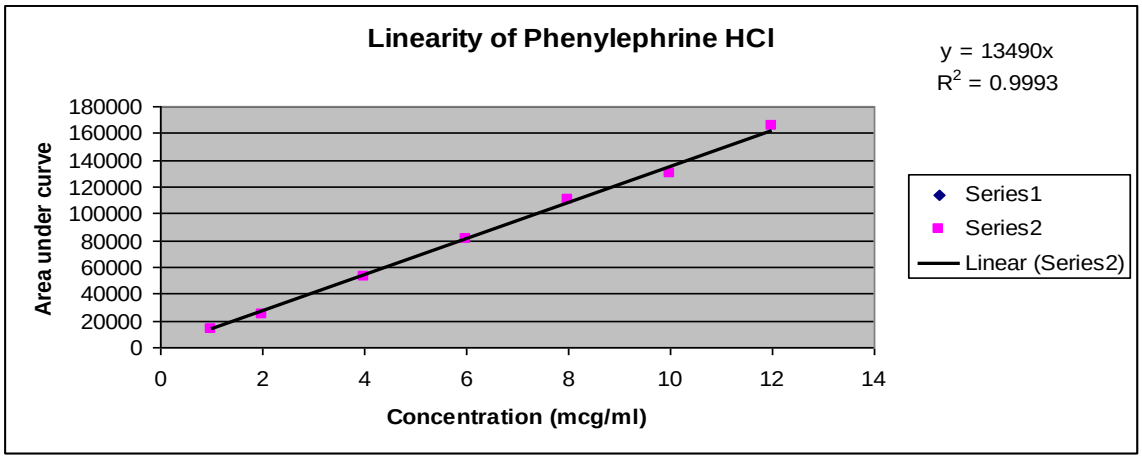


Figure 5(a): Calibration Curves of Phenylephrine HCl.

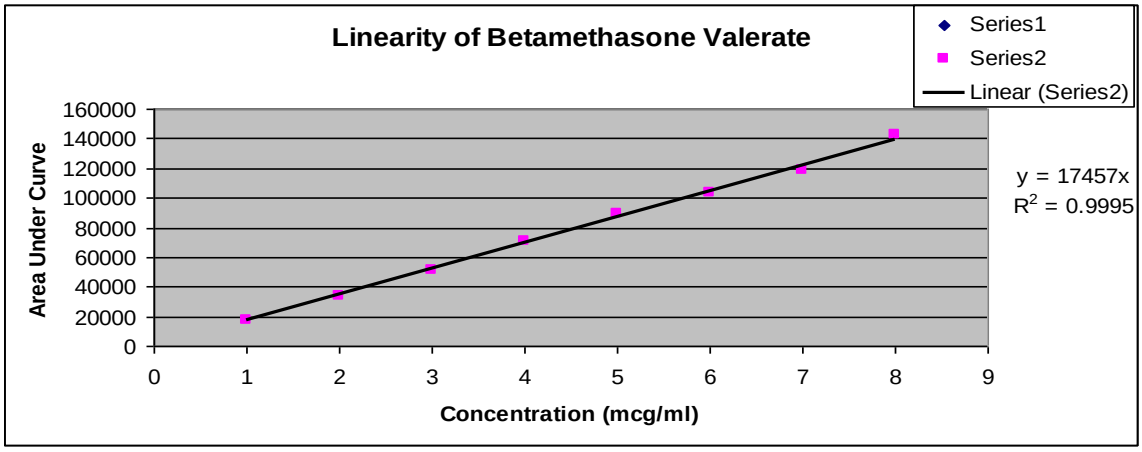


Figure 5(b): Calibration curves of Betamethasone Valerate

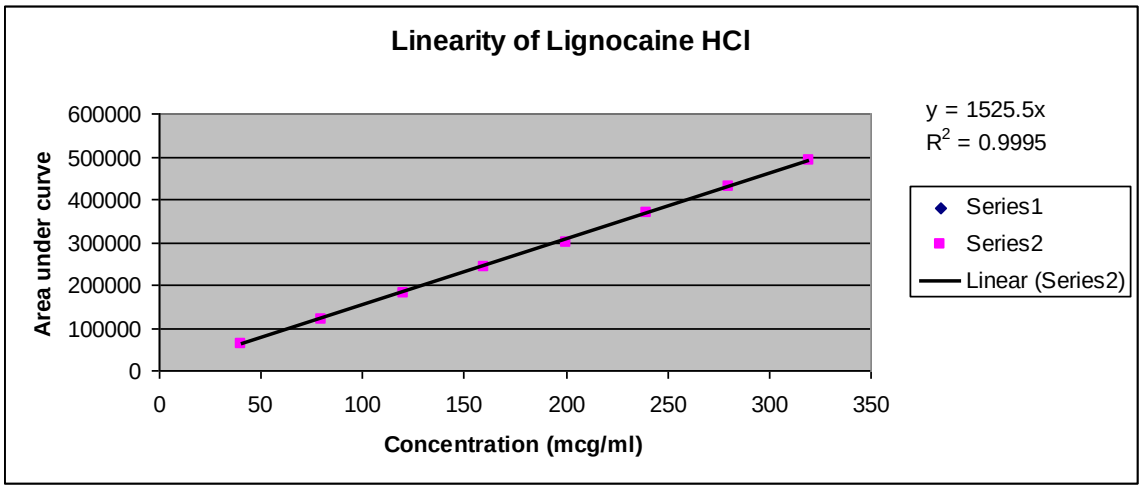


Figure 5(c): Calibration curves of Lignocaine HCl

Table 1: Regression characteristics of the proposed HPLC method.

Linearity Experiment	Phenylephrine Hydrochloride	Betamethasone Valerate	Lignocaine Hydrochloride
Range (mcg/ml)	1 to 12	40 to 320	40 to 320
Regression coefficient (r^2)	0.9993	0.9995	0.9995
Slope	13490	17457	1525.5

Precision

Precision was measured in terms of repeatability of measurement, performed by injecting the standard solution six times (n=6) and measuring the peak areas. The RSD was found to be 0.909, 0.804 and 1.093 for PH-HCl, L-HCl and B-Val respectively (Table.2). This shows that the precision of the method is satisfactory as relative standard deviation is not more than 2.0%.

Table 2: Results of Precision

Number of Injections	Area's of Active ingredients			% Assay		
	Phenylephrine HCl (PH-HCl)	Lignocaine HCl (L-HCl)	Betamethasone Valerate (B.Val)	(PH-HCl)	(L-HCl)	(B-Val)
Injection 1	110010.253	385422.104	89823.514	99.91	99.63	101.36
Injection 2	110209.223	385099.809	90890.098	100.09	99.54	102.57
Injection 3	109989.833	391999.981	87987.500	99.89	101.32	99.28
Injection 4	110201.199	384997.986	89535.098	100.08	99.51	101.04
Injection 5	112521.562	390899.900	89529.498	102.19	101.05	101.03
Injection 6	111218.259	386886.910	88890.395	101.01	100.01	100.24
LIMIT NMT 2. 0%	**Mean**			**100. 52**	**100.17**	**100. 92**
	SD			**0. 913**	**0. 805**	**1. 104**
	%RSD			**0. 909**	**0. 804**	**1. 093**

Intermediate Precision:

Intermediate precision also called as ruggedness of the method it was determined by analyzing standard solutions by two different analysts, using different instruments, in two different labs and on different days. The values of RSD obtained under set-I conditions were 0.747, 0.959 and 0.594 for PH-HCl, L-HCl and B-Val respectively. As the values of RSD for the two sets of conditions are below 2.0% for all the drugs, intermediate precision of the method is established.

Accuracy

The accuracy of the method was determined by recovery study carried out using standard addition method at three different concentration levels. The resulting spiked sample solutions were assayed in triplicate and the result obtained were compared with the expected results and expressed as percentage recovery. The mean percentage recoveries of **PH-HCl, L-HCl** and **B-Val** were found to be 100.94, 100.01 and 100.98 respectively which are within the acceptance limit.Table.3

Table 3: Results of Accuracy of Experiment

Amount of Sample			Amount of drug added			Amount recovered			% Recovery		
PH-HCl µg/ml	**L-HCl** µg/ml	**B-Val** µg/ml	**PH-HCl** µg/ml	**L-HCl** µg/ml	**B-Val** µg/ml	**PH-HCl** µg/ml	**L-HCl** µg/ml	**B-Val** µg/ml	**PH-HCl** µg/ml	**L-HCl** µg/ml	**B-Val** µg/ml
10	250	0.5	8	200	0.4	18.19	449.75	0.908	101.055	99.94	100.88
10	250	0.5	8	200	0.4	18.20	449.55	0.915	101.11	99.90	101.66
10	250	0.5	8	200	0.4	18.10	450.55	0.910	100.55	100.04	101.11
10	250	0.5	10	250	0.5	20.33	500.38	10.08	101.65	100.10	100.80
10	250	0.5	10	250	0.5	20.25	500.29	10.20	101.25	100.10	102.00
10	250	0.5	10	250	0.5	20.29	500.45	10.11	101.45	100.09	101.10
10	250	0.5	12	300	0.6	22.10	549.98	11.01	100.45	99.99	100.09
10	250	0.5	12	300	0.6	22.10	549.97	11.08	100.45	99.99	100.72
10	250	0.5	12	300	0.6	22.11	550.00	11.05	100.50	100.0	100.45
Mean % Recovery									**100.94**	**100.01**	**100.98**

Robustness

It is a measure of its capacity to remain unaffected by small but deliberate variations in the method parameters and provides an indication of its reliability during normal usage. To determine the robustness of the developed method, typical variations in analytical conditions were tested. Influence of flow rate, mobile phase composition and pH change were studied and retention time of PH-HCl, B-Val and L-HCl was noted. The factor selected were flow rate, pH and % Acetonitrile in the mobile phase. It was observed that there were no deliberate changes in the chromatogram, which demonstrated that the RP-HPLC method developed, are robust .Results describe in Table V.

Table 4: Robustness data

Factor	Level	Retention time		
		PH-HCl	**L-HCl**	**B-Val**
Flow rate ml/min				
1.4	-0.1	2.821	4.955	5.766
1.5	0	2.675	4.852	5.425
1.6	+0.1	2.524	4.732	5.310
pH of the mobile phase				
6.9	-0.1	2.670	4.845	5.560
7.0	0	2.675	4.852	5.425
7.1	+0.1	2.723	4.861	5.586
% Acetonitrile in the mobile phase				
53	-1	2.635	4.822	5.400
54	0	2.675	4.852	5.425
55	+1	2.680	4.873	5.455

VIII. Conclusion

The developed liquid chromatographic method is specific, precise, accurate and robust. It can be used as an alternative method for the rapid and routine simultaneous determination of Phenylephrine hydrochloride, Lignocaine hydrochloride and Betamethasone Valerate in semi solid pharmaceutical preparations.

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Devarajan PV, Adani MH, Gandhi AS.

Department of Chemical Technology, University of Mumbai, Matunga, India. pvd@pharma.udct.ernet.in.

AUTHORS

First Author – Safeena Sheikh, PGDAC, Unijules Life Science Ltd., ard@unijules.com.
Second Author – Suhail Asghar, M.Pharm. , Unijules Life Science Ltd., suhailasghar@unijules.com
Third Author – Showkat Ahmed Patni, PG. Unijules Life Science Ltd., research@unijules.com

Correspondence Author – Safeena Sheikh, PGDAC, Unijules Life Science Ltd., ard@unijules.com., Contact No. - 9371358783