Improved method by HPLC for Ibuprofen, Pseudoephedrine Hydrochloride and Chlorpheniramine maleate active ingredients and degradation products for the finished product in syrup dosage form

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The objective of this paper is to develop a simple, precise, accurate, and reproducible improved reversed phase high performance liquid chromatographic method for the quantitative determination of Chlorpheniramine Maleate, Ibuprofen and Pseudoephedrine Hydrochloride possible degradation product in a combined pharmaceutical dosage form.

Introduction

3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine is the IUPAC name for Chlorpheniramine Maleate. The empirical formula for Chlorpheniramine Maleate is $C_{20}H_{23}C_lN_2O_4$ *(Figure 1. chemical structure of Chlorpheniramine Maleate salt)*. Chlorpheniramine Maleate is a H-1 receptor blocker and acts as an antihistamine used to relieve symptoms of allergy, hay fever, and the common cold. These symptoms include rash, watery eyes, itchy eyes/nose/throat/skin, cough, runny nose, and sneezing. 2-[4-(2-methyl-propyl)phenyl]propanoic acid is the IUPAC name of the Ibuprofen. The empirical formula for Ibuprofen is $C_{13}H_{18}O_2$ *(Figure 2. chemical structure of Ibuprofen)*. Ibuprofen is a nonselective inhibitor of COX-2, an enzyme involved in prostaglandin synthesis of the arachidonic acid pathway. Its pharmacological effects are believed to be due to inhibition of COX-2 which decrease the synthesis of prostaglandin involved in mediating inflammation pain, fever, and swelling [1-5]. (1S,2S)-2-(methylamino)-1-phenylpropan-1-ol hydrochloride is the IUPAC name of the Pseudoephedrine Hydrochloride. The empirical formula for Pseudoephedrine Hydrochloride is $C_{10}H_{16}ClNO$ *(Figure 3. chemical structure of Pseudoephedrine Hydrochloride)* The most common use for Pseudoephedrine is as a decongestant, for conditions including nasal congestion, sinus congestion, and eustachian tube congestion, as it shrinks swollen nasal mucous membranes and reduces tissue hyperemia and edema [6] Other uses include vasomotor rhinitis, first-line treatment for priapism, and off-label use for hyperprolactinemia, while veterinarians often use pseudoephedrine off-label to treat incontinence in dogs and cats [6]. In conjunction with other medications, Pseudoephedrine is often used to treat allergic rhinitis, croup, sinusitis, otitis media, and tracheobronchitis [7].

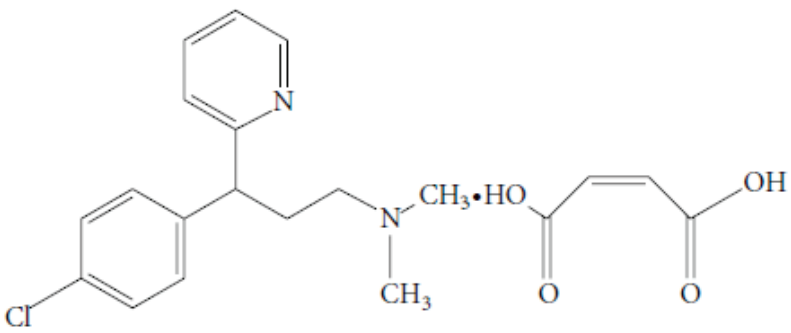

Figure 1. Chemical structure of Chlorpheniramine Maleate.

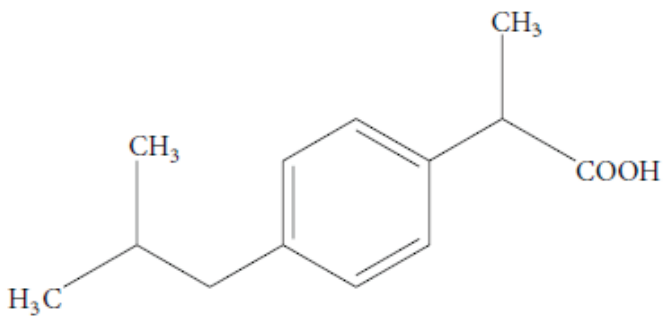

Figure 2. Chemical structure of Ibuprofen.

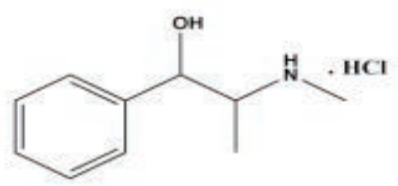

Figure 3. Chemical structure of Pseudoephedrine Hydrochloride.

There is no related substances method reported for the simultaneous estimation of Chlorpheniramine Maleate (CPM), Ibuprofen (IBU), and Pseudoephedrine Hydrochloride (PSEH) in combined dosage form. The present study involved the development and validation of RP HPLC method for the estimation of CPM, IBU, and PSEH in combined pharmaceutical dosage form.

Experimental

Reagents and Materials

Analytically pure standards of CPM, IBU, and PSEH were obtained from Supriya Lifescience (Mumbai, India), Hubei (Hubei, China) and Malladi (Guindy, Chennai) respectively. Analytically pure impurity standards of Ephedrine, Ibuprofen Impurity E and Chlorpheniramine Maleate Impurity C were obtained from Ph.Eur (European Pharmacopoeia), USP (United States Pharmacopeia) and Pharmaffiliates(India) respectively. HPLC grade Methanol and Acetonitrile were obtained from JT Baker. HPLC grade Decan^{-1} Sulphonic Acid Sodium Salt, Triethylamine and Phosporic Acid 85% were obtained from Merck. HPLC grade Sodium Hydroxide were obtained from Sigma Aldrich. HPLC grade Potassium Dihydrogen Phosphate were obtained from Panreac. The water was distilled and deionised by using Millipore Milli Q Ultrapure system. Syrup formulation (Pharmactive Pharmaceutical Company, Turkey) containing labelled amount of 2 mg of Chlorpheniramine Maleate, 200 mg of Ibuprofen, and 30 mg of Pseudoephedrine Hydrochloride was used for the study.

Apparatus and Chromatographic Conditions

The liquid chromatographic system consists of Shimadzu LC20 series equipped with a PDA detector, quaternary pump, and automatic injector, 20 µL fixed loop. The analytes were monitored at 215 nm for Ibuprofen and Pseudoephedrine Hydrochloride and 225 nm for Chlorpheniramine Maleate. Chromatographic analysis was performed using a Direct In-Line Filter and 1 cm Guard Cartridge holders guard column and YMC Triart C18 column (5 µm × 250mm × 4.6 mm). All the drugs and chemicals were weighed on Mettler Toledo XP26, XP2U,XP205 electronic balance. Chromatographic separation was achieved using timed gradient mode *(Table 1)*. The mobile phase consisted of A: pH 2.5 buffer (3.4 g KH_2PO_4, 5 g Decan-1-sulphonic acid sodium salt, and 5 mL of Triethylamine in 1000 mL deionised water adjusted to pH 2.5 with H_3PO_4) and B: Methanol (100%). The flow rate of the mobile phase was 0.7 mL/min. The column temperature was 45˚C and sample temperature was 15˚C. The injection volume was 20 µL. Diluent was pH:7.2 Buffer:Methanol (500:500 v/v).

Table 1. Gradient Mode.

Time (min.)	Mobile Phase A (%)	Mobile Phase B (%)	FlowRate
0.01	48	52	0.7
27.00	48	52	0.7
27.01	35	65	0.7
37.00	35	65	0.7
37.01	35	65	0.6
55.00	35	65	0.6
55.01	35	65	0.7
60.00	35	65	0.7
60.01	48	52	0.7
75.00	48	52	0.7

Diluent Preparation

Preparation of pH 7.2 Buffer: 6.8 g of Potassium Dihydrogen Phosphate and 1.2 g of Sodium Hydroxide were dissolved in 1000 mL of distilled water. pH is adjusted to 7.2 with ortho-phosphoric acid.

Preparation of Diuent: 500 mL of pH 7.2 Buffer and 500 mL of Methanol was mixed, filtered through 0.45 µm membrane filter, and degassed.

Standard Solution Preparation

PSEH stock standard solution was prepared by transferring an accurately weighed 9 mg of Pseudoephedrine Hydrochloride working standard into a 20-mL volumetric flask. 10 mL of diluent was added followed by sonication for 5 min using an ultrasonic source. The solution was brought up to final volume with the diluent. CPM stock standard solution was prepared by transferring an accurately weighed 6 mg of Chlorpheniramine Maleate working standard into a 100-mL volumetric flask. 60 mL of diluent was added followed by sonication for 5 min using an ultrasonic source. The solution was brought up to final volume with the diluent. IBU stock standard solution was prepared by transferring an accurately weighed 12 mg of Ibuprofen working standard into a 20-mL volumetric flask. 10 mL of diluent was added followed by sonication for 5 min using an ultrasonic source. The solution was brought up to final volume with the diluent. For preparation of standard solution, 2 mL of PSEH stock standard solution, 0.5 mL of CPM stock standard solution, 2 mL of IBU stock standard solution transferred into a 100-mL volumetric flask and the solution was brought up to final volume with the diluent ($C_{Ibuprofen}$= 0.012 mg/mL, $C_{Pseudoephedrine\ HCl}$= 0.009 mg/mL, $C_{Chlorphenamine\ Maleate}$= 0.0003 mg/mL).

Sample Solution Preparation

Approximately 3 mL syrup (equivalent to 60 mg Ibuprofen, 9 mg Pseudoephedrine HCl, and 0.6 mg Chlorpheniramine Maleate) was weighed into a 10-mL volumetric flask, dissolved with 5 mL of diluent was added followed by sonication for 2 min using an ultrasonic source. The solution was brought up to final volume with the diluent and vortex for 1 min and filtered through 0.22 µm PVDF filter and injected into the HPLC system. ($C_{Ibuprofen}$= 6.0 mg/mL, $C_{Pseudoephedrine\ HC}$l= 0.9 mg/mL, $C_{Chlorpheniramine\ Maleate}$= 0.06 mg/mL).

Method Validation

Precision

Precision was determined as both repeatability and intermediate precision, in accordance with ICH recommendations. Standard solution prepared at 100% working concentration was injected six times to evaluate the system precision. Peak areas obtained from injections and Relative Standard Deviation (RSD%) value between them were calculated. The RSD % of not more than 5% was recommended. Repeatability (precision of the method) and intermediate precision were also evaluated. For repeatability, 6 syrup spiked samples, which were prepared by adding known impurities at the limit concentration and the analysis carried out. For intermediate precision, 6 syrup spiked samples were prepared and analysed on a different day using a different device and column by a different analyst. Repeatability and intermediate precision results, for IBU, PSEH and CPM impurities were calculated. The difference between result means of the two analysts were also calculated.

Linearity and range

To prove the linear response relation, peak areas of the standard solutions between 0.015% LOQ (Limit of Quantification) % and 1.2% of specification at 7 different concentrations (0.015%, 0.1%, 0.3%, 0.5%, 0.8%, 1.0%, 1.2%) for PSEH, the standard solutions between 0.002 % LOQ (Limit of Quantification) % and 0.24% of specification at 7 different concentrations (0.002%, 0.05%, 0.06%, 0.1%,0.16%, 0.2%, 0.24%) for IBU, the standard solutions between 0.1% LOQ (Limit of Quantification) % and 0.6 % of specification at 6 different concentrations (0.1%, 0.15%, 0.25%, 0.4%, 0.5%, 0.6%) for CPM, the standard solutions between 0.015% LOQ (Limit of Quantification) % and 1.2% of specification at 7 different concentrations (0.015%, 0.1%, 0.3%, 0.5%, 0.8%, 1.0%, 1.2%) for Ephedrine, the standard solutions between 0.002% LOQ (Limit of Quantification) % and 0.24% of specification at 7 different concentrations (0.002%, 0.05%, 0.06%, 0.1%, 0.16%, 0.2%, 0.24%) for Ibuprofen Impurity E, the standard solutions between 0.1% LOQ (Limit of Quantification) % and 0.6% of specification at 6 different concentrations (0.1%, 0.15%, 0.25%, 0.4%, 0.5%, 0.6%) for Chlorpheniramine Maleate Impurity C were measured and linearity curves were plotted. The prepared linearity solutions were analysed, y=ax+b threshold was found and regression analysis was performed (y: area, a: slope, b: intercept, x: concentration, mg/mL). Correlation coefficient between concentration and areas of more than 0.99 was sought. The response factor for Ephedrine, Ibuprofen Impurity E, Chlorfeniramine Maleate Impurity C impurities with respectively calculated according to PSEH, IBU, CPM. Then this response factor was used in denomination of calculation formula of % Impurity.

Detection (LOD) and Quantification (LOQ) Limits

LOD and LOQ were determined by the Signal/Noise (S/N) method. All of the Impurities and active substances were injected and obtained S/N were evaluated.

Specificity

Specificity test was performed to demonstrate the ability of the analytical method to measure only the intended substances in a given sample. Standard, unspiked sample, placebo, impurity standards, spiked sample, diluent were injected into the HPLC system and analysed. Chromatogram of the injected solutions were evaluated.

Accuracy and recovery

Accuracy was determined by the standard addition method. Accuracy of method was perform at LOQ%, release limit level and 120% of the shelf life limit of the working concentration by adding Ephedrine, Ibuprofen Impurity E and Chlorpheniramine Maleate Impurity C to the sample solution. In totally, 9 samples were prepared (3 for each level) and injected triplicate. Recovery (%) and RSD (%) were calculated for each concentration.

Robustness

The robustness of the developed method was measured to evaluate the influence of deliberate variation in the chromatographic conditions. The robustness of the method was evaluated by changing the flow rate (0.6 and 0.8 mL/min), column temperature (40˚C and 50˚C) column brand (Inertsil ODS-4 column 250 x 4.6 mm 5 µm), and mobile phase pH change (pH:2.4-pH:2.6). Robustness testing was performed in order to obtain information about those critical parameters affecting the response (peak area, retention time).

Results and discussion

Precision

System precision was determined by performing injection repeatability standard solution and the obtained RSD values for PSEH, IBU and CPM were found 0.11%, 0.06% and 3.4% respectively. The low RSD values indicate that the system is precise. Also in comparison of repeatability and intermediate precision results of differences between means of two analysts sample results for the precision.

Table 2. Repeatability data of validated method (%).

Injection No	Ephedrine (%)	Ibuprofen Impurity E (%)	Chlorpheniramine Maleate Impurity C (%)
1	1.03	0.21	0.48
2	1.04	0.20	0.50
3	1.03	0.20	0.50
4	1.03	0.20	0.50
5	1.03	0.20	0.50
6	0.98	0.20	0.46
Average	1.02	0.20	0.49
SD	0.02	0.00	0.02
RSD (%)	2.1	2.0	3.4

Table 3. Intermediate precision data of validated method (%).

Injection No	Ephedrine (%)	Ibuprofen Impurity E (%)	Chlorpheniramine Maleate Impurity C (%)
1	1.05	0.20	0.50
2	1.05	0.20	0.51
3	1.04	0.20	0.48
4	1.04	0.20	0.49
5	1.03	0.20	0.48
6	1.02	0.20	0.51
Average	1.04	0.20	0.50
SD	0.01	0.00	0.01
RSD (%)	1.1	0.0	2.8

Table 4. Repeatability and Intermediate precision data mean and differences results between two analyst (%)

	Repeatability (%)	Intermediate Precision(%)	Differences (%)
Ephedrine	1.02	1.04	0.02
Ibuprofen Impurity E	0.20	0.20	0.00
Chlorpheniramine Maleate Impurity C	0.49	0.50	0.01

The method is found to be precise with respect to the criteria of the system precision, intermediate precision and repeatability.

Linearity and range

The calibration curve for PSEH was found to be linear in the range of 0.000135–0.010801 mg/mL with a correlation coefficient of 1.00. The calibration curve for Ephedrine was found to be linear in the range of 0.000135–0.010782 mg/mL with a correlation coefficient of 1.00. The calibration curve for IBU was found to be linear in the range of 0.000121–0.014537 mg/mL with a correlation coefficient of 1.00.

The calibration curve for Ibuprofen Impurity E was found to be linear in the range of 0.000120–0.014371 mg/mL with a correlation coefficient of 1.00. The calibration curve for CPM was found to be linear in the range of 0.0000610–0.000366 mg/mL with a correlation coefficient of 1.00. The calibration curve for Chlorpheniramine Maleate Impurity C was found to be linear in the range of 0.0000447–0.000268 mg/mL with a correlation coefficient of 0.99.

Table 5. Linearity Data for PSEH of validated method.

Concentration Level in (%)	Theoretical Concentration of PSEH (mg/mL)(x-value)	Response (Area) (y value)
0.015%	0.000135	5422.2
0.1%	0.000900	37886.2
0.3%	0.002700	108824.5
0.5%	0.004500	182038.8
0.8%	0.007201	289913.0
1.0%	0.009001	361572.8
1.2%	0.010801	434768.3

Table 6. Linearity Data for Ephedrine of validated method.

Concentration Level in (%)	Theoretical Concentration of Ephedrine (mg/mL) (x-value)	Response (Area) (y value)
0.015%	0.000135	5493.8
0.1%	0.000898	37196.8
0.3%	0.002696	106510.0
0.5%	0.004493	179369.3
0.8%	0.007188	284115.0
1.0%	0.008985	358511.0
1.2%	0.010782	428597.8

Table 7. Linearity Data for Ibuprofen of validated method.

Concentration Level in (%)	Theoretical Concentration of Ibuprofen (mg/mL) (x-value)	Response (Area) (y value)
0.002%	0.000121	12918.8
0.05%	0.003028	254683.8
0.06%	0.003634	296903.5
0.1%	0.006057	480526.3
0.16%	0.009691	782628.5
0.2%	0.012114	984041.7
0.24%	0.014537	1183409.2

Table 8. Linearity Data for Ibuprofen Impurity E of validated method.

Concentration Level in (%)	Theoretical Concentration of Ibuprofen Impurity E (mg/mL) (x-value)	Response (Area) (y value)
0.002%	0.000120	19923.0
0.05%	0.002994	252915.2
0.06%	0.003593	297940.5
0.1%	0.005988	486689.5
0.16%	0.009580	796728.5
0.2%	0.011976	1003007.7
0.24%	0.014371	1176968.8

Table 9. Linearity Data for Chlorpheniramine Maleate of validated method.

Concentration Level in (%)	Theoretical Concentration of CHL (mg/mL) (x-value)	Response (Area) (y value)
0.1%	0.000061	3566.0
0.15%	0.000091	5309.0
0.25%	0.000152	8992.7
0.4%	0.000244	14439.0
0.5%	0.000305	17768.8
0.6%	0.000366	22039.5

Table 10. Linearity Data for Chlorpheniramine Maleate Impurity C of validated method.

Concentration Level in (%)	Theoretical Concentration of Impurity C (mg/mL) (x-value)	Response CPM (Area) (y value)
0.1%	0.000045	2732.0
0.15%	0.000067	2912.5
0.25%	0.000112	5461.2
0.4%	0.000179	8322.5
0.5%	0.000223	11825.3
0.6%	0.000268	13031.3

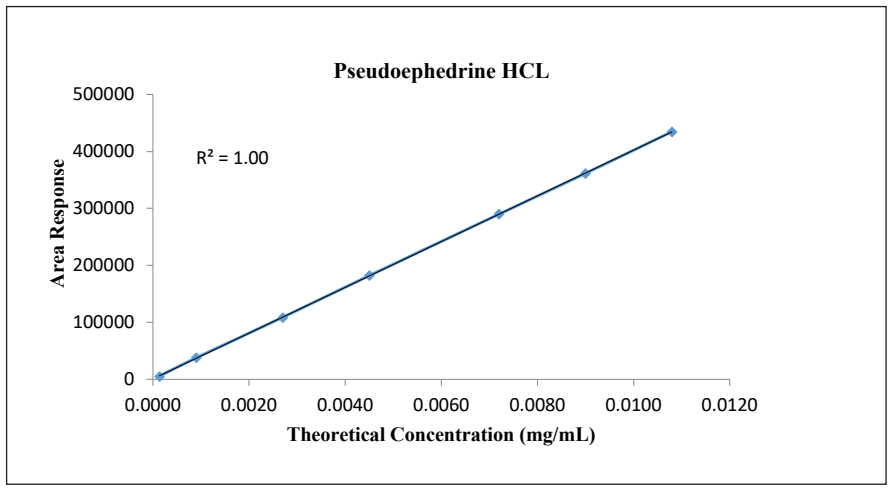

Figure 4. Linearity Plot of Pseudoephedrine HCl: A graph of Concentration (x value) against area response obtained (y- value) is plotted.

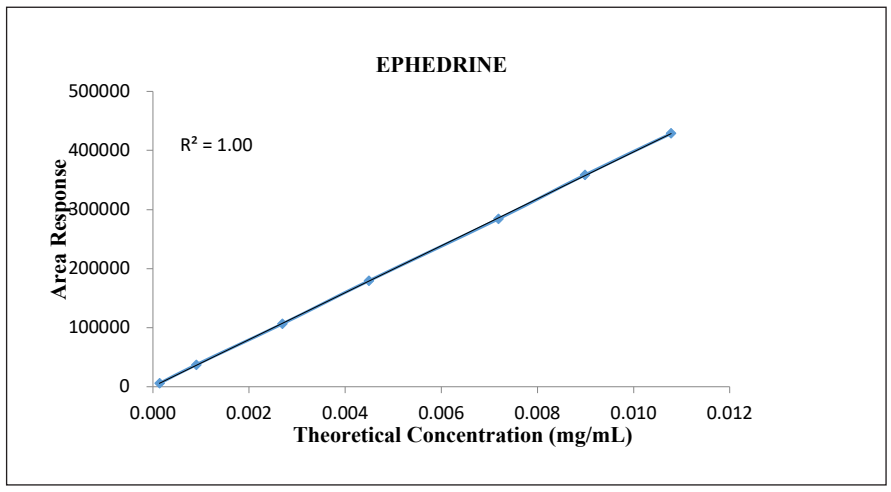

Figure 5. Linearity Plot of Ephedrine: A graph of Concentration (x value) against area response obtained (y- value) is plotted.

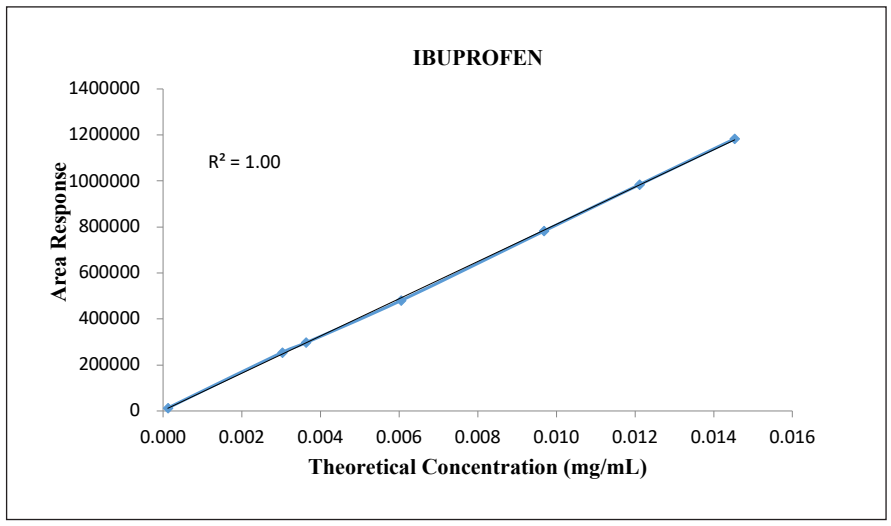

Figure 6: Linearity Plot of Ibuprofen: A graph of Concentration (x value) against area response obtained (y- value) is plotted.

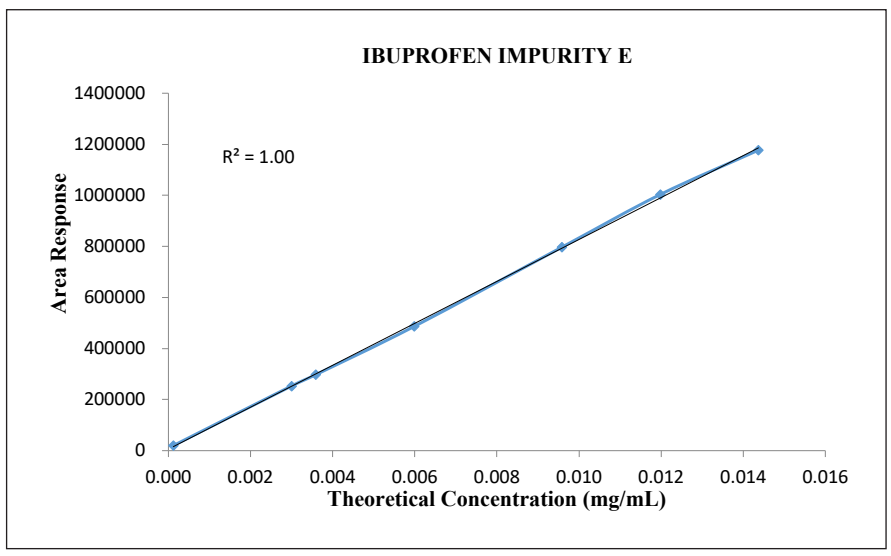

Figure 7: Linearity Plot of Ibuprofen Impurity E: A graph of Concentration (x value) against area response obtained (y- value) is plotted.

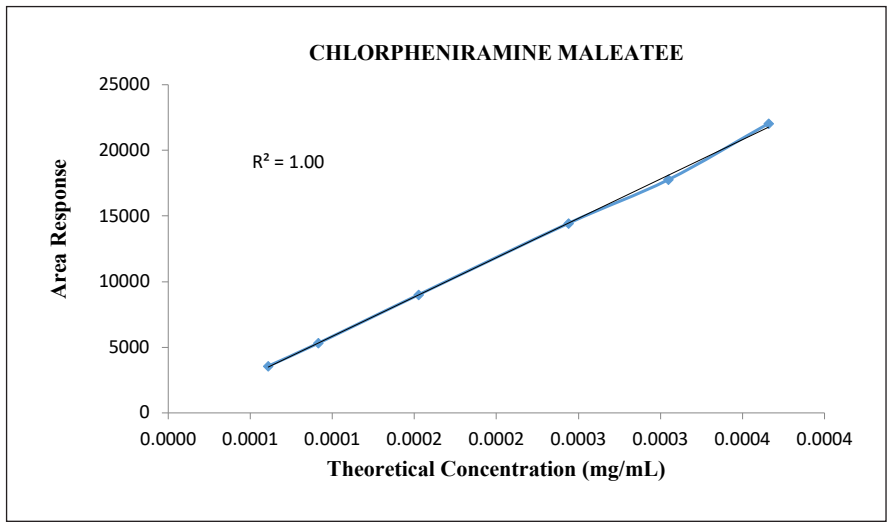

Figure 8: Linearity Plot of Chlorpheniramine Maleate: A graph of Concentration (x value) against area response obtained (y- value) is plotted.

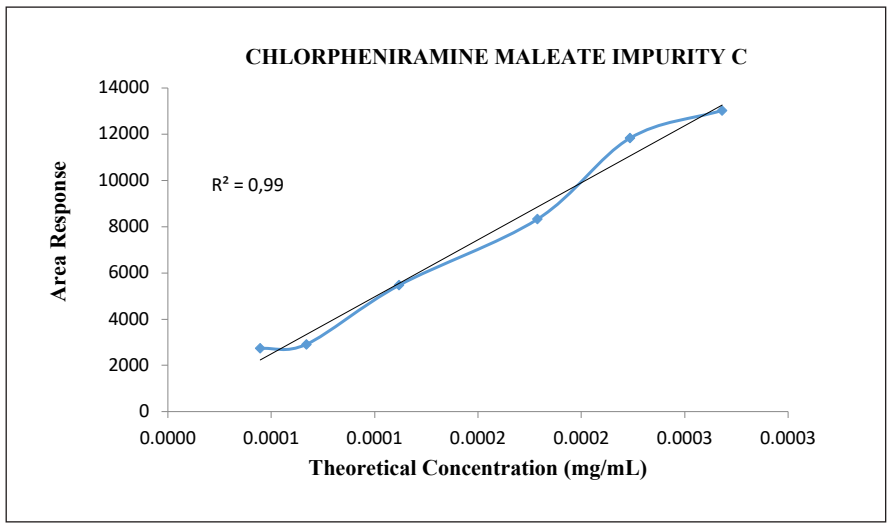

Figure 9: Linearity Plot of Chlorpheniramine Maleate Impurity C: A graph of Concentration (x value) against area response obtained (y- value) is plotted.

Table 11. Validation parameters for CPM, IBU, and PSEH.

Parameter	Pseudoephedrine	Ibuprofen	Chlorpheniramine Maleate
% Y-axis	802.27	1836.1	159.8
Intercept			
Slope	$4x10^7$	$8x10^7$	$6x10^7$
Response Factor for Division	1.00*	1.00*	1.00*

*It will be used for quantification of unknown impurities

Table 12. Response Factor of Impurities.

Parameter	Ephedrine	Ibuprofen Impurity E	Chlorpheniramine Maleate Impurity C
% Y-axis	407.61	5933.8	29.17
Intercept			
Slope	$4x10^7$	$8x10^7$	$5x10^7$
Response Factor for Division	1.01**	0.99***	1.21****

** Calculated based on Pseudoephedrine HCl
*** Calculated based on Ibuprofen
****Calculated based on Chlorpheniramine Maleate

Specificity

The applied method demonstrated excellent specificity. There are no interfering peaks in the chromatogram originating from the diluent, impurities and placebo. The spectrum of IBU, PSEH and CPM peaks did not interfere with the other peaks in Standard and Sample solution chromatograms. The selectivity of the method was proved. Spiked sample data is shown in *Figure 10*.

Accuracy and Recovery

Accuracy was determined by the standard addition method. Accuracy of the method was performed at LOQ%, release limit level and 120% of the shelf life limit of the working concentration by adding Ephedrine, Ibuprofen Impurity E and Chlorpheniramine Maleate Impurity C to the sample solution. In totally, 9 samples were prepared (3 for each level) and injected triplicate. Recovery (%) and RSD (%) were calculated for each concentration. Results are shown in *Table 13-15*. All results meet the acceptance criteria.

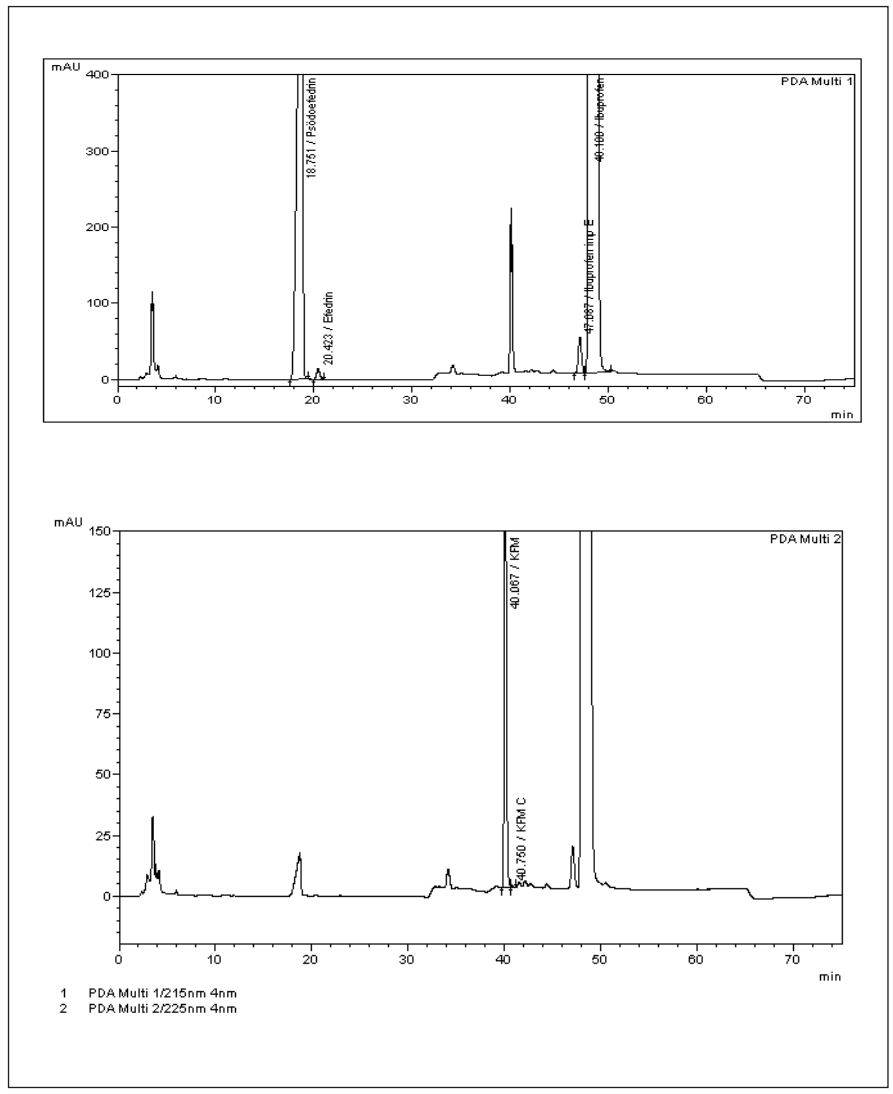

Figure 10. Chromatogram of Spiked Sample (RT: 18.75 for Pseudoephedrine, RT: 20.42 for Ephedrine, RT: 40.07 for Chlorpheniramine Maleate, RT: 40.8 for Chlorpheniramine Maleate Impurity C, RT: 47.08 for Ibuprofen Impurity E, RT: 48.1 for Ibuprofen)

Table 13. Recovery data of validated method (%).

Solution Name	Recovery of Ephedrine (%)
0.015 %	91.39
	90.09
	100.62
1.00%	99.99
	101.07
	100.96
1.20%	96.04
	98.18
	96.53
Average	97.21
SD	3.3
RSD %	3.4

Table 14. Recovery data of validated method (%).

Solution Name	Recovery of Ibuprofen Impurity E (%)
0.002%	101.87
	98.68
	90.66
0.2%	101.81
	97.56
	95.57
0.24%	96.03
	96.30
	97.92
Average	97.38
SD	3.4
RSD %	3.5

Table 15. Recovery data of validated method (%).

Solution Name	Recovery of Chlorpheniramine Maleate Impurity C (%)
0.1%	118.56
	106.95
	106.03
0.5%	103.69
	108.06
	109.68
0.6%	108.07
	107.32
	103.10
Average	107.94
SD	2.3
RSD %	2.1

Detection (LOD) and Quantification (LOQ) Limits

The LOD and LOQ of the method were show the *Table 16 and Table 17*. The method can be used for detection and quantification of trace amounts of Ibuprofen, Pseudoephedrine HCl, Chlorpheniramine Maleate impurities over a wide range of concentrations.

Table 16. LOD data of validated method (%).

Substances	LOD Concentration (mg/mL)	S/N (Signal/Noise)
Ephedrine	0.00000405	3.4
Ibuprofen Impurity E	0.000036	13.3
Chlorpheniramine Maleate Impurity C	0.000018	3.9
Pseudoephedrine HCl	0.00000405	3.4
Ibuprofen	0.000036	10.6
Chlorpheniramine Maleate	0.000018	4.5

Table 17. LOQ data of validated method (%).

Substances	LOQ Concentration (mg/mL)	S/N (Signal/Noise)
Ephedrine	0.0000135	12.5
Ibuprofen Impurity E	0.00012	46.0
Chlorpheniramine Maleate Impurity C	0.00006	10.6
Pseudoephedrine HCl	0.0000135	11.6
Ibuprofen	0.00012	26.9
Chlorpheniramine Maleate	0.00006	15.0

Robustness

With the Robustness parameter, the effect of changes in the method parameters and solution stability were examined. For this aim parameters which listed below were performed;

- Flow rate: 0.6 mL/min and 0.8 mL/min
- Column Temperature: 40°C and 50°C
- Mobile Phase pH Change: pH:2.3 and pH:2.5
- Column Brand Change: Inertsil ODS 4 250 mm x 4.6 mm x 5.0 µm

Table 18. Retention time of the Robustness of the method by small changes in chromatographic conditions.

Parameter	Ephedrine (min)	Ibuprofen Impurity E (min)	Chlorpheniramine Maleate Impurity C (min)
Normal	24.27	50.69	42.59
Flow Rate-0.6 mL/min	28.95	55.70	45.54
Flow Rate-0.8 mL/min	21.52	46.97	40.55
Column Temperature: 40°C	26.35	51.83	43.52
Column Temperature: 50°C	23.12	49.47	42.17
Mobile Phase pH Change: pH:2.3	24.00	50.44	42.75
Mobile Phase pH Change: pH:2.5	-	-	-
Different Column	20.65	47.19	40.82

Table 19. Retention time of the Robustness of the method by small changes in chromatographic conditions.

Parameter	Pseudoephedrine HCl (min)	Ibuprofen (min)	Chlorpheniramine Maleate (min)
Normal	21.64	52.09	41.64
Flow Rate-0.6 mL/min	25.72	57.12	44.81
Flow Rate-0.8 mL/min	19.13	48.26	39.66
Column Temperature: 40°C	23.20	53.37	42.70
Column Temperature: 50°C	20.73	50.82	41.14
Mobile Phase pH Change: pH:2.3	21.41	51.88	41.80
Mobile Phase pH Change: pH:2.5	-	-	-
Different Column	18.81	48.26	40.20

Table 20. Relative Standard Deviation of the standard solution by small changes in chromatographic conditions.

Parameter	Pseudoephedrine HCl (%)	Ibuprofen (%)	Chlorpheniramine Maleate (%)
Normal	0.27	0.21	1.52
Flow Rate-0.6 mL/min	0.12	0.16	0.80
Flow Rate-0.8 mL/min	0.15	0.08	0.76
Column Temperature: 40°C	0.12	0.16	3.71
Column Temperature: 50°C	0.09	0.13	0.76
Mobile Phase pH Change: pH:2.3	0.27	0.21	0.69
Mobile Phase pH Change: pH:2.5	-	-	-
Different Column	0.32	4.45	4.09

Obtained results showed that there was no significant change in system suitability parameters for any components except pH change. The method is robust in respect of changes to flow rate, column temperature, different manufacturer column. The method is sensitive to pH change

Stability

The stability of the drug in solution during analysis was determined by repeated analysis of the standard and spiked sample solutions at autosampler temperature (15°C). Peak areas were recorded and similarity % was calculated. It was proved that standard solution is stable at 15°C for 117 hours and sample solution is stable at 15°C for 49 hours.

Table 21. Stability of the Standard Solution for Pseudoephedrine HCl.

Time (hours)	Standard Solution (Pseudoephedrine)	Change % (Area)
0	300558	-
15	301195	0.2
24	301889	0.4
49	300232	0.1
83	303030	0.8
91	305897	1.8
117	300282	0.1

Table 22. Stability of the Standard Solution for Ibuprofen.

Time (hours)	Standard Solution (Ibuprofen)	Change % (Area)
0	933057	-
15	927982	0.5
24	928964	0.4
49	929594	0.4
83	930633	0.3
91	931667	0.1
117	937448	0.5

Table 23. Stability of the Standard Solution for Chlorpheniramine Maleate.

Time (hours)	Standard Solution (Chlorpheniramine Maleate)	Change % (Area)
0	16134	-
15	16000	0.8
24	16507	2.3
49	15953	1.1
83	16258	0.8
91	16469	2.1
117	16649	3.2

Table 24. Stability of the Spiked Sample Solution: Ephedrine.

Time (hours)	Ephedrine	Change % (Area)
0	309912	-
15	310737	0.3
24	310114	0.1
37	310348	0.1
49	311437	0.5
83	314325	1.4

Table 25. Stability of the Spiked Sample Solution: Ibuprofen Impurity E

Time (hours)	Ibuprofen Impurity E (Area)	Change %
0	1051222	-
15	1043505	0.7
24	1044317	0.7
37	1039344	1.1
49	914473	1.1
83	913500	1.2

Table 26. Stability of the Spiked Sample Solution: Chlorpheniramine Maleate Impurity C.

Time (hours)	Chlorpheniramine Maleate Impurity C (Area)	Change %
0	13080	-
15	13151	0.5
24	13036	0.3
37	12124	7.3
49	13124	0.3
83	11031	15.7

Conclusion

This HPLC method is sensitive, accurate, precise, reproducible, specific and stability-indicating. This method was found to be accurate and precise as indicated by the recovery studies and relative standard deviation. The Relative Response factor for impurities determined from validation can be used for quantification of impurities in Pseudoephedrine, Ibuprofen, Chlorpheniramine Maleate in routine testing, by which there is no need to use impurities' standard. Furthermore, the HPLC method could also be suggested for the routine analysis of Ibuprofen, Pseudoephedrine Hydrochloride, and Chlorpheniramine Maleate and their related substances in pharmaceutical dosage formulations.

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