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HPLC Method for Simultaneous Determination of Chlorpheniramine, Ibuprofen and Pseudoephedrine in Fixed-Dose Combination using Multiple Column Chemistries under Qbd Concept

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ABSTRACT

The concept of "Quality by Design" (QbD) is getting popularized in pharmaceutical manufacturing industry to understand the product and process to identify the risks involved during manufacturing. One of the perpetual quality attribute is to have robust analytical method that can provide consistent results though out the life cycle of the product. General considerations during analytical method validation is to perform robustness studies by deliberate changes made in pH of the buffer in mobile phase, change in organic ratio, change in column oven temperature, change in buffer strength and using different column lot numbers etc. However to improve the analytical quality standard, a novel method concept under QbD was introduced which uses single mobile phase for three drug components and estimates using different column chemistries used in pharmaceutical industry viz., C18, C8, phenyl and Cyano column. The validated RP-LC method was successfully applied to the quantitative determination of Chlorpheniramine, Ibuprofen and Pseudoephedrine in tablet dosage form, helping to improve quality control and to assure therapeutic efficacy using all column chemistries.

Keywords: Quality by Design (QbD), Validation, RP-LC, Stability Indicating, Fixed-dose combination and robust

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INTRODUCTION

Chlorpheniramine/Pseudoephedrine/Ibuprofen contains a nonsteroidal anti-inflammatory drug (NSAID), which relieves symptoms such as pain and sinus congestion due to colds, upper respiratory infections, and allergies. It is an antihistamine, analgesic, and decongestant combination. The antihistamine works by blocking the action of histamine, which helps to reduce symptoms such as watery eyes and sneezing[1]. The analgesic works in the brain to help decrease pain. The decongestant works by constricting blood vessels and reducing swelling in the nasal passages, which decreases stuffiness. OTC versions of Chlorpheniramine /Pseudoephedrine/ Ibuprofen tablets are available in 2mg/30mg/200mg in per tablet.

A combination drug most commonly refers to a fixed-dose combination (FDC), which is a formulation including two or more active pharmaceutical ingredients (APIs) combined in a single dosage form. Advantage of FDCs for Improved medication compliance by reducing the pill burden of patients[2-3]. Chlorpheniramine, Ibuprofen and Pseudoephedrine tablet dosage form is referred by United States Food and Drug Administration (USFDA) under over the counter (OTC) category. There were few numbers of analytical methods were available for quantification of this combination product[4-6]. For FDC products analytical challenge will always be there due to its variable strengths with different polarities of drug components. Getting shorter run methods is a difficult task. Reverse Phase Liquid Chromatography (RP-LC) is usually the analytical method of choice for quantification purpose. Getting robust and specific analytical method using different column chemistries available using single mobile phase is more challenging task to be considered to deliver acceptable chromatographic performance.

The aim of the present study is to develop a novel chromatographic method under QbD concept for simultaneous determination of Chlorpheniramine, Pseudoephedrine and Ibuprofen in fixed-dosage form using single mobile phase with different conventional column chemistries available in market. Hence this paper provides you simple, rapid, selective, and stability-indicating method for determining this fixed-dose combination product by using single mobile phase and with any conventional column chemistry. This method was fully validated as per USP and ICH guidelines[7-17].

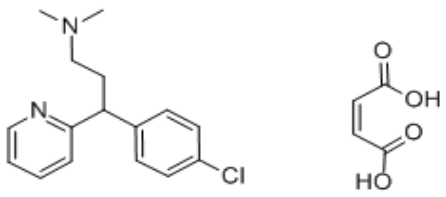


Figure1: chemical structure of Chlorpheniramine maleate

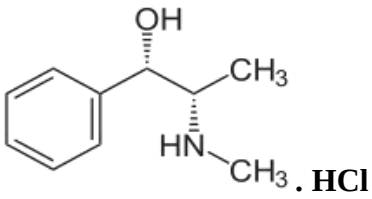


Figure2: chemical structure of Pseudoephedrine hydrochloride

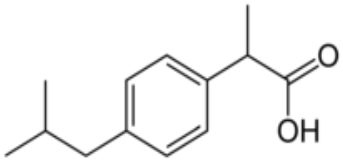


Figure3: chemical structure of Ibuprofen

MATERIALS AND METHODS

Instruments:

The LC systems, used for method development and method validation was Waters-Alliance HPLC equipped with separation module consisting of Quaternary gradient pump, Auto Sampler, thermostatted column compartment, Photo diode array detector (Model 2996), Auto sampler thermostatted, Computer with windows based Empower 2 Method validation manager software. Shimadzu prominence HPLC system equipped with separation module consisting of Quaternary gradient pump, Auto Sampler, thermostatted column compartment, Photo diode array detector (LC-20AT), Auto sampler thermostatted, Computer with windows based LC-Solution software. The output signal was monitored and processed using Empower 2 software and LC- Solution software. The columns used for validation were Phenomenex Luna 100 C18, (150mm x 4.6 mm id) with 5µm particle size (Column # 1), Phenomenex Luna 100 C8 (150 mm × 4.6 mm), 5 µm particle size(Column # 2), Zorbax SB- Phenyl (150 mm × 4.6 mm), 5 µm particle size (Column # 3) and Zorbax SB- CN (150 mm × 4.6 mm), 5 µm particle size (Column # 4).

Chemicals:

Chlorpheniramine maleate, Ibuprofen and Pseudoephedrine hydrochloride drug substance and Chlorpheniramine, Ibuprofen and Pseudoephedrine tablets (ADVIL ALLERGY SINUS tablets from Wyeth) were generously sponsored by Aurobindo Pharma Limited. Acetonitrile (HPLC grade, Merck chemicals), monobasic potassium phosphate (AR/GR grade, Merck chemicals) 1-Octane Sulfonic acid sodium salt (AR/GR grade, Merck chemicals) and Ortho phosphoric acid (AR/GR grade, Merck chemicals). Ultrapure water was prepared by using Millipore Milli-Q plus water purification system. All the chemicals and reagents were used as such without further purification.

METHOD DEVELOPMENT AND OPTIMIZATION FOR HPLC METHOD

The HPLC method carried out in this study aimed at developing chromatographic system capable of quantifying all three drug components of different polarity and strengths without compromising USP general system suitability criteria. The selection of this fixed-dose combination for analytical development is due to its nature polarity of each drug component. Pseudoephedrine hydrochloride is highly polar in nature. Chlorpheniramine maleate is mid polar in nature and Ibuprofen in non polar in nature.

Due to highly polar nature of Pseudoephedrine hydrochloride, it is difficult to retain the peak in conventional columns using normal buffers at different pH range. Based on this fact method should be developed either using polar embedded technology columns or by using ion pair buffers. For polar embedded technology different range of column chemistries are not available. Hence targeted to use ion pair buffer for chromatography to retain Pseudoephedrine hydrochloride in all types of conventional columns like C18, C8, Phenyl and Cyano. Based on type of column chemistry interaction, peaks will be retained on different columns at different retention times.

Pseudoephedrine hydrochloride and Chlorpheniramine maleate are basic in nature. These basic compounds can interact with underivatized free silanol groups of silica-based chemically bonded phases. It was observed that retention occurs by an ion-exchange process that involves protonated solutes and ionized silanols. This situation may leads to peak tailing, and poor column-to-column reproducibility. To reduce these interactions with the silanols, use mobile phases buffered at low pH, when silanol ionization is suppressed. For analysis of basic compounds anionic ion-pair reagents preferred to form neutral associates. Hence lower pH buffer was chosen for trail purpose along with 1-Octane sulfonic acid sodium salt as an ion pair reagent. 0.02M phosphate buffer containing 6.6g of ion pair at pH 2.5 buffer was finalized as mobile phase A and acetonitrile was mobile phase B. Mobile phase containing a degassed mixture of mobile phase A and Mobile phase B in the ratio of 55:45 v/v.

Method development work was targeted to develop on different column chemistries under QbD concept. Different available column chemistries were selected for development purpose and chosen Phenomenex Luna 100 C18, (150mm x 4.6 mm id) with 5µm particle size, Phenomenex Luna 100 C8 (150 mm × 4.6 mm), 5 µm particle size, Zorbax SB- Phenyl (150 mm × 4.6 mm), 5 µm particle size and Zorbax SB- CN (150 mm ×4.6 mm), 5 µm particle size for experimentation. In these columns all three components are well separated and there is a variation in retention

times observed in each column based on different types of interactions of drugs with the column chemistries.

Standard solution was prepared at 2 µg/mL for Chlorpheniramine maleate, 30 µg/mL for Pseudoephedrine hydrochloride and 200 µg/mL for Ibuprofen and injected in Diode Array detector at 30µL injection volume. From the spectral data of individual component it was observed that, the suitable wavelength to monitor all three components was at 215nm. Preferred mode for elution of drug components in ion pair chromatography is by isocratic. Hence the same was preferred. Retention times observed for Pseudoephedrine hydrochloride, Chlorpheniramine maleate and Ibuprofen is 1.70 min, 2.74 min and 12.79 min respectively in column # 1, 1.69min, 2.63min and 12.09 min respectively in column # 2, 1.87min, 2.91min and 5.03 min respectively in column # 3 and 1.68min, 2.27min and 3.14 min respectively in column # 4.

Finalized Chromatographic Conditions

The finalized chromatographic columns were Phenomenex Luna 100 C18, (150mm x 4.6 mm id) with 5µm particle size (Column # 1), Phenomenex Luna 100 C8 (150 mm × 4.6 mm), 5 µm particle size (Column # 2), Zorbax SB- Phenyl (150 mm × 4.6 mm), 5 µm particle size (Column # 3) and Zorbax SB- CN (150 mm × 4.6 mm), 5 µm particle size (Column # 4).

The mobile phase- A contains about 2.72 g of Mono basic potassium phosphate and 6.6 g of 1-Octane Sulfonic acid sodium salt in 1000 mL of Milli-Q water and mixed well. Adjust the pH to 2.50 ± 0.05 with diluted ortho phosphoric acid. Filter through 0.45µ Membrane filter. (Millipore PVDF or equivalent) and Acetonitrile was used as mobile phase-B. Mobile phase containing a degassed mixture of mobile phase A and Mobile phase B in the ratio of 55:45 v/v. The flow rate of the mobile phase was 1.2 mL/min with isocratic mode. The column temperature is maintained at 40°C and the detection is monitored at wavelength of 215 nm. The injection volume is 30 µL. Diluent-I consists of water and methanol in the ratio 50:50 v/v and Diluent-II consists of 6.8 g of Mono basic Potassium phosphate and 0.7 g of Sodium hydroxide in 1000 ml of water. Adjust the pH to 6.5 ± 0.05 with diluted ortho phosphoric acid solution.

PREPARATION OF SOLUTIONS

Preparation of standard solution

Initial Standard stock solutions of Chlorpheniramine maleate (0.102 mg/mL) and Pseudoephedrine hydrochloride (1.52 mg/mL) were prepared in Diluent-I. For Ibuprofen (0.20 mg/mL) were prepared by dissolving in 10 mL Diluent-I followed by addition of Chlorpheniramine maleate and Pseudoephedrine hydrochloride Standard stock solutions to

obtain a final concentration of 2 µg/mL for Chlorpheniramine maleate, 30 µg/mL for Pseudoephedrine hydrochloride and 200 µg/mL for Ibuprofen respectively using Diluent-II.

Preparation of sample solution

Weigh and make a fine powder of not less than 10 tablets using a suitable mortar and pestle. Transfer an accurately weighed quantity of the tablet powder equivalent to about 2 mg of Chlorpheniramine maleate into a 100 mL clean, dry volumetric flask, add about 75 mL of diluent-I and sonicate for about 45 minutes with intermittent shaking at room temperature. Allow the solution to cool to room temperature and dilute the volume with diluent-I and mix. Centrifuge a portion of the solution at 5000 rpm for about 5 minutes. Transfer 5 mL of clear, supernatant solution into a 50 mL volumetric flask, dilute to the volume with diluent-II and mix. Filter the solution through a suitable 0.45 µ membrane filter.

Chromatographic System suitability parameters

The column efficiency as determined from the peaks Chlorpheniramine, Pseudoephedrine and Ibuprofen is not less than 3000 USP plate count, the Symmetry factor for the same peaks is not more than 2.0 and the USP resolution between individual peaks should not be less than 3.0. RSD for corresponding peak areas of five replicate injections of the standard solution is not more than 2.0%. For data refer **Table 15**.

Table 15. Chromatographic system suitability data

Name of the component	USP Resolution	USP Tailing factor	USP Theoretical plats
Pseudoephedrine	--	1.1	3391
Chlorpheniramine	7.2	1.1	5424
Ibuprofen	29.8	0.9	9963
Pseudoephedrine	--	1.1	5260
Chlorpheniramine	9.3	1.2	7550
Ibuprofen	30.7	1.0	13197
Pseudoephedrine	--	1.1	3176
Chlorpheniramine	6.6	1.2	3173
Ibuprofen	10.9	0.9	9320
Pseudoephedrine	--	1.1	3178
Chlorpheniramine	4.8	1.1	4570
Ibuprofen	6.1	1.1	6336

Analytical method validation

OTC versions of Chlorpheniramine/Pseudoephedrine/Ibuprofen tablets are available 2mg/30mg/200mg in per tablet. The same strength was considered for entire validation. The developed method validated for Specificity (Stress studies), Precision, Intermediate precision, Linearity, Accuracy, solution stability and Robustness in Column #1, Column #2, Column #3 and Column #4 as per ICH guidelines.

Specificity and Stress studies

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The stress conditions used for degradation study are Acid hydrolysis (2M HCl / 65°C / 60 min), Base hydrolysis (5M NaOH / 65°C / 60 min), Oxidation (10% H2O2 / 65°C / 30 min), Thermal (70°C / 72 hours), Humidity (90%RH / 25°C / 72 Hours) and Photolytic (white fluorescent 1.2 million lux hours UV 200 watt hr/m^2 for 7 days). These stressed samples were analyzed on Column #1, Column#2, Column #3 and Column #4.

Precision

The precision of the method is checked by injecting six individual preparations at test concentration of 2µg/mL for Chlorpheniramine maleate, 30µg/mL for Pseudoephedrine hydrochloride and 200µg/mL for Ibuprofen. The percentage RSD for each component is calculated on all columns. The intermediate precision (Ruggedness) of the method was evaluated by different analyst using different column and different HPLC instrument on different day.

Linearity

Linearity curves were plotted from 50% level to 150% of the test concentration. The correlation coefficient, slope and Y-intercept of the Linearity curve are calculated for each component.

Accuracy

A known amount of each component of drug is added to the placebo at 50%, 100% and 150% of the analyte concentration. The % w/w of recoveries for all the components was calculated.

Solution Stability:

In order to demonstrate the stability of both reference and sample solutions, these solutions were injected immediately after preparation and at periodical intervals by maintaining room temperature.

Robustness

To determine the robustness of the developed method, experimental conditions are deliberately changed and the impact of the variation on each drug component was evaluated in all different column chemistries. The flow rate of the mobile phase is 1.2 mL/min. To study the effect of flow rate ± 0.1 unit was changed i.e., 1.1 and 1.3 mL/min. The effect of column temperature is studied at 35°C and 45°C instead of 40°C. For pH, the buffer is adjusted to pH 2.3 and pH 2.7 instead of pH 2.5. Organic composition was adjusted to 57:43 v/v and 53:47 v/v instead of 55:45 v/v. For wavelength variation, ± 5 nm was changed from the working wavelength i.e., 215nm.

RESULTS AND DISCUSSION

All degradations conditions were performed using finalized chromatographic conditions in each column.

Specificity and Stress studies

From diluent and placebo solution there were no peaks observed. Stress studies on Chlorpheniramine/Pseudoephedrine/Ibuprofen tablets under different stress conditions suggested the following degradation behavior.

Degradation in Acid stress condition

Significant degradation is observed in Chlorpheniramine, Pseudoephedrine and Ibuprofen drug components.

3.1.2 Degradation in Base stress condition

Ibuprofen significantly undergone degradation in Base stress condition. Whereas there is no considerable degradation observed for Chlorpheniramine and Pseudoephedrine.

Degradation in Peroxide stress condition

There is no significant degradation observed for Chlorpheniramine, Pseudoephedrine and Ibuprofen.

Degradation in Thermal stress condition

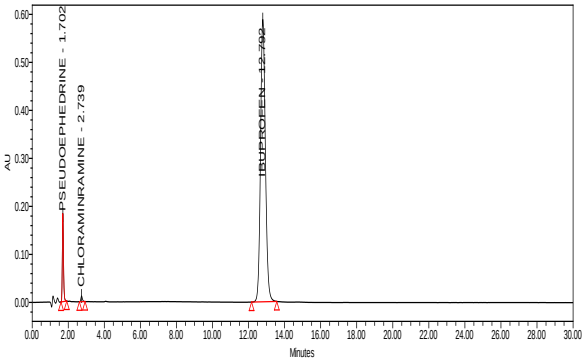
There is a slight degradation observed for Chlorpheniramine, Pseudoephedrine and Ibuprofen.

Degradation in Humidity stress condition

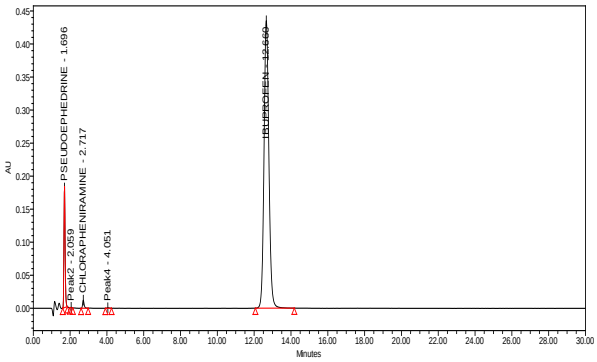
Ibuprofen and Pseudoephedrine significantly undergone degradation in Humidity stress condition. Whereas there is no considerable degradation observed for Chlorpheniramine.

Degradation in photolytic stress condition

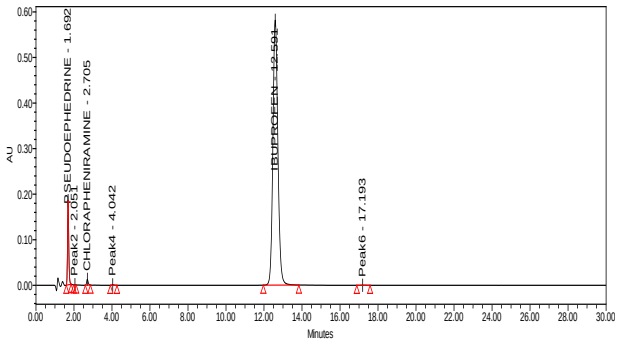
There is no significant degradation observed for Chlorpheniramine, Pseudoephedrine and Ibuprofen. (**Figure 4, Table 1 to Table 4**)



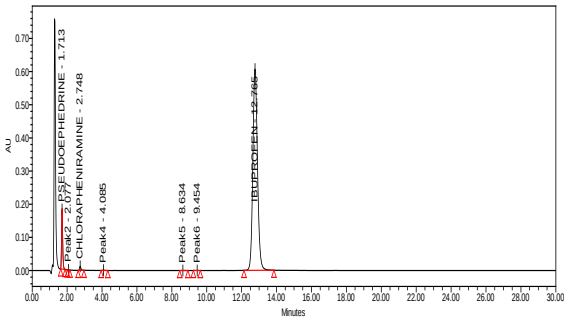
Standard solution



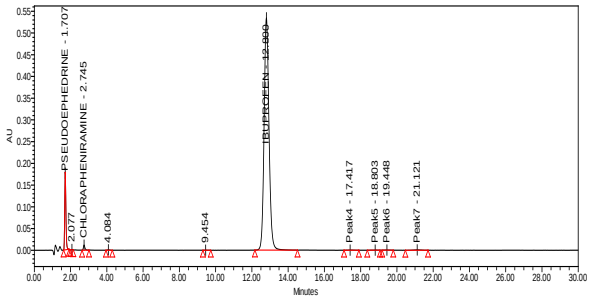
Acid Degradation sample



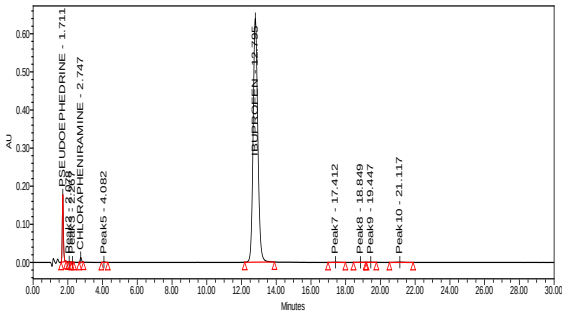
Base Degradation sample



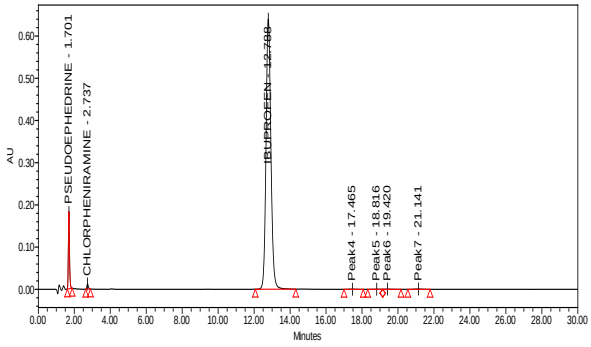
Peroxide degradation sample



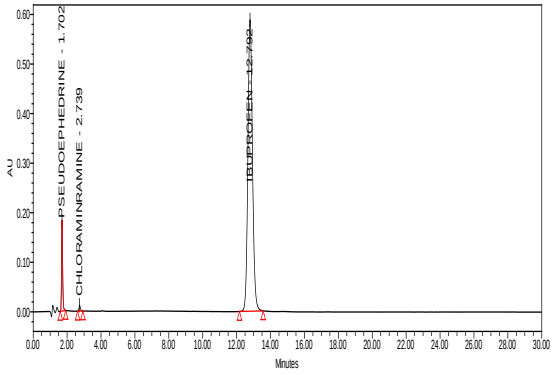
Thermal degradation sample



Humidity degradation sample

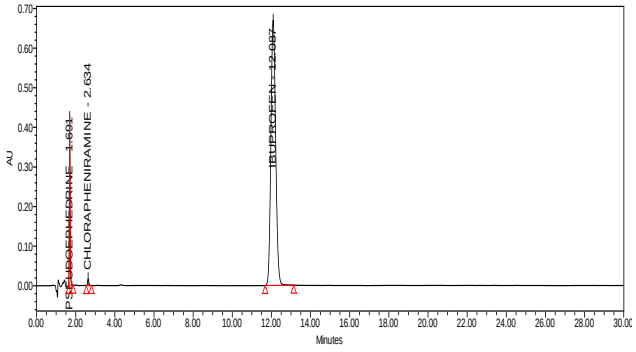


Photolytic degradation sample

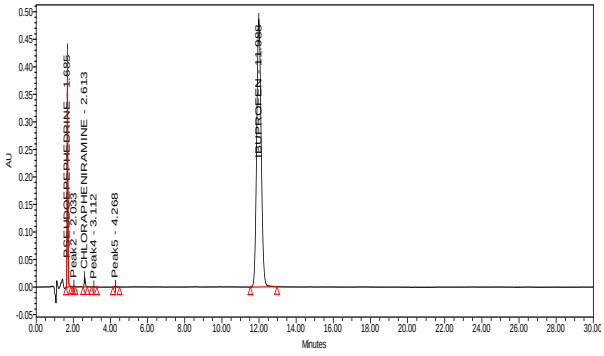


Undegraded sample

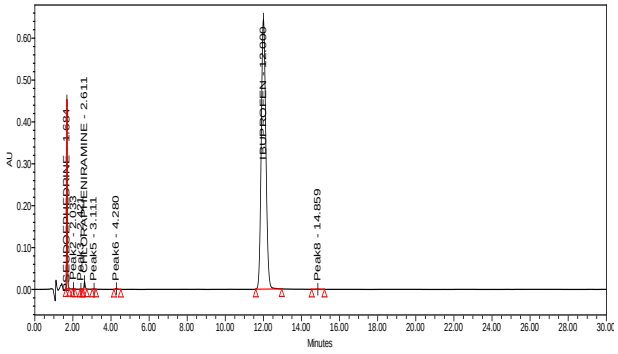
a) Standard and Stress sample chromatograms data in column #1



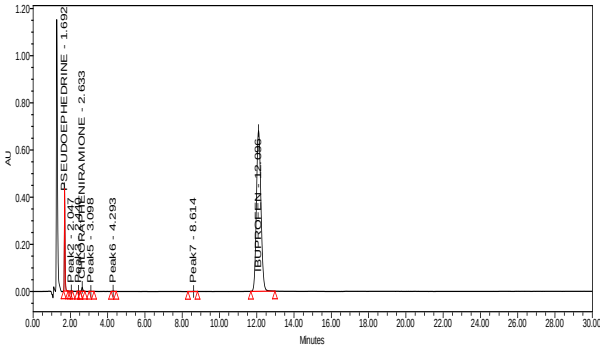
Standard solution



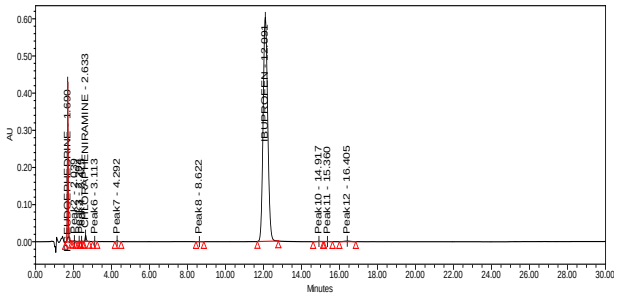
Acid Degradation sample



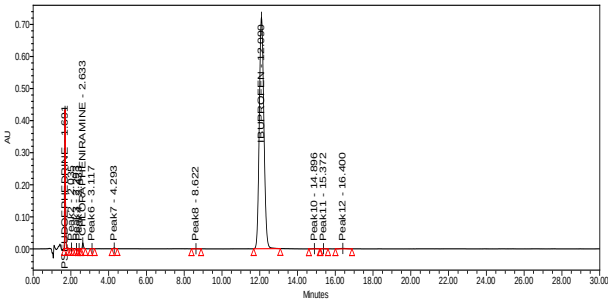
Base Degradation sample



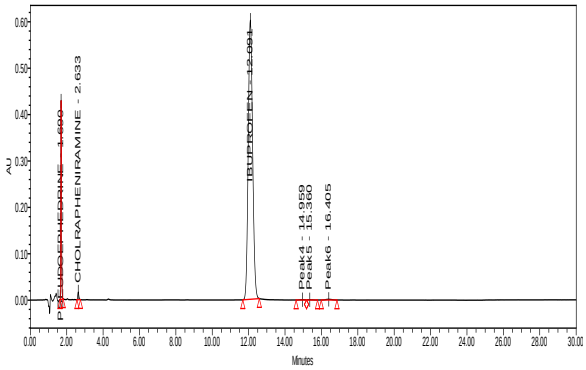
Peroxide degradation sample



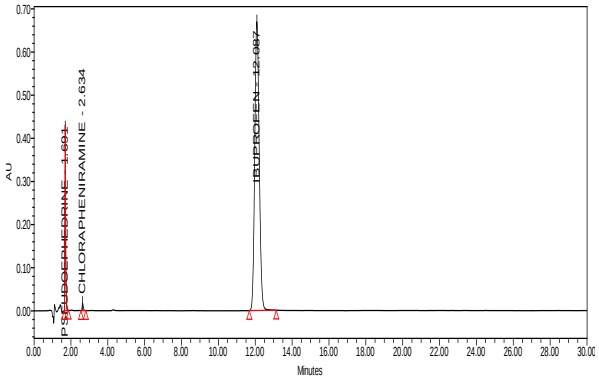
Thermal degradation sample



Humidity degradation sample

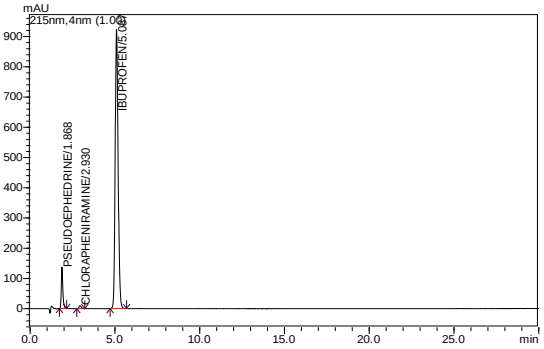


Photolytic degradation sample

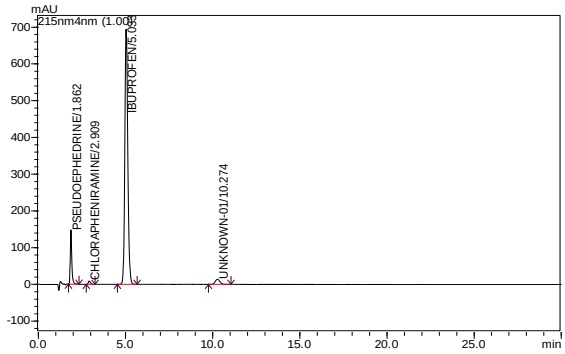


Undegraded sample

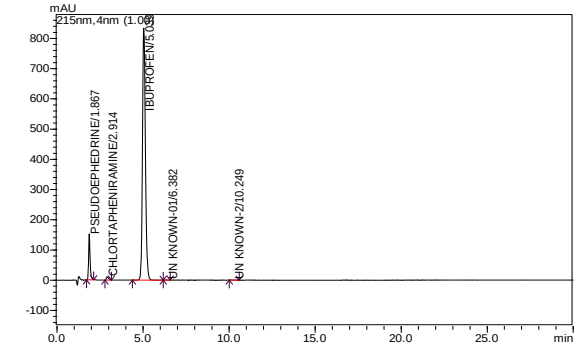
b)Standard and Stress sample chromatograms in column #2



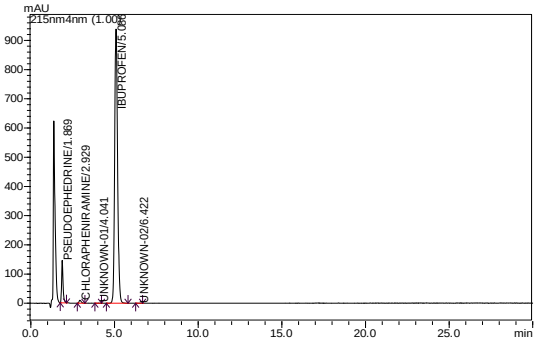
Standard solution



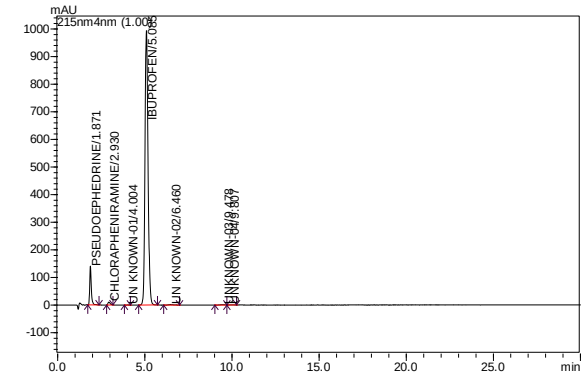
Acid Degradation sample



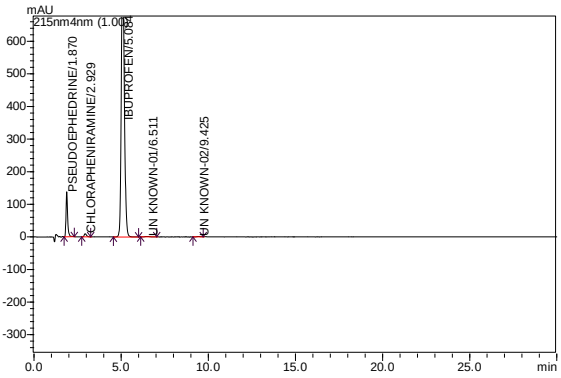
Base Degradation sample



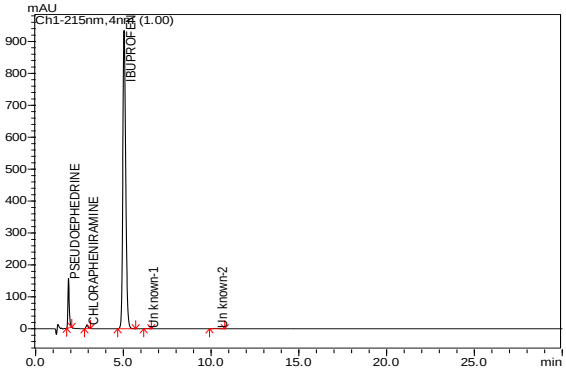
Peroxide degradation sample



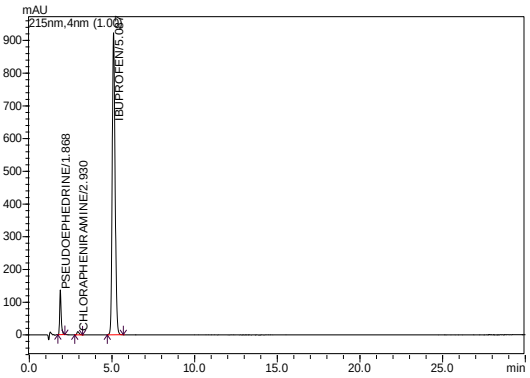
Thermal degradation sample



Humidity degradation sample

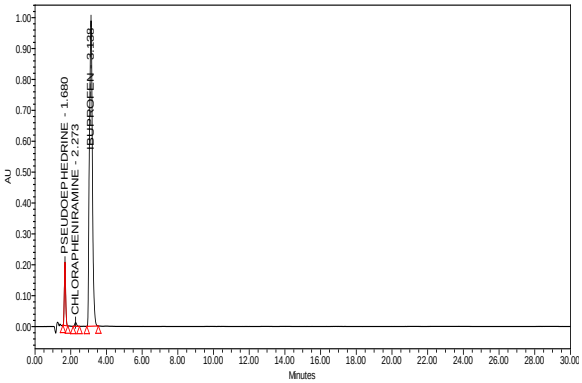


Photolytic degradation sample

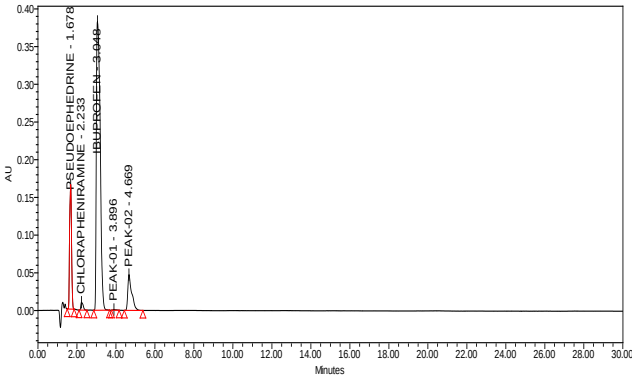


Undegraded sample

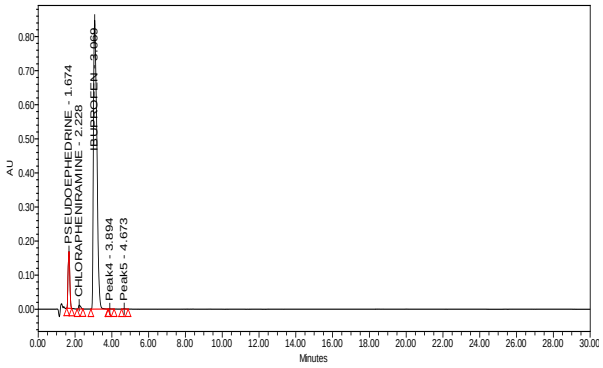
c)Standard and Stress sample chromatograms in column #3

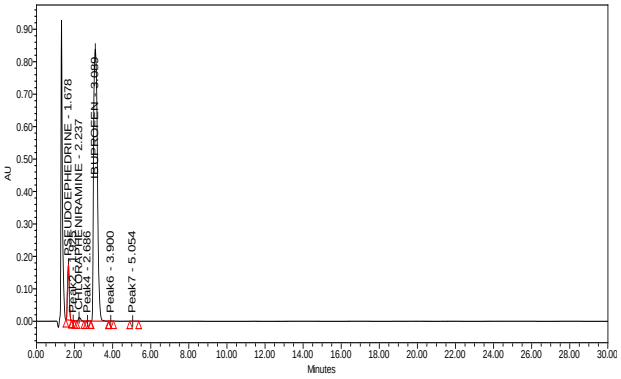


Standard solution

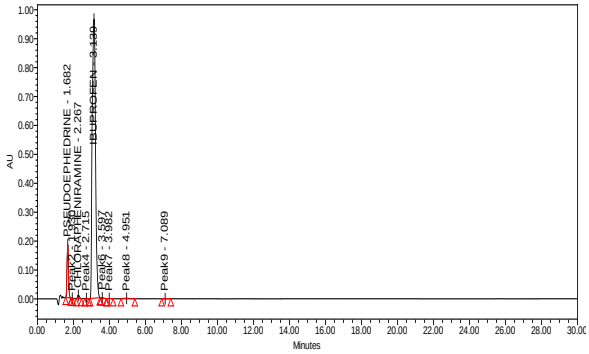


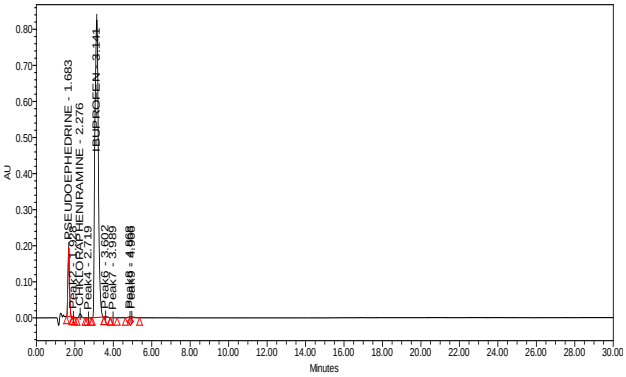
Acid Degradation sample



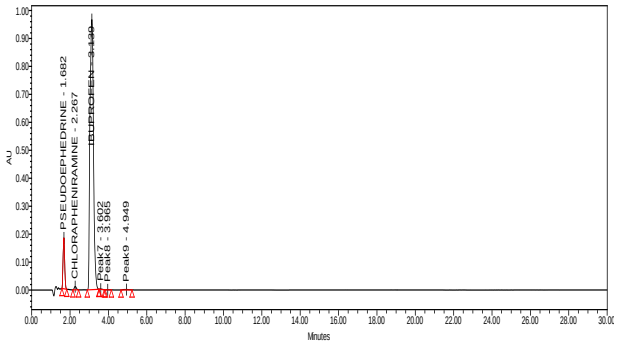


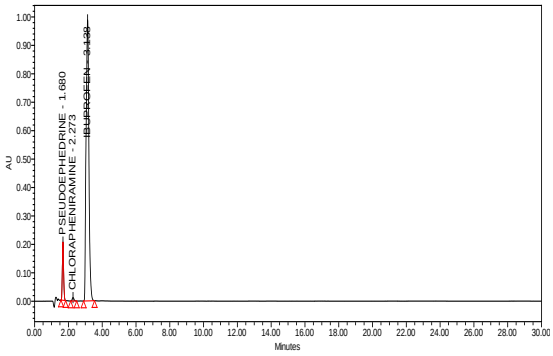
Base Degradation sample **Peroxide degradation sample**





Thermal degradation sample **Humidity degradation sample**





Photolytic degradation sample **Undegraded sample**

Figure 4: Typical chromatogram of Standard solution & Stress sample chromatograms on Column #1, 2, 3 &4.

Table 1. Summary of Forced degradation study in Column#1

Stress condition	Content in mg/unit			% Labeled amount			% Degradation		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Undegraded Sample	30.8	2.08	202.71	102.7	104.0	101.4	0.0	0.0	0.0
Acid degradation sample	30.42	1.99	142.16	101.4	99.5	71.1	1.2	4.3	29.9
Base degradation sample	31.06	2.04	190.42	103.5	102.0	95.2	0.0	1.9	6.1
Peroxide degradation sample	29.87	2.02	200.16	101.0	99.6	100.1	1.7	4.4	1.3
Thermal degradation sample	30.29	2.05	202.31	101.0	102.5	101.2	1.7	1.4	0.2
Humidity degradation Sample	30.36	2.02	181.45	101.2	101.0	90.7	1.4	2.9	10.5
Photolytic degradation Sample	30.98	2.07	202.28	103.3	103.5	101.1	0.6	0.5	0.3

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen

Table 2. Summary of Forced degradation study in Column#2

Peak Purity Data in Column#1 Stress condition	*Ps		*Ch		*Ib	
	#PA	#PT	#PA	#PT	#PA	#PT
Undegraded Sample	0.128	0.304	0.679	0.897	0.044	0.260
Acid degradation sample	0.307	1.098	1.832	6.178	0.122	0.504
Base degradation sample	0.174	0.277	0.430	0.602	0.043	0.256
Peroxide degradation sample	0.114	0.271	0.519	0.626	0.042	0.254
Thermal degradation sample	0.360	0.401	0.393	0.703	0.135	0.289
Humidity degradation Sample	0.330	0.379	0.598	0.828	0.156	0.283
Photolytic degradation Sample	0.235	1.094	0.404	1.433	0.046	1.019

#PA – Purity Angle, #PT – Purity Threshold Acceptance criteria – Purity Angle should be less than purity thresh

Table 3. Summary of Forced degradation study in Column#3

Stress condition	Content in mg/unit			% Labeled amount			% Degradation		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Undegraded Sample	31.45	2.07	201.78	104.8	103.5	100.9	0.00	0.00	0.00
Acid degradation sample	30.52	1.94	141.05	101.7	97.2	70.5	3.00	6.30	30.10
Base degradation sample	31.2	2.03	188.39	104.0	101.5	94.2	0.80	1.90	6.60
Peroxide degradation sample	30.81	1.98	200.27	102.7	99.0	100.1	2.00	4.30	0.70
Thermal degradation sample	30.52	2.01	197.17	101.7	100.5	98.6	3.10	2.90	2.30
Humidity degradation Sample	30.84	2.12	192.27	102.8	100.0	96.1	4.80	0.00	4.80
Photolytic degradation Sample	31.12	2.05	202.19	103.7	102.9	101.1	1.0	0.6	0.2

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen

Stress condition	Peak Purity Data in Column#2 *Ps		*Ch		*Ib	
	#PA	#PT	#PA	#PT	#PA	#PT
Undegraded Sample	0.128	0.304	0.361	1.582	0.811	1.024
Acid degradation sample	0.351	1.137	0.338	1.686	0.318	1.045
Base degradation sample	0.653	1.135	0.326	1.536	0.415	1.035
Peroxide degradation sample	0.173	1.065	0.394	1.433	0.687	1.019
Thermal degradation sample	0.461	1.713	0.357	1.78	0.891	1.09
Humidity degradation Sample	0.180	1.049	0.362	1.600	1.001	1.009
Photolytic degradation Sample	0.167	1.060	0.233	1.657	0.058	1.026

#PA – Purity Angle, #PT – Purity Threshold Acceptance criteria – Purity Angle should be less than purity thresh

Stress condition	Content in mg/unit			% Labeled amount			% Degradation		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Undegraded Sample	31.08	2.02	201.06	103.6	101.0	100.5	0.00	0.00	0.00
Acid degradation sample	30.89	1.55	142.95	103.0	78.5	71.5	0.60	22.50	28.9
Base degradation sample	31.46	2.06	189.61	104.9	103.0	94.8	0.00	0.00	5.70
Peroxide degradation sample	30.28	2.03	199.45	100.9	101.5	99.7	2.60	0.00	0.80
Thermal degradation sample	30.67	2.04	198.98	102.2	102.0	99.5	0.0	1.0	1.00
Humidity degradation Sample	30.61	2.03	181.56	102.0	101.5	90.8	0.00	0.00	9.70
Photolytic degradation Sample	30.83	2.04	201.29	102.8	102.0	100.6	0.8	1.0	0.1

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen,

Stress condition	Peak Purity Data in Column#3		
	*Ps MPPI#	*Ch MPPI#	*Ib MPPI#
Undegraded Sample	76	5529	56
Acid degradation sample	61	4950	23
Base degradation sample	50	732	21
Peroxide degradation sample	76	3784	29
Thermal degradation sample	62	1915	22
Humidity degradation Sample	99	9792	84
Photolytic degradation Sample	406	2511	145

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen, MPPI# - Minimum peak purity index (value should be positive – then there no co elution been observed)

Table 4. Summary of Forced degradation study in Column#4

Stress condition	Content in mg/unit			% Labeled amount			% Degradation		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Undegraded Sample	31.5	2.05	201.15	105.0	102.5	100.6	0.00	0.00	0.00
Acid degradation sample	30.22	1.96	140.83	100.7	98.0	70.4	4.3	4.40	30.00
Base degradation sample	29.64	2.04	188.38	98.8	102.0	94.2	6.2	0.50	6.30
Peroxide degradation sample	31.04	1.98	198.45	103.5	99.0	99.2	1.5	3.40	1.30
Thermal degradation sample	31.02	2.06	199.15	103.4	103.0	99.6	1.5	0.00	1.00
Humidity degradation Sample	29.85	1.97	179.34	99.5	98.5	89.7	5.5	3.9	10.80
Photolytic degradation Sample	31.21	2.06	202.36	104.0	103.0	101.2	1.0	0.5	0.6

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen

Stress condition	Peak Purity Data in Column#4					
	*Ps		*Ch		*Ib	
	#PA	#PT	#PA	#PT	#PA	#PT
Undegraded Sample	0.346	2.057	2.013	2.674	0.068	2.011
Acid degradation sample	0.275	2.614	1.550	9.426	0.038	2.155
Base degradation sample	0.207	2.094	1.568	2.970	0.056	2.020
Peroxide degradation sample	0.310	2.069	1.793	2.772	0.045	2.013
Thermal degradation sample	0.875	2.223	1.592	3.468	0.063	2.025
Humidity degradation Sample	0.640	2.397	1.617	5.868	0.058	2.059
Photolytic degradation Sample	0.632	1.035	1.227	1.247	0.063	1.004

#PA – Purity Angle, #PT – Purity Threshold Acceptance criteria – Purity Angle should be less than purity thresh

Precision

The percentages RSD for each component in all columns were found to be less than 1.0%. The average results between method precision and intermediate precision has also shown less than 1.0% RSD **(Table 7 to Table 14)**.

Table 7. Method precision data in Column #1

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib

Preparation #1	30.4	1.996	202.1	101.3	99.8	101.1
Preparation #2	30.5	2.005	204.3	101.7	100.3	102.2
Preparation #3	30.3	1.993	202.7	101.0	99.7	101.4
Preparation #4	30.5	2.009	203.4	101.7	100.5	101.7
Preparation #5	30.3	1.991	202.1	101.0	99.6	101.1
Preparation #6	30.2	1.989	202.0	100.7	99.5	101.0
Average	30.4	1.997	202.8	101.2	99.9	101.4
SD	0.120	0.01	0.92	0.410	0.40	0.46
%RSD	0.4	0.5	0.5	0.4	0.4	0.5

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen

Table 8. Method precision data in Column #2

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	30.1	1.980	201.8	100.4	99.0	100.9
Preparation #2	30.5	2.001	203.4	101.7	100.1	101.7
Preparation #3	30.4	1.996	202.7	101.3	99.8	101.4
Preparation #4	30.5	2.001	203.7	101.6	100.1	101.9
Preparation #5	30.3	2.005	202.3	101.0	100.3	101.2
Preparation #6	30.2	1.975	202.3	100.7	98.8	101.2
Average	30.3	1.993	202.7	101.1	99.7	101.4
SD	0.150	0.010	0.710	0.510	0.630	0.370
%RSD	0.5	0.5	0.4	0.5	0.6	0.4

Table 9. Method precision data in Column #3

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	29.8	1.991	197.0	99.3	99.6	98.5
Preparation #2	30.2	2.007	197.7	100.7	100.4	98.8
Preparation #3	30.0	1.999	198.3	100.0	100.0	99.2
Preparation #4	30.2	2.015	198.4	100.7	100.8	99.2
Preparation #5	30.1	1.993	198.0	100.3	99.7	99.0
Preparation #6	30.0	1.993	197.7	100.0	99.7	98.8
Average	30.1	2.000	197.9	100.2	100.0	98.9
SD	0.150	0.010	0.52	0.530	0.480	0.270
%RSD	0.5	0.5	0.3	0.5	0.5	0.3

Table 10. Method precision data in Column #4

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	30.0	1.960	197.8	100.0	98.0	98.9
Preparation #2	30.3	2.013	199.5	100.9	100.7	99.7
Preparation #3	30.0	1.989	198.4	100.1	99.5	99.2
Preparation #4	30.3	1.996	199.5	101.0	99.8	99.7
Preparation #5	29.9	1.998	198.3	99.7	99.9	99.2
Preparation #6	29.9	2.001	198.0	99.7	100.1	99.0
Average	30.1	1.993	198.6	100.2	99.7	99.3
SD	0.170	0.020	0.74	0.580	0.910	0.340
%RSD	0.6	1.0	0.4	0.6	0.9	0.3

Table 11. Intermediate Method precision (Ruggedness) data in Column #1

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	30.1	1.990	198.4	100.3	99.5	99.2
Preparation #2	30.3	1.999	199.5	101.0	100.0	99.8
Preparation #3	30.1	1.989	198.5	100.3	99.5	99.3
Preparation #4	30.0	1.987	199.5	100.0	99.4	99.8
Preparation #5	29.9	1.983	198.6	99.7	99.2	99.3
Preparation #6	30.0	1.985	199.1	100.0	99.3	99.6
Average	30.1	1.989	198.9	100.2	99.5	99.5
SD	0.14	0.01	0.27	0.44	0.28	0.27
%RSD	0.5	0.5	0.3	0.5	0.3	0.3

*Ps – Pseudoephedrine, *Ch- Chlorpheniramine, *Ib – Ibuprofen

Table 12. Intermediate Method precision (Ruggedness) data in Column #2

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	30.1	2.002	198.3	100.3	100.1	99.2
Preparation #2	30.3	2.009	199.5	101.0	100.5	99.8
Preparation #3	30.4	1.990	199.0	101.3	99.5	99.5
Preparation #4	30.5	2.013	200.2	101.7	100.7	100.1
Preparation #5	30.3	2.011	199.1	101.0	100.6	99.6
Preparation #6	30.4	2.019	199.4	101.3	101.0	99.7
Average	30.3	2.007	199.3	101.1	100.4	99.7
SD	0.14	0.01	0.63	0.47	0.53	0.30
%RSD	0.5	0.5	0.3	0.5	0.5	0.3

Table 13. Intermediate Method precision (Ruggedness) data in Column #3

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	30.0	1.997	198.1	100.0	99.9	99.1
Preparation #2	30.2	2.007	199	100.7	100.4	99.5
Preparation #3	30.1	2.003	198.5	100.3	100.2	99.3
Preparation #4	30.2	2.005	199.2	100.7	100.3	99.6
Preparation #5	30.0	1.992	198.2	100.0	99.6	99.1
Preparation #6	30.1	2.004	198.8	100.3	100.2	99.4
Average	30.1	2.001	198.6	100.3	100.1	99.3
SD	0.09	0.01	0.44	0.31	0.30	0.21
%RSD	0.3	0.5	0.2	0.3	0.3	0.2

Table 14. Intermediate Method precision (Ruggedness) data in Column #4

Preparation	Content in mg/unit			% Labeled amount		
	*Ps	*Ch	*Ib	*Ps	*Ch	*Ib
Preparation #1	30.0	1.988	198.3	100.0	99.4	99.2
Preparation #2	30.2	2.005	198.8	100.7	100.3	99.4
Preparation #3	30.1	1.995	198.4	100.3	99.8	99.2
Preparation #4	30.2	1.999	199.1	100.7	100.0	99.6
Preparation #5	30.1	1.994	198.1	100.3	99.7	99.1
Preparation #6	30.2	2.002	198.5	100.7	100.1	99.3

Average	30.1	1.997	198.5	100.5	99.9	99.3
SD	0.08	0.01	0.36	0.29	0.32	0.18
%RSD	0.3	0.5	0.2	0.3	0.3	0.2

Linearity

Calibration curve obtained by the least square regression analysis between peak area and concentration showed linear relationship with a correlation coefficient of greater than 0.99 over the calibration ranges tested. Linear calibration plot for the Assay is obtained over the range 50 to 150% against test concentration. The results show an excellent correlation obtained between peak area and concentration of Pseudoephedrine, Chlorpheniramine and Ibuprofen. (**Table 5**)

Table 5. Summary of Linearity table

Column	Name of the component	Trend line equation	Range (µg/mL)	Correlation coefficient	Intercept	Residual sum of squares
Column #1	Pseudoephedrine	y = 34809x + 15902	15.23 – 45.69	0.99867	15901.9	5816.1
	Chlorpheniramine	y = 34821x – 775.2	1.0045-3.093	0.99958	-775.198	708.1591
	Ibuprofen	y = 53967x + 14643	100.23 – 304.99	0.9999	146432.7	51952.3
Column #2	Pseudoephedrine	y = 33979x – 6426	15.23 – 45.69	0.99955	-6426.7	10493.6
	Chlorpheniramine	y = 36599x – 961.9	1.0045-3.054	0.99964	-961.939	674.9524
	Ibuprofen	y = 55871x + 22762	100.23 – 300.69	0.99995	22761.8	38774.7
Column #3	Pseudoephedrine	y = 26699x – 9298	15.23 – 46.3	0.99971	-9298.2	6652.1
	Chlorpheniramine	y = 38860x – 487.8	1.0045-3.014	0.99955	-487.89	786.8158
	Ibuprofen	y = 53273x + 21965	100.23 – 304.7	0.99994	219656.1	40060.4
Column #4	Pseudoephedrine	y = 35465x + 12064	15.23 – 46.3	0.99959	12064.1	10562
	Chlorpheniramine	y = 34353x – 855.7	1.0045-3.094	0.99967	-855.709	614.6554
	Ibuprofen	y = 60391x -83704	100.23 – 300.69	0.99942	-83703.7	139321.7

Accuracy

Accuracy was assessed at three different levels including 50%, 100% and 150% of the test concentration level for each component. The observed recovery results were found in the range between 98 to 102%, demonstrating that the method is accurate within the desired range. (**Table 6a, Table 6b and Table 6c**)

Table 6a. Table for Accuracy study for Pseudoephedrine

Column	Sample accuracy at level	Amount added (%w/w)	Amount recovered (%w/w)	% Recovery
Column #1	At 50% Level	15.53	15.81	101.8
	At 100% Level	30.48	30.56	100.3
	At 150% Level	45.67	45.87	100.4
Column #2	At 50% Level	15.53	15.79	101.7
	At 100% Level	30.48	30.11	98.8
	At 150% Level	45.67	45.39	99.4
Column #3	At 50% Level	15.53	15.79	101.7
	At 100% Level	30.48	30.11	98.8
	At 150% Level	45.67	45.39	99.4
Column #4	At 50% Level	15.53	15.81	101.8
	At 100% Level	30.48	29.97	98.3
	At 150% Level	44.91	44.14	98.3

Table 6b. Table for Accuracy study for Chlorpheniramine

Column	Sample accuracy at level	Amount added (%w/w)	Amount recovered (%w/w)	% Recovery
Column #1	At 50% Level	1.0059	1.0222	101.6
	At 100% Level	2.0118	2.0134	100.1
	At 150% Level	3.0176	3.0743	101.9
Column #2	At 50% Level	1.0059	0.9974	99.2
	At 100% Level	2.0118	1.9937	99.1
	At 150% Level	3.0176	2.9919	99.1
Column #3	At 50% Level	1.0059	1.0179	101.2
	At 100% Level	2.0118	2.0054	99.7
	At 150% Level	3.0176	2.9906	99.1
Column #4	At 50% Level	1.0059	1.0101	100.4
	At 100% Level	2.0118	1.9999	99.4
	At 150% Level	3.0176	3.0741	101.9

Table 6c. Table for Accuracy study for Ibuprofen

Column	Sample accuracy at level	Amount added (%w/w)	Amount recovered (%w/w)	% Recovery
Column #1	At 50% Level	100.23	102.15	101.9
	At 100% Level	200.92	202.68	100.9
	At 150% Level	299.7	305.51	101.9
Column #2	At 50% Level	100.23	101.8	101.6
	At 100% Level	200.92	201.15	100.1
	At 150% Level	299.7	302.67	101
Column #3	At 50% Level	100.23	101.13	100.9
	At 100% Level	200.92	199.61	99.3
	At 150% Level	299.7	300.51	100.3
Column #4	At 50% Level	100.23	101.37	101.1
	At 100% Level	200.92	197.02	98.1
	At 150% Level	290.66	285.66	98.3

Solution Stability:

No significant changes are observed in the area of Pseudoephedrine, Chlorpheniramine and Ibuprofen during solution stability experiment in all columns. The solution stability experiment data confirms that standard and sample solutions are stable up to the study period of 28 hours.

Robustness

Close observation of analysis results for deliberately changed chromatographic conditions Flow rate, column temperature, pH of the buffer, Organic ratio and wave length which revealed that there is no significant change observed in the retention times of the main analytes on different columns illustrating the robustness of the method.

CONCLUSION

The proposed HPLC method enables the simultaneous quantitative determination of Pseudoephedrine, Chlorpheniramine and Ibuprofen in tablets. The developed method was validated as per ICH requirements. The stress studies indicated that method is selective and stability indicating. UV detection at 215nm was found to be suitable without any interference from excipients. All the calibration curves obtained were found linear with values of correlation coefficients greater than 0.99. Recovery tests confirmed the accuracy of the method. The proposed HPLC method is precise, accurate, robust and efficient to determine the components in all proposed column chemistries of C18, C8, Phenyl and Cyano. It will perfectly fit into the concept of QbD.

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