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Simultaneous Determination of Cetirizine and Pseudoephedrine Combined in Tablet Dosage Form by High Performance Liquid Chromatography

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ABSTRACT

This study develops and validates an efficient, sensitive and simple method for the simultaneous determination of cetirizinedihydrochloride and pseudoephedrinecombined in tablet dosage form by high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector.The validation of this method was carried out according to ICH and USP guidelines. In this study, the mobile phase used wasacetonitrile: water (530:470 (v/v)) with 200mg sodium heptane sulfonic acid and the pH value was adjusted to 2.5 with sulfuric acid. The limit of detection and quantification for cetirizinedihydrochloride were 0.805 and 2.685 µg/mL,respectively,and the limit of detection and quantification for pseudoephedrinewere17.976 and 59.921 µg/mL, respectively. The linearity was studied in the concentration range of 12.2 and 36.5 µg/mL forcetirizinedihydrochloride and 295.91 and 861.73 µg/mL for pseudoephedrine. The recovered amounts of cetirizinedihydrochloride and pseudoephedrinewere98.2% -102.9% and99.5%- 102.4%, respectively.

Keywords: cetirizinedihydrochloride, pseudoephedrine, validation,ICH, USP,andHPLC..

INTRODUCTION

Cetirizine (CET) (Figure 1-A) is the carboxylated metabolite of hydroxyzine, and it has a high specific affinity for histamine H1 receptors.[1]Pseudoephedrine (PSE) (Figure 1-B) is a sympathomimetic drug that acts directly on alpha-adrenergic receptors.[2]The combination of pseudoephedrine and cetirizine with a long acting antihistaminic and slow release pseudoephedrine, which is a sympathomimetic,is a decongestant widely used in the comprehensive management of allergic rhinitis.

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Cetirizine is a relatively new second-generation antihistamine in the market. It has, however, been foundthat cetirizine has both sedative and anti-cholinergic effects, though to a smaller extent than that seen in the first generation antihistamines.[4]

A literature survey reveals a variety of analytical methods for the analysis of cetirizine and pseudoephedrine.A rapid, selective, and stable methodindicating high performance thin layer chromatography was developed and validated by Makhijaet al.for the simultaneous estimation in pharmaceutical dosage forms. The method employed TLC aluminum plates pre-coated with silica gel 60F-254 as the stationary phase. The solvent system consisted of ethyl acetate–methanol–ammonia (7:1.5:1, v/v/v).[5]

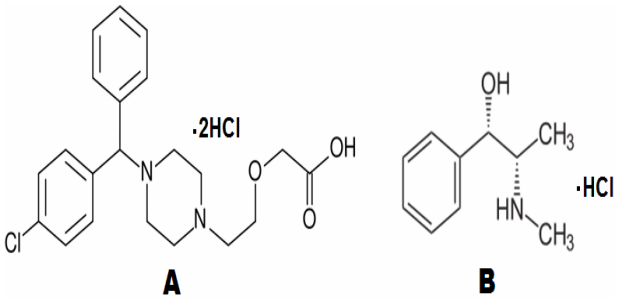

Figure 1: The chemical structure of (A)cetirizine dihydrochlorideand (B) pseudoephedrinehydrochloride

Tan et al. presented a liquid chromatography–ion trap mass spectrometry method coupled with electrospray ionization (HPLC–ESI–ion trap mass spectrometry) for simultaneous determination ofcetirizineandpseudoephedrinein human plasma.[6]

A liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed and evaluated to determine simultaneously the concentrations ofpseudoephedrine andcetirizinein human plasma. The procedure is only a one-step protein precipitation.[7]

A dissolution test for a once daily combination tablet containing 10 mg ofcetirizine dihydrochloride (cetirizine HCl) for immediate release and 240 mg ofpseudoephedrinehydrochloride (pseudoephedrine HCl) for extended release was developed and validated by Likaret al.according to ICH and FDA guidelines.[8]

A reversed-phase HPLC method and subsequent validation using an ICH suggested approach for the determination of antihistaminic-decongestant pharmaceutical dosage forms containing binary mixtures ofpseudoephedrinehydrochloride (PSE) with fexofenadine hydrochloride (FEX) orcetirizinedihydrochloride (CET) was developed by Karakuşet al.[9]

A stability-indicating reversed-phase high-performance liquid chromatography (RP-HPLC) method has been developed by Hadadet al.The method can separate quantitiesof paracetamol, dantrolene,cetirizine,andpseudoephedrine. The method was validated for the purpose of conducting stability studies in quality control (QC) laboratories. Quantitation was achieved with UV detection at 214 nm, based on a peak area.[10]

Moreover, other authors proposed amethod to determinepseudoephedrine hydrochloride (PSE) and cetirizinein plasma to investigate the pharmacokinetics profile.[11]A reversed phase high performance liquid chromatographic method was developed using a Shimadzu HPLC-VP series, LC-10 ATV pump, SPD10 AVP, and C8 column, for simultaneous determination of pseudoephedrine hydrochloride and cetirizine hydrochloride in 3 marketed tablet formulations (extended release). The mobile phase consistedof a phosphate buffer of pH 7.0 and acetonitrile HPLC grade in the ratio of 1:1. The flow rate was maintained at 1 mL/min, and the UV detection was done at 242 nm, which is the isosbestic point.[12]

Zarapkaret al.developed a reversed-phase HPLC method for the simultaneous determination of cetirizine and pseudoephedrine in tablets. A Hypersil BDS C18 (5 µm, 25 cm*3.9 mm) column in isocratic mode, with the mobile phase H_2O/MeCN/Et_3N (63:37:0.2),was used and the pH was adjusted to 7.5 with 5% phosphoric acid. The flow rate was 1 mL/min. and the effluent was monitored at 254 nm.[13]

The objective of this study is to develop and validate an efficient, sensitive, and simple HPLC method with ultraviolet (UV) detection for the simultaneous determination of cetirizine dihydrochloride and pseudoephedrinehydrochloride as a combination in film coated tablet dosage forms.

EXPERIMENTAL

Chromatographic Conditions

An HPLC systemtype Shimadzu with LC-2010C pump and degasser connected to a UV detector was used. The injections were performed using the auto sampler model. The instrument wasconnected to class VP.The chromatographic column was a 250*4.6 mm C18with 5.0 µm particle size. The mobile phase was acetonitrile: water (530:470) with a buffer solution containing200 mg

sodium heptane sulfonic acid. The pH value was adjusted to 2.5 with sulfuric acid. The flow rate was 1 mL/min at room temperature and the eluent was monitored at a wavelength of 220 nm. The injection volume was 20 µl injected separately for samples, and standards used a back pressure of 10 MPa. The total run time was 9 min.

Reagents and Tools

Pseudoephedrine working standard with a purity of 100% and cetirizine dihydrochloride working standard with a purity of 100% were obtained from Middle East Pharmaceuticals. Tablets of Cirrus(the commercial name of the tablet containing 5 mg cetirizine dihydrochloride and 120 mg pseudoephedrine) were used in the validation solutions. Sodium heptane sulfonic acid reagent grade and sulfuric acid HPLC grade were purchased fromScharlau, Spain. Highly pure water was prepared by using Millipore Millie Q plus purification system.Acetonitrile HPLC grade was obtained from Arcos Lab. Side-tests were performed by UV spectrophotometer,type Beckman- DV-650I, pH meter type Orion-420A, and an analytical balance,type Ohaus. The adjustable pipettesandall glassware used (pipettes, beakers, cylinders, and volumetric flasks) wereof class A grade with high purity and accuracy in measuring volumes.

Standard Preparation

The standard solution of cetirizine dihydrochloride was prepared by dissolving 50 mg of cetirizine dihydrochloride in 50 ml water. Thecetirizine dihydrochloride working solution was prepared from the stock solution by taking 2.5 mL and dilutingup to 100 mL with mobile phase using a volumetric flask. A concentration of 25 µg/mLwas obtained.

The standard solution of pseudoephedrinehydrochloridewas prepared by dissolving 300 mg of pseudoephedrinehydrochloride in 50 ml water. The pseudoephedrinehydrochloride working solution was prepared from the stock solution by taking 10.0 mL and dilutingup to 100 mL with mobile phase using a volumetric flask. A concentration of 600µg/mLwas obtained.

Sample Preparation

The sample solution for cetirizine dihydrochlorideand pseudoephedrinehydrochloridewas prepared by dissolving 1.9 g of Cirrus tablets to be tested in 50 mL water, and then 1.0 mL of the resulting solution was diluted to 20 mL with the mobile phase.

RESULTS AND DISCUSSION

Choice of Detection Wavelength

A UV scan (200.0-300.0nm) was applied for each cetirizine dihydrochloride and pseudoephedrine raw material and samples. Maximum absorbance was observed between 210 and 250 nm. A wavelength of 220 nm was selected as the optimum wavelength for HPLC analysis for both cetirizine dihydrochloride and pseudoephedrine and gave a flat baseline.

Choice of Mobile Phase

The structure, polarity, and solubility of both cetirizine dihydrochloride and pseudoephedrine were investigated. The solubility of the compounds was tested by trying different solvent systems.The initially used mobile phase was acetonitrile: water (550:450 (v/v)), and then changed to acetonitrile: buffer solution (550:450) at pH= 6.0. The ratios of the solvents were changed. Then the sodium heptane sulfonic acid solution was tested with a C_8 column, and the results still were unsatisfactory.

Finally, a mobile phase of amixture of acetonitrile: water (530:470(v/v)) with 200mg/L sodium heptane sulfonic acid was chosen. The pH value was changed from neutral to acidic for improving the separation of these drugs. ThepH of the mobile phase was adjusted to 2.5 by using sulfuric acid at a flow rate of 1 mL/min. The elution time for cetirizine dihydrochloride and pseudoephedrine was 5.4 and 2.9 min, respectively. A C18 column, which provides a minimum noise in the base line,was chosen.

Validation Parameters

The validation of this method was carried out according to ICH and USP guidelines. In this work the method precision, method accuracy, linearity, stability, recovery, robustness, ruggedness, and selectivity parameters were tested.

Method Precision

Standard solutions of 25 and 600µg/mL ofcetirizine dihydrochloride and pseudoephedrine hydrochloride, respectively,wereprepared according to the test method and injected in seven replicates in the chromatograph.

Standard and Sample Precision

System suitability tests are an integral part of a liquid chromatographic method.They were used to verify that the proposed method was able to produce a good resolution between the peaks of interest with high reproducibility. The system suitability was determined by performing seven replicate injections from freshly prepared standard solutions and analyzing each solute for their peak areas. The RSD value of the peak areas was less than 2%. The results of the system suitability test are shown in table 1. According to the results presented, the proposed method fulfills these requirements within the accepted limits (ICH, 2000;USP, 2007; and EP, 2007).Also, the method's precision of the sample was performed by preparing seven replicate injections, and the RSD value was less than 2%.The results are not shown here.

Table 1: **Results ofcetirizine dihydrochloride and pseudoephedrine hydrochloridestandard solution test.**

Injection no.	Cetirizine dihydrochloride		Pseudoephedrine hydrochloride	
	Peak area	Concentration (ppm)	Peak area	Concentration (ppm)
1	403695	28.6	2962066	628.8
2	404076	28.6	2962140	628.8
3	403271	28.6	2962830	628.8
4	403434	28.6	2958599	628.8
5	402586	28.2	3017333	632.0
6	402314	28.2	3015845	632.0
7	402502	28.2	3017199	632.0
Average	403125	28.4	2985145	630.2
RSD%	0.17	0.77	0.99	0.27

Method Reproducibility (Robustness)

Several experimental parameters, like buffer pH, detection wavelength, mobile phase, flow rate and the analyst were varied around the value set in the method to reflect changes likely to arise in different test environments. Analyses were carried out in triplicate and only one parameter was changed in the experiments at a time. The results are shown as a recovery %.

Analysts'Variation

Three more samples were prepared and injected into the chromatograph by another analyst from the same batch. The results are shown in table 2.

Wavelength Variation

Slight variations in wavelength were made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by small variations. The wavelength was changed from 220 nm to 225 nm. The results are shown in table 3.

Flow Rate Variation

To study the effect of small changes in the flow rate, it was changed from 1 to 1.2 ml/min. The results are shown in table 4.

PH of the Mobile Phase Variation

When the pH of the mobile phase changed from 2.5 to 2.6, the method gave the same results as shown in table 5.

It is concluded that upon changing the flow rate, the pH of the mobile phase,the wavelength, and the analyst, a slight variation witha RSD% value less than 4.0%appeared on the percentage assay keeping it within the accepted limits.Figure 2(A and B) show the chromatograms of the mentioned variations.

Table 2: Results of injections of standard and sample solutions by two different analysts.

Injection no.	**Cetirizine *dihydrochloride***		**Pseudoephedrine *hydrochloride***	
	Assay%		**Assay%**	
	Analyst(1)	**Analyst(2)**	**Analyst(1)**	**Analyst(2)**
1	101.22	100.03	99.91	101.95
2	101.03	100.01	99.80	100.97
3	101.06	100.23	99.04	100.68
Average	101.10	100.09	99.59	101.20
RSD%	0.10	0.12	0.47	0.66

Table 3: Results of injections of standard and sample solutions upon changing in the wavelength.

Injection no.	**Cetirizine** di***hydrochloride***		**Pseudoephedrine *hydrochloride***	
	Assay%		**Assay%**	
	220nm	225nm	220nm	225nm
1	100.03	99.8	99.13	100.45
2	100.01	99.51	99.80	100.02
3	100.23	99.65	99.04	100.07
Average	100.09	99.66	99.32	100.18
RSD%	0.12	0.15	0.42	0.24

Table 4: Results of injections of standard and sample solutions with variation in the flow rate of mobile phase.

Injection no.	**Cetirizine *dihydrochloride***		**Pseudoephedrine***hydrochloride*	
	Assay%		**Assay%**	
	1 ml/min	1.2 ml/min	1 mL/min	1.2 mL/min
1	100.03	99.72	99.91	100.77
2	100.01	99.43	99.80	100.42
3	100.23	98.87	99.04	100.99
Average	100.09	99.34	99.59	100.73
RSD%	0.12	0.43	0.47	0.29

Table 5: Results of injections of standard and sample solutions with changing the pH of the mobile phase

	Cetirizine *dihydrochloride*		**Pseudoephedrine *hydrochloride***	
	Assay%		**Assay%**	
	pH=2.5	pH=2.6	pH=2.5	pH=2.6
1	100.03	100.90	99.25	99.75
2	100.01	100.40	99.45	99.84
3	100.23	100.02	99.35	99.68
Average	100.09	100.45	99.35	99.76
RSD%	0.12	0.45	0.10	0.08

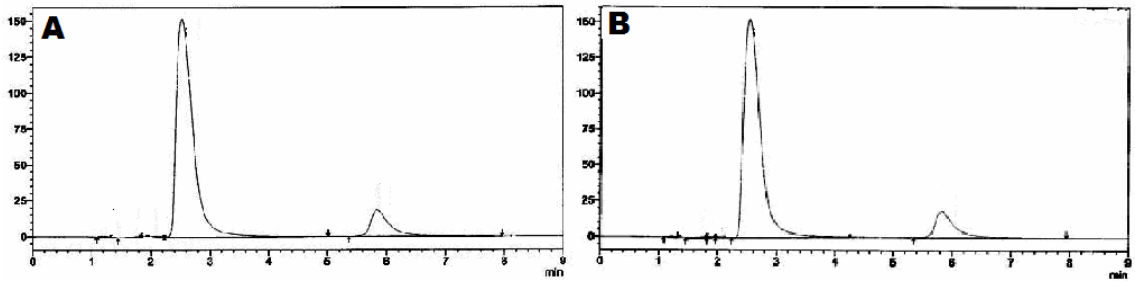

Figure 2: Chromatograms of method robustness;A: wavelength variation, B: pH of mobile phase variation

Linearity

Linearity of CetirizineDihydrochloride

Linearity was demonstrated by preparing five different standards with concentrations of 60%, 80%, 100%, 120%, and 130% w/v of the stated concentration in the tested method. Results are shown in table 6.

Table 6:Results of injection of calibration curve for linearity ofcetirizine dihydrochloride.

Parameters	**Cetirizine dihydrochloride**
Linearity range	12.2 -36.5 ppm
Regression equation results:	
Slope	13065
S.D of slope	168205.2
Intercept	29373
S.D of intercept	3507.503
Correlation coefficient(r)	0.9993
Quantitation limit	2.7µg/mL
Detection limit	0.805 µg/mL

The accepted limits of R should be greater than 0.999according to ICH,2000;USP, 2007;and EP, 2007.

The method is linear over the range of 12.2 - 36.5µg/mL for cetirizine dihydrochloride. This attests to

the linearity of the method as shown in figure 3.

Linearity of PseudoephedrineHydrochloride

Linearity was demonstrated by preparing and analyzing five different µg/mLstandard solutions with concentrations of 50%, 60%, 80%, 100%, and 130% of the stated concentration in the test method. Results are shown in table 7.

Table 7:Results of injection of calibration curve linearity parameters for pseudoephedrine hydrochloride.

Parameters	Pseudoephedrinehydrochloride
Linearity range	295.91 – 861.73
Regression equation results:	
Slope	3888.2
S.D of slope	48164.41
Intercept	300961
S.D of intercept	23298.22
Correlation coefficient(r)	0.9993
Quantitation limit	59.92 µg/mL
Detection limit	17.98 µg/mL

The method is linear over the range of295.91 - 861.73µg/mLfor pseudoephedrine hydrochloride;this attests to the linearity of the method as shown in figure 4.

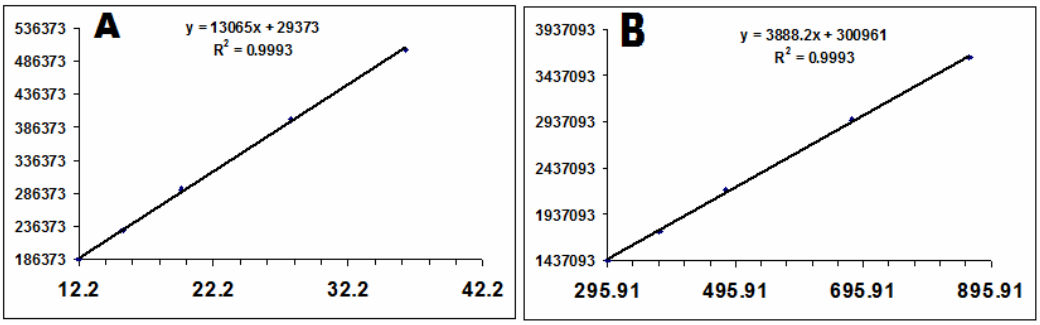

Figure 3: Calibration curve plotted between the concentration and peak area;A: Cetirizine dihydrochloride, concentration range 12.2 - 36.5,B: Pseudoephedrine hydrochloride, concentration range 295.91 - 861.73 µg/mL.

Recovery

Recovery of CetirizineDihydrochloride

A placebo was prepared and spiked with known concentrations of the active ingredient at three different levels 75%, 100%, and 150% (w/v) of the concentration stated in the test method. The results are shown in table 8.

Table 8: Recovery results at three different levels (75%, 100%, and 150%) (w/v) for cetirizine dihydrochloride

Level	Sample #	Spike concentration. (ppm)	Peak area	Measured concentration	Recovery %	Bias
75%	1	16.5	239417	16.22115	98.31	-1.69
	2	16.4	238069	16.10972	98.23	-1.77
	3	16.6	242422	16.41408	98.88	-1.12
Average	------	16.5	239969.3	16.24832	98.47	
RSD%		0.58	0.93	0.94771602	0.36	
100%	1	26.5	398180	26.90545	101.53	1.53
	2	26.2	397409	26.84452	102.46	2.46
	3	26.1	397469	26.84646	102.86	2.86
Average	--------	26.3	397686	26.86548	102.28	
RSD%		0.77	0.11	0.12890711	0.67	
150%	1	40.56	784209	40.74658	100.46	0.46
	2	40.6	785074	40.79082	100.47	0.47
	3	40.9	796986	41.37035	101.15	1.15
Average	--------	40.7	788756.3	40.96925	100.69	
RSD%		0.51	0.91	0.84958272	0.39	

The recovery experiments, placebo solution containing cetirizine dihydrochloride standards, showed mean recoveries of 100.5 ± 1.7 with R.S.D. values of 1.7% for cetirizine dihydrochloride.The accepted limits of recovery should be within 96.0%-104.0% of the spiked amount (ICH, 2000; USP,2007; and BP, 2007). Figure 5 indicates a good recovery value.

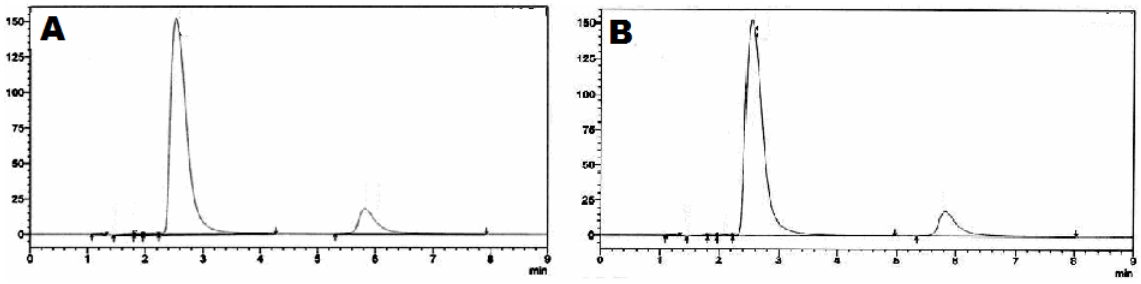

Figure 5: Chromatograms of method robustness;A: wavelength variation, B: pH of mobile phase variation

Recovery of PseudoephedrineHydrochloride

A placebo was prepared in the research and development lab and spikedwith a known concentration of the active ingredient at three different levels:75%, 100%, and 150% of the concentration stated in the test method. The results are shown in table 9.

Table 9:Recovery results at three different levels (75%, 100%, and 150%) (w/v) for pseudoephedrine hydrochloride

Level	Sample #	Spike concentration. (mg/mL)	Peak area	Measured concentration	Recovery %	Bias
75%	1	379	1804555	385.5946	101.74	1.74
	2	380	1799702	384.56	101.2	1.2
	3	0.382	1796363	383.8336	100.48	0.48
Average	------	380	1800207	384.66273	101.14	
RSD%		0.400	0.23	0.2300674	0.62	
100%	1	613	2859972	611.0997	99.69	-0.31
	2	609	2876635	614.6637	100.93	0.93
	3	611	2882419	615.888	100.8	0.8
Average	--------	611	2873009	613.8838	100.48	
RSD%		0.33	0.41	0.40522273	0.68	
150%	1	911	4257947	909.8157	99.87	-0.13
	2	915.4	4264874	911.2807	99.55	-0.45
	3	916.2	4317674	922.6134	100.7	0.7
Average	--------	914.2	4280165	914.56993	100.04	
RSD%		0.31	0.76	0.7658522	0.59	

The recovery experiments, using a placebo solution containing pseudoephedrinehydrochloride,cetirizine dihydrochloride standards, showed mean recoveries of 100.6 ± 0.7 with R.S.D. values of 0.7% for pseudoephedrine.Results in figure 5 indicate a good recovery value.

Selectivity

The standard, sample, solvent, and placebo solutions were injected into the chromatograph according to the parameters stated under the tested method. It was found that there was no interference between the analytes and both the solvent or placebo,so the method is selectiveand valid for this criteria.

Stability of Solutions

The standard and sample solutions were prepared as stated in the proposed method and they were stored at room temperature. The results are shown in tables 10 and 11. Three more samples were prepared and injected into the chromatograph at0, 24, and 48 hours at room temperature. The results indicate that the method is stable for variation of the time.The RSD value is less than 4.0%.

Table 10: Results of standard stored at room temperature at 0, 24, and 48 hrs.

	Cetirizine di*hydrochloride*		Pseudoephedrine *hydrochloride*	
Time (hr)	Peak area	Assay%	Peak area	Assay%
0	402787	101.34	2981079.5	98.84
24	405363	102.02	2971887.5	98.54
48	403524	101.56	3008035.5	99.73
RSD%	0.33	0.34	0.63	0.63

Table 11: Results of sample stored at room temperature for 0, 24, and 48 hrs.

	Cetirizine di*hydrochloride*		**Pseudoephedrine*hydrochloride***	
Time (hr)	Peak area	Assay%	Peak area	Assay%
0	404258	101.82	2973845	98.87
24	405626.5	102.51	3000933	99.77
48	406657	102.77	2975342	98.92
RSD%	0.30	0.44	0.51	0.51

The standard and sample solutions were prepared as stated in the test method and stored in the refrigerator at a temperature of 5 °C. Results are shown in tables 12and 13. Three more samples were prepared and injected into the chromatograph at0, 24, and 48 hours at refrigerator. The results indicate that the method is stable for variation of the time at refrigerator.The RSD value is less than 4.0%.

Table 12: Results of standard stored in the refrigerator at 0, 24, and 48 hrs.

	Cetirizine dihydrochloride		Pseudoephedrine***hydrochloride***	
Time (hr)	Peak area	Assay%	Peak area	Assay%
0	404263.5	101.62	2970776.5	98.50
24	404150	101.71	3027939.5	100.39
48	405256	102.0	3006956.5	99.70
RSD%	0.15	0.20	0.96	0.96

Table13: Results of sample stored in the refrigerator at 0, 24, and 48 hrs.

	cetirizine dihydrochloride		***Pseudoephedrine hydrochloride***	
Time (hr)	Peak area	Assay%	Peak area	Assay%
0	403202.5	101.62	2971199	98.78
24	404599	102.24	2974810	98.90
48	406083.5	102.62	2986862	99.30
RSD%	0.36	0.50	0.28	0.28

Placebo, standard and production sample solutions were exposed to drastic conditions like heat, 0.1 M HCI, and 0.1 MNaOH to test any degradation products and to study the method for resolving the active component from any degradation products that may be obtained.

Upon exposing the placebo, cetirizine dihydrochloride

working standard and the sample solutions to the conditions of pure water, 0.1M HCl, and 0.1MNaOH with heat, it was found that no degradation occurred.

Therefore, the proposed method was able to resolve cetirizine dihydrochloride and pseudoephedrine hydrochloride from degradation products, as shown in figure6.

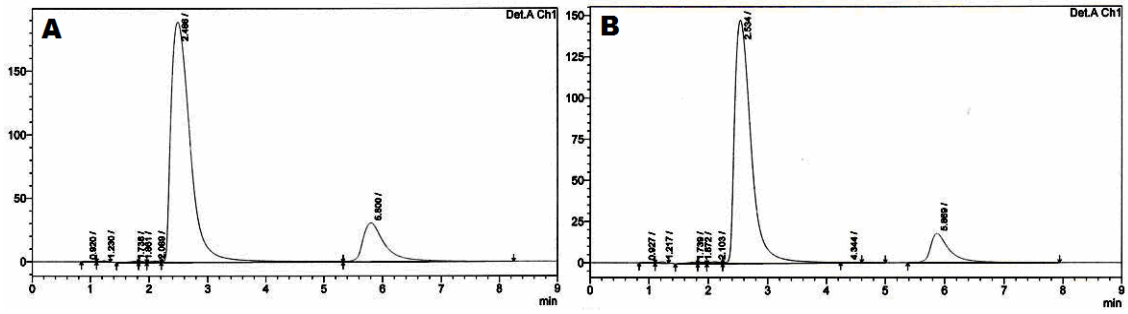

Figure 6: Chromatogram for the *effect of exposing cetirizine dihydrochloride*and pseudoephedrine hydrochloride working standard solution to A: 0.1 M*HCI* and heat andB:*0.1 MNaOH* and heat.

ANOVA Single Factor Test for CetirizineDihydrochloride Validation Data

The raw data was forced to ANOVA Single Factor statistical analysis to define the significance changes in the data obtained by the proposed method. The P value is the probability, with a value ranging from zero to one. If the P value is small, then the difference between sample means is unlikely to be a coincidence. Instead the populations have different means.Variance within a group is when individual scores within each group vary around the mean of group. SS is the sum of squares between groups or within groups. It is the degree of freedom between and within groups which is used to calculate the mean square (MS) between and within groups.

ANOVA Single Factor Test Method Reproducibility: Variation of the Analysts

When introducing the data obtained by the variation of the analyst (table 2 up) to ANOVA single factor tests, the results in Table 14 were obtained.

Table 14:Results of ANOVA Single Factor for Variation of the analysts for cetirizine dihydrochloride

Groups	*Count*	*Sum*	*Average*	*Variance*		
Assay of analyst 1	3	300.28	100.09	0.015		
Assay of analyst 2	3	303.31	101.10	0.010		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.53	1	1.53	119.73	0.0004	7.71
Within Groups	0.0511	4	0.012771			
Total	1.5801	5				

The data intable14 shows that the P-value is less than 0.05 which indicates that there werenosignificantchanges when the analyst was changed and $F_{1,\ 8\ Cal}$ (119.73) $>F_{crit}$ (7.71), which means that there was nosignificant

difference between the assay of analyst I and the assay of analyst II for method reproducibility.

The variation of other factors (wavelength, flow rate, and pH value) were performed but not shown here. The results were satisfactory.

ANOVA Single Factor Test Stability of Solutions: Storing Sample Solution inRefrigerator

When introducing the data obtained by storage in refrigerator for 0, 24 and 48 hrs (table 12) to ANOVA single factor tests, the results in table 15were obtained.

Table 15:Results of ANOVA Single Factor for storage in refrigerator for 0, 24 and 48 hrs for cetirizine dihydrochloride

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
0	2	203.23	101.62	0.07		
24	2	204.48	102.2399843	0.002		
48	2	205.25	102.6229007	0.004		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.03	2	0.52	20.29	0.018	9.55
Within Groups	0.08	3	0.03			
Total	1.11	5				

The data intable15 shows that the P-value < 0.05 which indicates that there werenosignificantchanges when analyzing the sample solution atdifferent times and $F_{2,\ 6\ Cal}$ (20.29) >F_{crit} (9.55), which means that there was nosignificant difference between the assay of a solution at 0 h, 24 h and 48 h when storing the sample solution in arefrigerator for the stability of solutions.

The storing sample solution at room temperature was performed but not shown here. The results were satisfactory.

ANOVA Single Factor Test for PseudoephedrineHydrochloride Validation Data

ANOVA Single Factor Test Method Reproducibility: Variation of the Analysts

When introducing the data obtained bya variation of the analyst (table 2) to ANOVA single factor tests, the results in table 16 were obtained.

Table 16: Results of ANOVA Single Factor for Variation of the analysts for pseudoephedrine hydrochloride

Groups	***Count***	***Sum***	***Average***	***Variance***		
Analyst 1 Assay sample	3	298.76	99.59	0.22		
Analyst 2 Assay sample	3	303.61	101.20	0.44		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.92	1	3.92	11.75	0.027	7.71
Within Groups	1.33	4	0.33			
Total	5.25	5				

The data in table16 shows that the P-value <0.05 which indicates that nosignificantchanges were observed when the analyst was changed and $F_{1, 8\ Cal}$ (11.75) >F_{crit} (7.71).This means that there was nosignificant difference between the assay of analyst I and the assay of analyst II for method reproducibility. The variation of other factors (wavelength, flow rate and pH value) were performed but not shown here. The results were satisfactory.

ANOVA Single Factor Test Stability of Solutions for PseudoephedrineHydrochloride: Storing Sample Solution at Refrigerator

When introducing the data obtained by storage in a refrigerator for 0, 24 and 48 hrs (table 12) to ANOVA single factor tests, the results in table 17were obtained.

Table 17: Results of ANOVA Single Factor for storage in refrigerator for 0, 24 and 48 hrs for pseudoephedrine hydrochloride

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
0	2	199.57	99.78	0.013
24	2	198.45	99.23	0.019
48	2	199.18	99.59	0.004

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.32	2	0.16	13.40	0.032	9.55
Within Groups	0.036	3	0.012			
Total	0.36	5				

The data in the tableshows that the P-value < 0.05 which indicates that nosignificance changes were observed when analyzing the sample solution atdifferent times and $F_{2, 6\ Cal}$ (13.40) >F_{crit} (9.55), which means that there was nosignificant difference between the assay of solution at 0 h, 24 h and 48 h when storing the sample solution in arefrigerator for the stability of solutions.The storing sample solution at room temperature was performed but not shown here. The results were satisfactory.

CONCLUSION

The proposed method was developed and successfully applied for the simultaneous determination of cetirizine dihydrochloride and pseudoephedrinehydrochloride combined in tablet dosage form. The method development and validation employed an HPLC method with a UV detector.The method was found to be simple, sensitive, and selective. The validation parameters,namely, linearity, accuracy, precision,stability, recovery, and selectivity,wereperformed according to the ICH, USP, and EP guidelines.

Under severe degradation conditions, cetirizine dihydrochloride and pseudoephedrinehydrochloride were found to be stable. The method was found to be rugged and robust.

The limit of detection and quantificationfor cetirizine dihydrochloridewere0.805 and2.685 µg/mL,respectively. The limit of detection and quantification for pseudoephedrinehydrochloride were 17.976 and59.921 µg/mL,respectively. The linearity was studied in the concentration range of 12.2 - 36.5 µg/mL for cetirizine dihydrochloride and 295.91 - 861.73 µg/mLpseudoephedrinehydrochloride.

The results were tested by the ANOVA test and the P-value was found to be less than 0.05 which means that the data was not changed upon changing the conditions.

Based on the above details,it can be concluded that the method is precise, accurate, and sensitive.In addition, the preparation procedure is simple and the retention timeis short which make it suitable for studying cetirizine dihydrochloride and pseudoephedrinehydrochloride in tablet dosage form combination.

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التحديد المتزامن للستريزين والسودوفيدرين في الأقراص باستخدام الفصل الكروماتوغرافي عالي الكفاءة

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ملخـص

لقد تم تطوير طريقه سهلة ودقيقة وحساسة لتحليل الستريزين والسودوفيدرين بشكل متزامن باستخدام الفصل عالي الكفاءة موصولا بمحساس فوق بنفسجي على طول موجه220 نانومترا وذلك في الاقراص الدوائية. لقد تم استخدام عمود من نوع كربون 18 بطول 25 سم ونصف قطر داخلي 4.6 مليمتر. إن الشكل المتحرك في النظام هو اسيتو نيترايل مع ماء بنسبة 540 إلى 470 إضافة إلى 200 مللي غرام من هبتان الصوديوم سلفونيك أسيد ومن ثم تعديل الأس الهيدروجيني إلى 2.5 باستخدام حامض الكبريتيك. لقد كانت نسبة الاسترجاع للستريزين والسودوفيدرين 98.2 الى 102.9 % æ 99.5 Åái 102.4 % ÚáìÇáÊæÇíÇ. لقد كان منحنى المعايرة للستريزين من 12.2 الى 36.5 مايكروجرام لكل مليلتر بينما كان منحنى المعايرة للسودوفيدرين من 295.91 إلى 861.73 مايكروجرام لكل مليلتر. لقد كان الحد الادنى للقياس 0.805 مايكروجرام لكل مللتر للستريزين بينما كان الحد الادنى للقياس للسودوفيدرين هو 17.978 مايكرو جرام لكل مليلتر وقد كان الحد الادنى لتحديد ومشاهدة الستريزين والسودوفيدرين على الجهاز 2.685 و 69.921 مايكروجرام لكل مليلتر على التوالي.

الكلمـات الدالـة: الستريزين ، السودوفيدرين، جهاز الفصل الكروماتوغرافي عالي الكفاءة.

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