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Original Research Article

VALIDATED METHOD FOR SIMULTANEOUS DETERMINATION OF PSEUDOEPHEDRINE HYDROCHLORIDE, CODEINE PHOSPHATE, AND TRIPROLIDINE HYDROCHLORIDE USING HPLC

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Corresponding Author:
Ali E. Karim,
Email: ali.i@uodiyala.edu.iq

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Ali E. Karim[1]

[1]Department of Physiology, Biochemistry and medicine, College of Veterinary Medicine, Diyala University, Baquba, Iraq

Abstract

The paper describes the development of method for the simultaneous estimation of triprolidine hydrochloride, pseudoephedrine hydrochloride, and codeine phosphate in syrup pharmaceutical form. This method separates the three active constituents very well. The HPLC method uses the Shimadzu HPLC system with EC 150/4.6 nucleodur 100-5 as the column. A mixture of ethanol and 0.4 % ammonium acetate solution (840- 160) was the mobile phase. Isocratic mode has been used and the flow rate was 1.5 ml/min. Detection was carried out using 254 nm. The retention time of pseudoephedrine hydrochloride, codeine phosphate and triprolidine hydrochloride was 3.72, 5.56, 6.72 respectively. This method was developed and validated with respect to linearity, accuracy, precision, and robustness. This method is rapid, simple, and accurate. This validated method can be used for determination of these active ingredients in syrup pharmaceutical form.

INTRODUCTION

Cough and cold medication comes in many different forms (tablets, syrups, suspension, sachets, capsules etc. and commonly have a complex combination of nitrogenous compounds as the active ingredients.[1,2] The preparations involve complex formulations which contain many active materials and different excipients for example flavoring agents, and preservatives.[3]

Triprolidine, 2-[(1E)-1-(4-methylphenyl)-3-(pyrrolidin-1-yl) prop-1-en- 1-yl] pyridine Anti allergic which blocks endogenous histamine action , that relief the negative symptom. It can be used to treat perennial allergic rhinitis or non-allergic rhinitis.[4,5]

Pseudoephedrine Hydrochloride -2-(methylamino)-1-phenylpro¬pan-1-ol, it uses as adrenergic agents, Sympa¬thomimetics, Bronchodilator Agent. It is used to treat nasal congestion, sinus congestion, and vasomotor rhinitis.[6,7]

Codeine phosphate known as 7,8-didehydro-4, 5epoxy 3 methoxy 17methylmorphinan 6 ol dihydrogen phosphate hemihydrate. It can be used as narcotic analgesic.[8]

In the literature, method for determination of pseudophedrine HCl, codeine phosphate, and triprolidine HCl has been published individually and with other ingredients. These methods include UV,[9-11] and HPLC.[12-20]

In this paper, HPLC method for estimation of the combination of pseudophedrine HCl, codeine phosphate, and triprolidine HCl in liquid form is introduced. Validation of the method has been done according to ICH guidelines and applied for quantification of all combination of pseudophedrine HCl, codeine phosphate, and triprolidine HCl in syrup form.

Experimental part

Reagents

Standards are obtained from Wadi al- rafidain for pharmaceutical Baghdad -Iraq. Pseudoephedrine hydrochloride purity is (99.61%). Codeine phosphate purity is (99.51%). triprolidine HCl purity is (99.25%). Ammonium acetate and ethanol have been purchased from Sigma Aldrich. Chemicals were purchased HPLC grade.

Instrumentation

The HPLC instrument has been used SHIMADZU, Japan with a diode array detector. The spectrophotometer was UV-Vis Spectrophotometer Shimadzu 1800 with UV probe software. Analytical balance was used Shimadzu. pH meter (WTW Germany) was used. All the glass wares used were purchased from ISOLAB.

Standard solution

Stock solution of pseudoephedrine hydrochloride has been prepared by dissolving 200 mg in 50 ml of 0.01N HCl to obtain a concentration of 4 mg/ml.

Stock Solution of triprolidine HCl was prepared by dissolving 125 mg with 0.01N HCl in 100 ml

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volumetric flask to get a concentration of 1.25 mg/mL. Stock Solutions for Codein phosphate was prepared by dissolving 50 mg with 0.01N HCl in 50 ml volumetric flask to get a solution of 1 mg/ml.
Different concentrations of the standards solutions have been prepared by diluting stock solution with concentration range of 0.3 mg/mL –2.4 mg/mL for pseudoephedrine HCl, 0.1 mg/mL - 1 mg/mL Codein phosphate, and 0.025 mg/mL -0.25 mg/mL triprolidine HCl. Samples in triplicates have been made for each concentration. The calibration curves have been prepared by plotting the concentrations verses the peak areas.

Procedure

10 ml syrup equivalent to 60 mg pseudoephedrine hydrochloride, 20 mg codein phosphate, and 2.5 mg triprolidine HCl was dissolved in a 50 mL volumetric flask using 0.01 N HCl. The solution was sonicated for 10 min and filtered using 0.45μm syringe filter. The solution was injected, and peak area was measured.

RESULTS

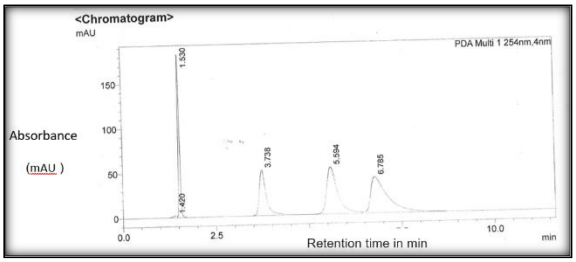

Figure 1: Standard of Pseudoephedrine, codeine phosphate, and triprolidine

Method Development: Many chromatographic conditions were examined in each experiment to achieve the best result. Parameters such as wavelength, column, and mobile phase composition have been optimized. A mixture of ethanol and 0.4 % ammonium acetate solution (840- 160) has been selected to be the mobile phase. Isocratic elution mode has been used with flow rate of 1.5 mL/min. The wavelength was selected at 254 nm which is the best detector response has been achieved for all active ingredient.

Validation: This method has been validated according to the United States Pharmacopeia and ICH guidelines.[8,21]

Selectivity: As shown in [Figure 1], the active ingredients have been separated from its excipients nicely. This method was found to be selective.

Linearity: The calibration curves were linear in the tested ranges {0.3 –2.4 mg/mL of pseudoephedrine HCl, 0.1 - 1 mg/mL for codein phosphate,and 0.025 -0.25 mg/mL of triprolidine HCl} as shown in [Figures 2-4].

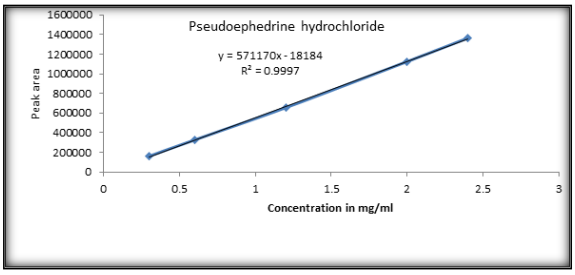

Figure 2: Calibration curve of pseudoephedrine hydrochloride

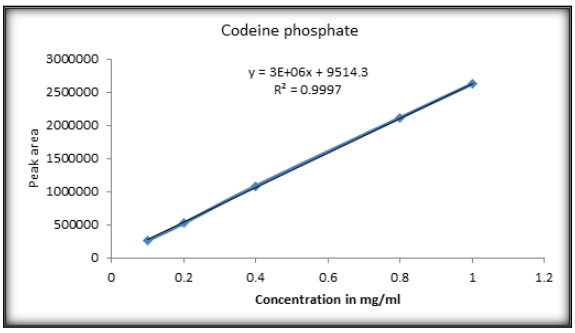

Figure 3: Calibration curve of codein phosphate

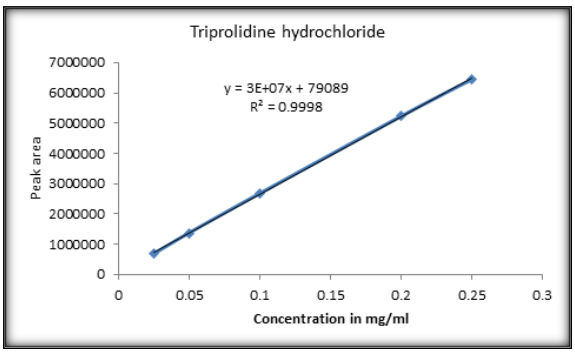

Figure 4: Calibration curve of triprolidine hydrochloride

Accuracy

The method has been validated by using recovery studies. The result of the evaluation of accuracy shows high accuracy as shown in [Table 1,2, and 3].

Table 1: Accuracy of pseudoephedrine hydrochloride.

Concentration. mg/mL	Calculated Conc in mg/mL	Recovery	AVR
1	0.9915	99.15	99.218
1	0.9931	99.31	
1	0.9905	99.05	
1	0.9912	99.12	
1	0.9946	99.46	
1.2	1.196	99.66	99.546
1.2	1.191	99.25	
1.2	1.190	99.16	
1.2	1.202	100.16	

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1.2	1.194	99.50	
1.4	1.392	99.42	99.81
1.4	1.388	99.14	
1.4	1.408	100.57	
1.4	1.405	100.35	
1.4	1.394	99.57	

Table 2: Accuracy of codeine phosphate.

Concentration mg/mL	Calculated Conc in mg/mL	Recovery	AVR
0.32	0.3221	100.62	99.82
0.32	0.3191	99.71	
0.32	0.3187	99.59	
0.32	0.3182	99.43	
0.32	0.3193	99.78	
0.4	0.3982	99.55	99.22
0.4	0.3968	99.2	
0.4	0.3972	99.3	
0.4	0.3959	98.97	
0.4	0.3965	99.12	
0.48	0.4790	99.79	99.46
0.48	0.4771	99.39	
0.48	0.4758	99.12	
0.48	0.4766	99.29	
0.48	0.4788	99.75	

Table 3: Accuracy of triprolidine hydrochloride.

Concentration mg/mL	Calculated Conc in mg/mL	Recovery	AVR
0.04	0.03962	99.05	99.26
0.04	0.03959	98.97	
0.04	0.03968	99.20	
0.04	0.03979	99.47	
0.04	0.03986	99.65	
0.05	0.05018	100.36	99.69
0.05	0.05006	100.12	
0.05	0.04982	99.64	
0.05	0.04952	99.04	
0.05	0.04966	99.32	
0.06	0.05981	99.68	99.48
0.06	0.05992	99.86	
0.06	0.05978	99.63	
0.06	0.05961	99.35	
0.06	0.05934	98.9	

Precision

Precision of this method has been validated through injecting the same standard concentration six times. Result in table 4 shows that the RSD % for pseudoephedrine HCl 0.184 %, codeine phosphate 0.446 %, and triprolidine HCl 0.35 is less than 1 % which indicates this method is precise.

Table 4: Precision of pseudoephedrine HCl, codeine phosphate, and triprolidine HCl.

Injection number	pseudoephedrine HCl		codeine phosphate		triprolidine HCl	
	Concentration in mg/mL	Area	Concentration in mg/mL	Area	Concentration in mg/mL	Area
1	1.2	653425	0.4	1086834	0.05	1370453
2	1.2	652364	0.4	1079766	0.05	1368482
3	1.2	654192	0.4	1076482	0.05	1377448
4	1.2	651266	0.4	1088362	0.05	1364296
5	1.2	653828	0.4	1076742	0.05	1368266
6	1.2	654392	0.4	1082284	0.05	1374959
AVR		653244.5		1081745		1370651
SD		1206.425		4831.66		4807.829
RSD%		0.184682		0.446654		0.35077

Table 5: Results of (TRIACIN-C-) obtained using this method.

Active component	Triprolidine hydrochloride	Codeine phosphate	Pseudoephedrine hydrochloride
Stated amount	1.25 mg/5ml	10 mg/5ml	30 mg/5ml
Result	1.247	9.932	29.826
Result % ± (SD)	99.76 (± 0.52)	99.32(± 0.31)	(± 0.38)

Robustness

Mobile phase composition has been changed {±2 %} to check the robustness of this method and no

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affect was shown. Also, wavelength has been changed {±2 nm} and no difference was shown which indicates that this method is robustness.

Specificity

The syrup was analyzed and peaks were separated with high resolution. No interferences were shown between the peaks. Also, No interfaces between the ingredients and its excipients were found and that indicate specificity of this method.

System Suitability

The wavelength and the flow rate in this method should not be changed. It is necessary to use the same wavelength and the flow rate when applying the reported method.

Active ingredients concentration in syrup (TRIACIN-C-)

TRIACIN-C- is syrup produced STI Pharma LLC that contains triprolidine hydrochloride 1.25 mg/ 5ml, pseudoephedrine hydrochlorides 30 mg/ 5ml and 10mg/5ml codeine phosphate. TRIACIN-C- was used to examine this method. The result obtained using this method was accurate as shown in

[Table 5].

CONCLUSION

A rapid HPLC method was developed and validated. The reported method is rapid, precise, accurate and simple for simultaneous assay of pseudoephedrine HCl, codeine phosphate and triprolidine HCl. This validated method can be used in a daily work in quality control lab. This method was used to analyze syrup containing pseudoephedrine HCl, codeine phosphate and triprolidine HCl.

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