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Simultaneous determination of Guaifenesin, codeine phosphate, phenylephrine hydrochloride, and sodium benzoate in syrup pharmaceutical form by RP-HPLC

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Keywords: (RP-HPLC, method validation, Guaifenesin, codeine phosphate, phenylephrine, sodium benzoate.

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Abstract:

This work describes the development of the RP-HPLC method for the simultaneous estimation of Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate. This method separates Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate nicely. This RP-HPLC method uses the Shimadzu HPLC instrument with Chromegabond WR C18 column 5 µm 120 A 30 cm * 3.9 mm. The mobile phase is a mixture of 1% o-phosphoric acid, methanol: acetonitrile (80 : 10: 10), and the pH was adjusted to 3.1. Isocratic elution mode was used with 1 ml/min as the flow rate. The detection for all compounds is carried out at 254 nm. The retention time of Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate was 14.11, 5.75, 3.9, and 20.52, respectively. The method has been validated concerning accuracy, linearity, and precision. This method is simple, accurate, and reproducible. This validated method was used to estimate these drugs in syrup pharmaceutical form.

Keywords: RP-HPLC, method validation, Guaifenesin, codeine phosphate, phenylephrine, sodium benzoate.

طريقة لتقدير كوايفنيسين، وفوسفات الكوديين، والفينيليفرين، وبنزوات الصوديوم في شراب صيدلاني بواسطة كروموتغرافيا السائل عالي الأداء ذات الطور المعكوس

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الخلاصة:

يصف هذا العمل تطوير طريقة لكروموتغرافيا السائل عالي الأداء ذات الطور المعكوس لتقدير كوايفنيسين، وفوسفات الكوديين، والفينيليفرين، وبنزوات الصوديوم في وقت واحد. تفصل هذه الطريقة بين الكوايفنيسين وفوسفات الكوديين والفينيليفرين وبنزوات الصوديوم بشكل جيد. تستخدم هذه الطريقة جهاز *Shimadzu HPLC* مع عمود *Chromegabond WR C18 5* . لطور المتحرك يتكون من خليط من ١٪ حمض الفوسفوريك: ميثانول: أسيتونتريل بنسبة (٨٠: ١٠: ١٠) وتم ضبط الرقم الهيدروجيني إلى ٣,١. تم استخدام زمن الجريان ١ مل/دقيقة كمعدل التدفق. يتم الكشف عن جميع المركبات عند ٢٥٤ نانومتر. وجد أن زمن الاحتجاز بالكوايفنسين، فوسفات الكوديين، الفينيلفرين وبنزوات الصوديوم، ١٤,١١، ٥,٧٥، ٣,٩، و٢٠,٥٢ على التوالي.

تم التحقق من صحة الطريقة فيما يتعلق بالدقة والاستقامة الخطية والاستردادية. وقد وجد أن هذه الطريقة بسيطة ودقيقة وقابلة للاستخدام المستمر. تم استخدام هذه الطريقة المعتمدة لتقدير هذه المواد الفعالة الموجودة في شكل شراب دوائي.

الكلمات المفتاحية: كروموتغرافيا السائل عالي الأداء، الكوايفنيسين، فوسفات الكوديين، الفينيليفرين، وبنزوات الصوديوم.

1. Introduction:

Guaifenesin, 3-(2-methoxyphenoxy)-propane-1,2-diol, is used as an expectorant for the symptomatic relief of respiratory problems. Its formula is $C_{10}H_{14}O_4$. Its molecular weight is 198.21. It is white or slightly crystalline with a grey color [1]. Codeine phosphate is known as 7,8-dihydro-4, 5-epoxy-3-methoxy-17-methylmorphinan-6-ol dihydrogen phosphate hemihydrate. It is used as a narcotic analgesic [2]. Phenylephrine hydrochloride is 1-(3-hydroxyphenyl)-2-methylamino-ethanol hydrochloride. The formula is C9H13NO2HCl. P.H.E. decreases nasal congestion and improves drainage of sinus cavities [2-4]. Sodium benzoate is a chemical that is used in pharmaceutical preparation as a preservative [2, 3] to decrease the microorganism development [5-7]. The literature review reveals many HPLC methods are reported for the determination of Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate individually and in a mixture of Guaifenesin alone and in combination with other drugs [8-12]. Phenylephrine hydrochloride alone and mixing with other drugs [13-14] Codeine phosphate alone and in combination with other drugs [15-18]. The combination of

Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate has not been published in U.S. and B.P. pharmacopeia. No RP-HPLC method has been published for the simultaneous estimation of Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate. Therefore, it is necessary to come up with a rapid method for the analysis of these drugs in liquid pharmaceutical formulations. Two main aspects that are important in the determination of the shelf life of the drug are the concentration of the active ingredient and the stability study. A stability test sample was done using this method, according to the guidelines of the International Conference on Harmonization (ICH) **[19]**. This method will be used to separate Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate from each other. This method has been validated and successfully applied for quantification of the active ingredient and the preservative in liquid pharmaceutical form. The aim of this research is to develop an RPHPLC method for the simultaneous determination of Guaifenesin, codeine phosphate, phenylephrine hydrochloride, and sodium benzoate.

2. Experimental

2.1 Chemicals and reagents

The working standards are obtained from Wadi al- rafidain for pharmaceutical products – Iraq- Baghdad. Guaifenesin purity is (99.93%). Codeine phosphate purity is (99.51%). Phenylephrine HCl purity is (99.34%). Sodium benzoate purity is (99.58%). O-phosphoric acid was purchased from Sigma Aldrich. Acetonitrile and methanol were purchased from ISOLAB GmbH, Germany. All chemicals were purchased HPLC grade.

2.2 Instrumentation

The HPLC instrument has been used in SHIMADZU, Japan, with a diode array detector. The spectrophotometer was UV-Vis Spectrophotometer Shimadzu 1800 with U.V. probe software. Analytical balance was used by Shimadzu. pH meter was used by W.T.W. Germany. All the glass wares used were from ISOLAB.

2.3 Standard solution

A Standard Stock Solution of Guaifenesin was prepared by using 200 mg and dissolving it with mobile phase in a 50 ml volumetric flask to obtain a concentration of 4 mg/ml. A stock solution of sodium benzoate was prepared by dissolving 100 mg with mobile phase in a 50 ml volumetric flask to obtain a concentration of 2 mg/ml. Standard Stock Solutions for Codeine phosphate and Phenylephrine HCl were prepared using mobile phase to obtain concentration (1mg/mL). A standard solution of 2 mg/mL Guaifenesin, 0.2 mg/mL codeine phosphate,0.1 mg/mL Phenylephrine HCl, and 0.5 mg/mL sodium benzoate was prepared from the stock

solutions. Calibration standards solutions at five different concentrations have been prepared by diluting stock standard solutions with a concentration range of 0.25 mg/mL –4 mg/mL for Guaifenesin, 0.05 mg/mL - 1 mg/mL Codeine phosphate, 0.025 mg/mL -0.5 mg/mL Phenylephrine HCl, and 0.125 mg/mL - 2 mg/mL for sodium benzoate. Samples in triplicates have been made for each concentration. The calibration curve was prepared by plotting the peak areas versus the concentrations.

2.4 Assay Procedure for Syrup

10 ml syrup equivalent to 200 mg guaifenesin, 20 mg codeine phosphate, 10 mg Phenylephrine HCl, and 50 mg sodium benzoate were dissolved in a 100 volumetric flask using mobile phase. The solution was sonicated for 10min and filtered using 0.45μm syringe filter. The solution was injected, and peak areas were measured.

3. RESULTS AND DISCUSSION

3.1 Method Development

Several chromatographic conditions were changed in each experiment to achieve the best efficiency. Parameters like wavelength, column, mobile phase, and pH have been optimized. Different proportions of buffer and solvents have been experimented with to achieve the best mixture of the mobile phase. The mixture of 1% o-phosphoric acid: methanol: acetonitrile (80: 10: 10) and pH adjusted to 3.1 has been selected to be the mobile phase. Isocratic elution mode was used with a flow rate of 1 mL/min. The optimum wavelength was selected at 254 nm, which is the best detector response that has been achieved for all drugs.

3.2 Selectivity

The result shows that drugs have been separated from the excipients nicely, as shown in **Figure 1**. This HPLC method has been found to be selective.

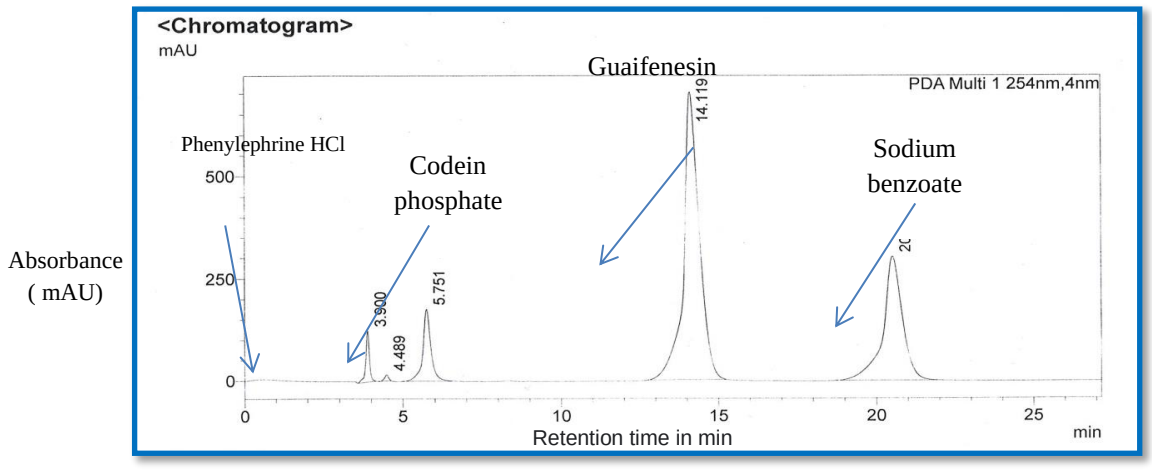

Figure1. RPHPLC chromatogram of Guaifenesin, Codeine phosphate, Phenylephrine HCl, and sodium benzoate.

3.3 Linearity

The linearity of calibration curves was checked in the ranges of 0.25 –4 mg/ml for Guaifenesin, 0.05 - 1 mg/ml Codeine phosphate, 0.025 -0.5 mg/ml Phenylephrine HCl, and 0.125 - 2 mg/ml for sodium benzoate. Results in **Figure** (**2**,**3**,**4**,**5**) show that the calibration curves have been linear in the tested range.

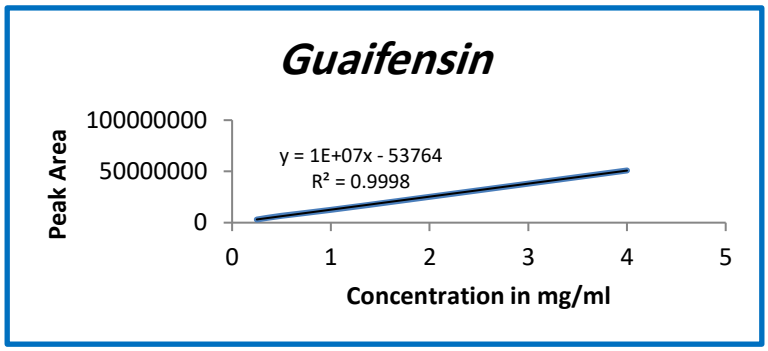

Figure2. Calibration curve of Guaifenesin

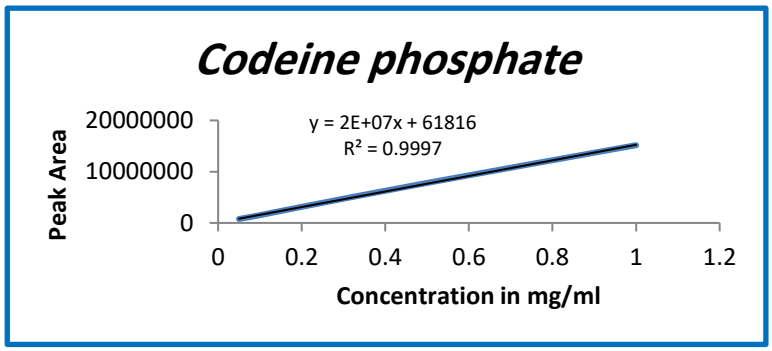

Figure3. Calibration curve of Codeine phosphate

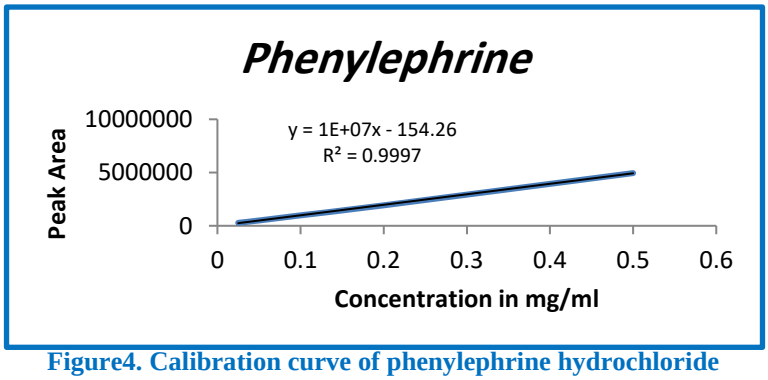

Figure4. Calibration curve of phenylephrine hydrochloride

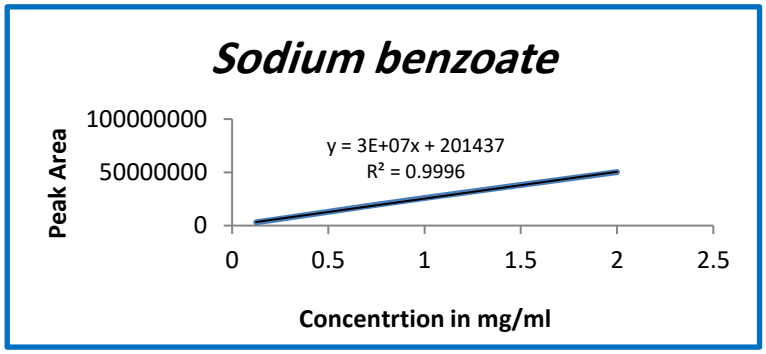

Figure5. Calibration curve of sodium benzoate.

3.4 Accuracy

The accuracy of this reported method has been validated using recovery studies. Results in **Tables 1,2, 3**, and 4 show that this method is significant.

Table 1: Accuracy for Guaifenesin.

Concentration mg/mL	Calculated Conc in mg/mL	Recovery	A.V.R. Recovery%
1.6	1.588	99.25	99.675
1.6	1.592	99.50	
1.6	1.590	99.375	
1.6	1.596	99.75	
1.6	1.608	100.5	
2	1.995	99.75	100.06
2	2.011	100.55	
2	2.006	100.30	
2	1.996	99.80	
2	1.998	99.90	
2.4	2.396	99.833	100.058
2.4	2.412	100.5	
2.4	2.394	99.75	
2.4	2.397	99.875	
2.4	2.408	100.333	

Table 2: Accuracy for codeine phosphate.

Concentration mg/mL	Calculated Conc in mg/mL	Recovery	A.V.R. Recovery%
0.16	0.1596	99.75	100.046
0.16	0.1611	100.68	
0.16	0.1593	99.56	
0.16	0.1607	100.43	
0.16	0.1597	99.81	
0.2	0.1992	99.6	99.86
0.2	0.1996	99.8	
0.2	0.2014	100.7	
0.2	0.1991	99.55	
0.2	0.1993	99.65	
0.24	0.2386	99.41	99.73
0.24	0.2406	100.25	
0.24	0.2391	99.62	
0.24	0.2396	99.83	
0.24	0.2389	99.54	

Table 3: Accuracy for phenylephrine hydrochloride.

Concentration mg/mL	Calculated Conc in mg/mL	Recovery	A.V.R. Recovery%
0.08	0.07982	99.77	99.63
0.08	0.07936	99.2	
0.08	0.07932	99.15	
0.08	0.08016	100.2	
0.08	0.07988	99.85	
0.1	0.1012	101.2	99.96
0.1	0.09964	99.64	
0.1	0.09982	99.82	
0.1	0.09924	99.24	
0.1	0.09992	99.92	
0.12	0.1191	99.25	99.51
0.12	0.1190	99.16	
0.12	0.1196	99.66	
0.12	0.1193	99.41	
0.12	0.1201	100.08	

Table 4: Accuracy for sodium benzoate.

Concentration mg/mL	Calculated Conc in mg/mL	Recovery	A.V.R. Recovery%
0.4	0.3972	99.3	99.27
0.4	0.3968	99.2	
0.4	0.3962	99.05	
0.4	0.3992	99.8	
0.4	0.3960	99	
0.5	0.4992	99.84	99.68
0.5	0.4984	99.68	
0.5	0.5022	100.44	
0.5	0.4972	99.44	
0.5	0.4950	99	
0.6	0.5962	99.36	99.33
0.6	0.5944	99.06	
0.6	0.5988	99.8	
0.6	0.5966	99.43	
0.6	0.5942	99.03	

3.5 Robustness

Robustness was determined using different experimental conditions. The flow rate and the pH were changed on the same day to obtain the best result.

3.6 Specificity

The syrup was analyzed and separated with good resolution. No interferences were shown between the peaks, and that indicates that the method is specific. No interferences are shown between the active ingredients and the excipients.

3.7 Precision

Six injections of the same standard concentration have been injected to check the precision of this method. As shown in **Table 5**, the R.S.D. % for Guaifenesin 0.18 %, codeine phosphate 0.21 %, phenylephrine 0.157, and sodium benzoate 0.314 is not more than 1%, and that indicates that this method is precise.

Table 5. Precision of the method

Repeatability								
Injection number	guaifenesin		codeine phosphate		phenylephrine		sodium benzoate	
	Conc mg/ml	Area	Conc mg/ml	Area	Conc mg/ml	Area	Conc mg/ml	Area
1	2	24692488	0.2	3136006	0.1	980413	0.5	12916302
2	2	24631822	0.2	3129028	0.1	981934	0.5	12816336
3	2	24611646	0.2	3139982	0.1	981906	0.5	12886284
4	2	24682942	0.2	3130116	0.1	979218	0.5	12926269
5	2	24571458	0.2	3134176	0.1	979122	0.5	12906348
6	2	24643064	0.2	3121036	0.1	982802	0.5	12866312
AVR		24638903.3		3131724		980899.1667		12886308.5
SD		45099.9164		6582.24606		1544.433154		40482.20481
RSD%		0.18		0.21		0.157		0.314

3.8 Concentration of active ingredients in syrup form (Pulmocodain Syrup)

Pulmocodain Syrup is syrup produced by The State Company for Drugs Industry and Medical Appliances Samarra/ Iraq. It contains guaifenesin 100 mg/5mL, Codeine phosphate 10 mg/5mL, Phenylephrine HCl 10mg/5mL, and Sodium benzoate 25mg/5mL. This syrup was

injected to check the validation of this method. As presented in **Table 6**, the result shows this method is specific and accurate.

Table 6: Results of Pulmocodain Syrup obtained by using this method.

Active component	Guaifenesin	Codeine phosphate	Phenylephrine HCl	Sodium benzoate
Stated amount	100 mg/5ml	10 mg/5ml	5 mg/5ml	25 mg/5ml
Result found	99.46 mg/5ml	9.92 mg/5ml	4.975 mg/5ml	25.34 mg/5ml
Result Found % ± (S.D.)	99.46 (± 0.32)	99.2 (± 0.18)	99.5 (± 0.54)	101.36 (± 0.44)

4. Conclusion

This work aims to build and validate the HPLC method to determine the concentration of Guaifenesin, codeine phosphate, phenylephrine, and sodium benzoate in liquid pharmaceutical form. The validation result shows good accuracy, precision, and selectivity. This method is easy, and it can be used to determine the assay of four compounds in only one run. This method is rapid, accurate, selective, precise, and robust. It can be used for everyday work in a quality control laboratory. There is no reported method for this mixture of active ingredients in liquid pharmaceutical form.

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