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Simultaneous RP-HPLC Determination of Chlorpheniramine Maleate and Paracetamol in Pharmaceutical Formulations

Dhilal Sukban Alwan [*,1], **Khalaf F. AL-Samarray**[1]

[1]*Chemistry Department, College of Education for Pure Science, University of Samarra, 34010 Salah Aldeen, Iraq.*
[2]*Al-Hadhara University College, Salah Aldeen, Iraq.*
***Corresponding author:**
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ABSTRACT

The quality control of chlorphenamine maleate (CPM) and paracetamol (PCM) in pharmaceutical preparations needs a reliable, rapid, and cost-effective analytical technique. The study aimed to develop and optimize a reversed-phase high-performance liquid chromatography (HPLC) method for the simultaneous determination and separation of these two frequently utilized pharmaceuticals. To do chromatographic separation on a C18 column (250x4.6 mm, 5mm), an isocratic mobile phase of phosphate buffer (pH 6), water, acetonitrile, and ethanol (70:15:15 v/v) was used at a flow rate of 0.8 ml/min, with justification in terms of λmax and sensitivity. Even though chlorpheniramine maleate and paracetamol had absorption maxima of 262 nm and 249 nm respectively, 228 nm was chosen as a compromise wavelength since it gave both analytes good absorbance and good detector response since it has good absorbance and good response characteristics of a stable baseline. The procedure was confirmed with pure substances as well as pharmaceutical preparations using standard guidelines, which evaluated linearity, accuracy, detection limit (LOD), and quantification limit (LOQ). The process had the capability of separating CPM and PCM with a 3.2 min retention time and 5.3 min retention time respectively and analysis time was below 10 minutes. The procedure exhibited a good linearity in the concentration ranges of 10-120 µg/mL of CPM (R2=1) and 10-110 µg/mL of PCM (R2=0.9999). It showed high accuracy and reliability. The LOD was 0.06 µg/mL for CPM and 0.27 µg/mL for PCM, while the LOQ was 0.18 µg/mL for CPM and 0.82 µg/mL for PCM. The developed HPLC method is rapid, sensitive, precise, and economical. It is therefore suitable for routine quality control analysis and for the simultaneous quantification of chlorphenamine maleate and paracetamol in combined pharmaceutical dosage forms.

Keywords: Chlorpheniramine malate, Paracetamol, Pharmaceutical, RP-HPLC

INTRODUCTION

Multi-component pharmaceutical preparations are among the most widespread pharmaceutical forms due to their therapeutic efficacy resulting from containing more than one active ingredient. However, analyzing these preparations presents an analytical challenge because of the need to simultaneously and accurately determine multiple components. Therefore, the development of modern analytical techniques has led to the advancement of advanced methods, with high-performance liquid chromatography (RP-HPLC) being a prominent example due to its high efficiency in the simultaneous separation and determination of compounds [1,2].

The chemical substance known as Paracetamol is scientifically identified as (N-(4-hydroxyphenyl) acetamide. It appears in the form of white solid crystals. Chemically, its molecular formula is ($C_8H_9NO_2$) and it has a molecular weight of 151.16 g/mol. This substance is characterized by specific physical properties, notably its melting point, which ranges between 169°C and 170°C [3]. Paracetamol is a main ingredient in many cold and flu medications [4]. It consists of a benzene ring attached to a nitrogen atom and is a white, poorly water-soluble substance [5,6]. Chlorpheniramine maleate is a low-cost antihistamine found in many over-the-counter medications used to relieve cold and allergy symptoms. It is known as 3-(4-Chlorophenyl)-N, N-dimethyl-3-(pyridin-2-yl)-propan-

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1-amine, with the molecular formula $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$, is a white, odorless crystalline powder with a molecular weight of 390.9 g/mol. It has a melting point of approximately 135 °C and exhibits good solubility in water, ethanol, and methanol, while being slightly soluble in ether and benzene and is soluble in water and ethanol [7,8].

Paracetamol has previously been analyzed by spectrophotometric and chromatographic methods [9,10]. Chlorphenamine has also been determined by several spectroscopic and chromatographic methods [11,12].

Many industries that use HPLC technology, especially in the pharmaceutical, food, and environmental science sectors, do so because it is an excellent and vital technique for drug analysis, enabling separation and simultaneous quantification of drugs from multiple substances [13]. The precision of this technique makes it essential in research and industry for drug discovery and formulation, as well as for quality control. The process is carried out by injecting the liquid sample, which is carried by the mobile phase, into the separation column containing small particles that serve as the stationary phase. The success of the separation process depends on the difference in retention times between the components of the sample [14].

The most common type is reversed-phase liquid chromatography, which uses a liquid mobile phase that is more polar than the stationary phase [15]. The HPLC system comprises of a solvent tank, a pump, an injector, a column, a detector and a sample collection and display system. The column does the separation which is the primary part of the system [16]. High-performance liquid chromatography (HPLC) offers specific details on how to identify, quantify, and determine the accuracy of drugs. Technology finds extensive applications in forensics, medicine, food safety, pesticide control, herbal research, and in checking on the presence of biological contamination [17,18].

The recent studies affirm the esteemed position of HPLC technology as an analytical tool in the different sectors such as pharmaceuticals, food safety and monitoring the environment. Its use in the identification of contaminants in food products, biochemical and clinical studies has been noted, and it is proven to be effective in comparison to other techniques [19, 20, 21].

This study was proposed with the following objectives: (a) To prepare and optimize the reverse-phase high-performance liquid chromatography (RP-HPLC) technique in determining both paracetamol and chlorpheniramine maleate by investigating the different analytical parameters, including pH, flow rate, composition of the mobile phase (organic to aqueous solvent ratio), and the nature of the chromatographic column. (b) To investigate the impact of various analytical conditions on separation efficiency, such as retention time, tailing factor, and number of theoretical plates to obtain the best separation possible between the two compounds. (c) Confirm the soundness of the analytical procedure as per ICH guidelines in the sense of linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision, and selectivity.

MATERIALS AND METHODS

Materials

All materials used were of high purity; therefore, they did not undergo further purification. The solvents were of analytical grade and supplied by CHEM-LAB (Belgium). Distilled water, acetonitrile, and ethanol were 99.9% pure.

Chromatographic Conditions and Instrumentation

The pharmaceutical compounds chlorphenamine maleate and paracetamol were separated using a Shimadzu Lab-20AD system (Shimadzu Corporation, Japan) equipped with a C18 column (250 × 4.6 mm, 5 µm, Macherey-Nagel, Germany). The mobile phase consisted of aqueous 0.02 M phosphate buffer (70%), acetonitrile (15%), and ethanol (15%) (v/v/v). The pH was adjusted to 6.0 prior to use

The mobile phase was degassed for three minutes in an ultrasonic bath (Power-Sonic-420) and then filtered through a 0.45 µm filter. We injected 20 microliters of the samples at a rate of 0.8 ml/min and watched them at 228 nm. We figured out what CPM and PCM were by comparing their retention times to those of reference standards. We used a UV-Vis-1800 spectrophotometer with a quartz cuvette (1 cm path length) to do the spectroscopic characterization. All analyses were conducted at the SDI Samarra facility in Iraq.

Standard stock solution

To prepare a standard stock solution (1000 µg/mL), 0.1 g of each active ingredient was weighed and put in a 100 mL volumetric flask. 20 ml of the solution moved to a different 100 ml volumetric flask and solvent added until it reached the mark. This made a 200 µg/ml solution of each drug. From this, more dilutions were made.

Dilutions of Solutions

Different concentrations of CPM and PCM were prepared one after the other from the 200 µg/ml stock solution. Then 0.5 to 6 ml of CPM and 0.5 to 5 ml of PCM moved to 10 ml volumetric flasks and enough water added to reach the mark. This made the final concentrations of CPM 10 to 120 µg/ml and PCM 10 to 110 µg/ml.

Pharmaceutical Tablet Assay

Commercial pharmaceutical preparations were ground, and the equivalent of one tablet was dissolved in a 70% water:15% acetonitrile:15% ethanol solution in a 100 ml flask. The solution was sonicated, diluted to volume, and filtered. The filtrate was further diluted to obtain the required concentrations for analysis.

RESULTS AND DISCUSSION

Detection of Wavelength and Method Development

Solutions containing 20 µg/mL of CPM and PCM were scanned between 200 and 400 nm, explanationAlthough chlorpheniramine maleate and paracetamol showed individual absorption maxima at 262 nm and 249 nm, respectively, 228 nm was selected as a compromise wavelength because it provided adequate absorbance for both analytes, enhanced sensitivity, and suitable detector response for simultaneous analysis with stable baseline characteristics .20 µL of the standard sample solution was injected at 228 nm, resulting in higher chromatogram heights, clear drug separation, and shorter retention times. The results showed that chlorphenamine maleate (CPM) had a shorter retention time on the column than paracetamol (PCM). Due to the high solubility of chlorphenamine maleate in its salt form in water and its partial charge, which increases its polarity, its association with the stationary phase is weaker, so it separates in a shorter time than paracetamol. Figure 1a shows the wavelength of the two drugs.

Several solvent mixtures were tested. The best mobile phase was a mixture of 70% water, 15% acetonitrile and 15% ethanol (pH 6.0+0.2). A number of solvent mixtures were experimented with. The best mobile phase was a blend of 70 per cent water, 15 per cent acetonitrile and 15 per cent ethanol (pH 6.0+0.2). The mobile phase was prepared by mixing HPLC-grade water, acetonitrile, and ethanol in the ratios (70:15:15, v/v/v), respectively. Sodium dihydrogen phosphate was then added to the mixture at a concentration of 0.02 M to ensure pH stability . The mobile phase was prepared by mixing HPLC-grade water, acetonitrile, and ethanol in the ratios (70:15:15, v/v/v), respectively. Sodium dihydrogen phosphate was then added to the mixture at a concentration of 0.02 M to ensure pH stability. The solution was subsequently placed in an ultrasonic bath for 3 minutes to ensure complete homogeneity and degassing, followed by filtration through a 0.45 µm membrane filter before final adjustment of the pH value with high precision. This buffer concentration was specifically selected to maintain a stable acidic environment during the separation process . A flow rate of 0.8 ml/min with a 20 µL injection volume provided excellent separation. No interference from excipients was observed. Also, the retention times are excellent for both CPM and PCM. Table 1 provides a comprehensive list of optimal chromatographic conditions, along with notes on column efficiency. Figures 1b and 1c show the chromatograms of each drug individually, while Figure 1d shows a standard combination of CPM and ACA drugs under optimal conditions

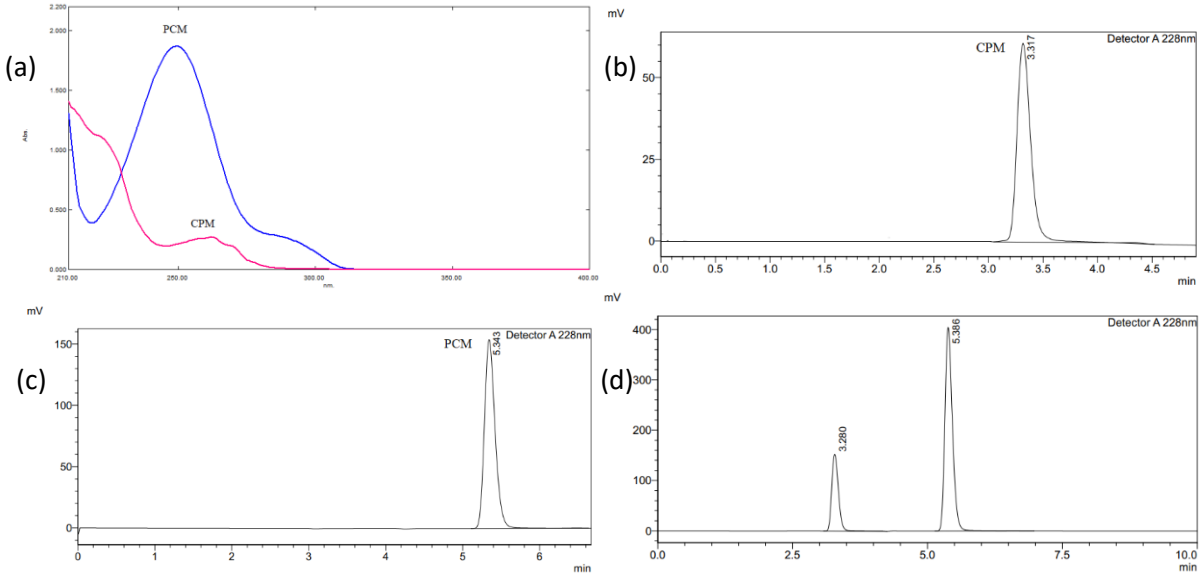

Figure 1: (a) The wavelength of the two drugs (CPM, PCM). HPLC chromatograms for common medications: (b) CPM (20 µg /ml), (c) PCM (20 µg /ml). (d) Typical drug combinations HPLC chromatograms.

Table 1: The optimized chromatographic conditions.

Predicate optimum	Parameters results
Composition of eluent phase	PH 0.02M H_2O70:ACN15:EOH15 V/V
Column Types	MN, C18 (250 cm /4.6, 5µm)
Flow rate	0.8 (mL/ min)
Samples Temperature	Ambient
Injection volume	20 (µL)
Column Temperature	25 ± 1 (°C)
Run Times	10.00 (min)
Detection wavelengths	228 (nm)
Retention time	3.2 CPM and 5.3 PCM

Method validation

Standard solutions were prepared by diluting the stock solution with the same mobile phase (water and acetonitrile in the right amount), that gave us a calibration range of 10–120 µg/mL of the pharmaceutical standard. The chromatographic analysis commenced with the injection of a mobile phase sample to confirm the absence of impurities in the column, succeeded by the injection of an additional paracetamol sample at a concentration of 20 µg/mL, as depicted in Figures 2a and 2b.

The proposed RP-HPLC method exhibited excellent linearity over the concentration ranges of 10–120 µg/mL for chlorpheniramine maleate (CPM) and 10–110 µg/mL for paracetamol (PCM). The regression equations obtained were y = 24377x – 40194 (R^2 = 0.9999) for CPM and y = 72250x – 29815 (R^2 = 1) for PCM. These findings confirm a strong and statistically significant linear relationship between analyte concentration and detector response across the studied range, indicating the suitability of the method for quantitative analysis. As illustrated in Figures 2c and 2d

The objective of this validation was to demonstrate the closeness of agreement between the measured values obtained using the proposed method and the true concentrations. Accordingly, three concentration levels (25, 65, and 95 µg/mL) within the Beer's law range were analyzed for both CPM and PCM, as summarized in Table 2.

The limits of detection (LOD) and quantification (LOQ) were found by serially diluting the stock solution until we got the smallest response that could be detected. The values were subsequently calculated in accordance with ICH guidelines, where LOD is defined as 3.3 × (SD/S) and LOQ as 10 × (SD/S), with S representing the slope of the calibration curve and SD the standard deviation of the intercept [(22)], as summarized in Table 3.

Specificity was further investigated to confirm the absence of interference from potential degradation products or excipients. A placebo solution, free from active pharmaceutical ingredients, was analyzed under the same chromatographic conditions. No significant interfering peaks were observed at the retention times of the analytes. Comparative evaluation of chromatograms obtained from the placebo and CPM standard solutions demonstrated consistency in peak behavior, while the high coefficient of determination (R^2) further supports the reliability and accuracy of the proposed method. Figures 2c and 2d present the chromatograms of the blank and CPM standard solutions, respectively.

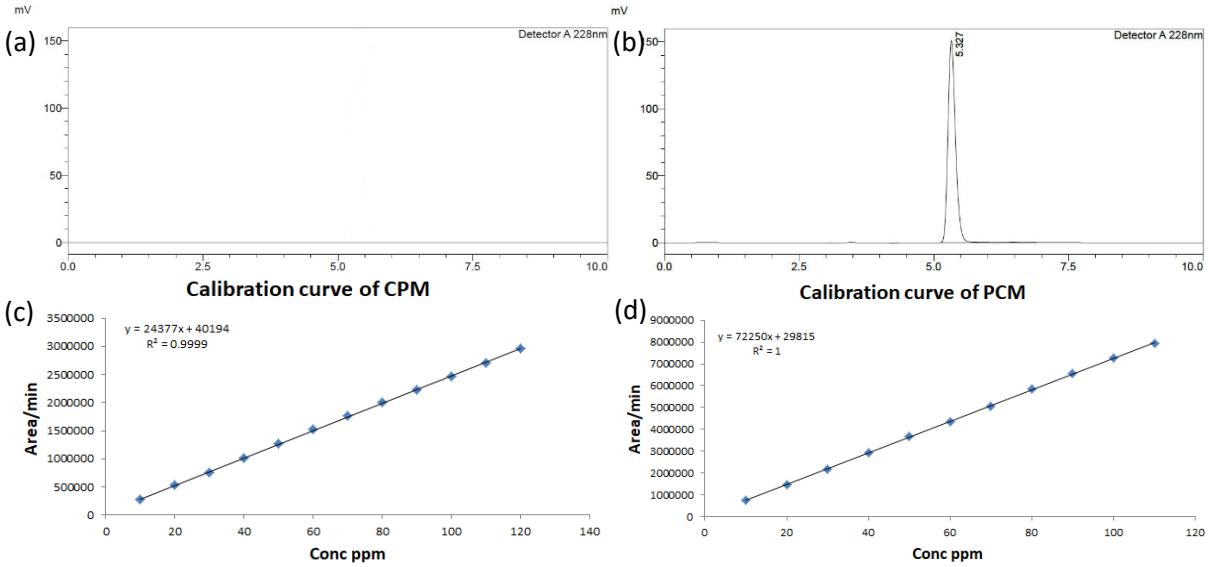

Figure 2: (a) Calibration curve of CPM. (b) Calibration curve of PCM. The blank placebo chromatogram is shown in (c), prepared with (H_2O:EOH: ACN, 70:15:15 v/v), and (d) represents the standard PCM solution (20 µg /ml).

Table 2: Accuracy and Precision of the Method.

CPM µg /ml	**Recovery%**	**RSD %, n=3**		
Taken	Found			
25	25.00	100.02	Mean = 100.17	0.02
65	65.24	100.38		0.79
95	95.10	100.11		0.34
PCM µg /ml	Recovery%	RSD %, n=3		
Taken	Found			
25	25.00	100.02	Mean =100.22	0.20
65	65.34	100.52		0.97
95	94.26	99.22		0.88

Table 3: Summary of Method Validation Parameters of the method.

S. N	**Predicate optimum**	**Parameters results**
1	Linearity Range	10– 120 of CPM and 10-110 of PCM µg/mL
2	Straight line equation	y =24377x - 40194 of CPM
3	Straight line equation	y = 72250x - 29815 of PCM
4	R^2	0.9999 CPM, 1.000 PCM
5	LOD	0.06 of CPM and 0.27 of PCM µg/mL
6	LOQ	0.18 of CPM and 0.82 of PCM µg/mL
7	Accuracy (Rec%)	CPM, 99.46 and PCM, 100.22

Application in Real Samples

Pharmaceutical formulation FUL-OUT in ten tablets was finely powdered and homogenised. The mean weight of each of the ten tablets was calculated to be 7.253 g. The specified weight of one tablet (0.7253 g) of the powder was precisely weighed and dissolved in the necessary solvent through constant stirring in order to completely dissolve the powder. The solution that was obtained was filtered and poured into a 100 mL volumetric flask and diluted to the mark with the same solvent to produce stock concentrations of 3500 ppm PCM and 20 ppm CPM. This solution was then employed to ascertain the true content of the active pharmaceutical ingredients (APIs) in each tablet.

Further dilution was done to obtain the desired concentrations of PCM and CPM analysis. In the case of the exact analysis of the changes in CPM, the weight of four tablets was subjected to the same procedure in a 100 mL volumetric flask. The developed sample was carefully injected (20 µL) into the HPLC system.

In order to show the practicability of the elaborated RP-HPLC method, the samples were examined with the UV detection at 228 nm with a flow rate of 0.8 mL/min. A solution of water, acetonitrile and ethanol (70:15:15, v/v/v) with the addition of 0.02 M phosphate buffer was used as the mobile phase. With such optimized conditions, the two analytes were separated efficiently in a total of 10 minutes with full separation being attained in less than 6 minutes.

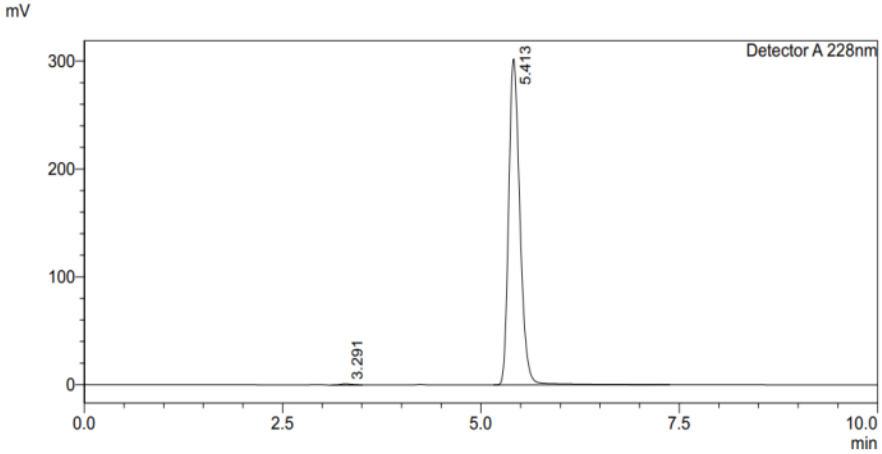

Figure 3: Chromatogram of drugs in the mixed formulation sample (FLO-OUT) showing separation of CPM and (PCM 40 µg /ml).

Table 4: Accuracy and Precision of the method.

Company name	Drug Type	Concentration µg /mL		RSD %, n=3
		Taken	Found	
FUL-OUT	CPM	75	74.92	0.85
	PCM	40	40.07	0.98

CONCLUSION

This experiment has been able to provide a sound, well-validated RP-HPLC procedure of the analysis of chlorpheniramine maleate and paracetamol together. The invented method turned out to be very effective, providing a fast analysis time, and good sensitivity with no complicated sample preparation. It is a perfect and economical selection to use in pharmaceutical companies in routine quality control due to its proven accuracy and precision. Moreover, the flexibility of such an approach underscores the possibility of integrating it with more advanced detection technologies, like mass spectrometry, to enable more complex analytical uses in the future. High-Performance Liquid Chromatography (HPLC) is still among the best techniques of measuring drugs in pharmaceutical preparations. The validity data were high accuracy and great consistency, making HPLC a well-known method of both qualitative and quantitative analysis of active ingredients. This study points out the extended use of HPLC especially in complicated analytical procedures.

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CONFLICTS OF INTEREST

The authors state that they do not have any conflicts of interest in the publication of this manuscript.

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ETHICS STATEMENTS

In this study, there is no human research or use of animals; thus, ethical approval was not needed.

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التحديد المتزامن لماليات الكلورفينيرامين والباراسيتامول في المستحضرات الصيدلانية باستخدام (RP-HPLC) كروماتوغرافيا السائل عالية الأداء عاكسة الطور

ظلال صكبان علوان،[1]، خلف السامرائي [2]

[1]قسم الكيمياء، كلية التربية للعلوم الصرفة، جامعة سامراء،صلاح الدين، العراق

[2]كلية جامعة الحضارة، صلاح الدين، العراق

الخلاصة :

تطلب عملية الرقابة النوعية لماليات الكلورفينيرامين والباراسيتامول في المستحضرات الصيدلانية تقنية تحليلية موثوقة، سريعة، واقتصادية. هدفت هذه الدراسة إلى تطوير وتحسين طريقة كروماتوغرافيا السائل عالية الأداء عاكسة الطور لغرض الفصل والتحديد المتزامن لهذين المركبين الدوائيين الشائعي الاستخدام .ولإجراء الفصل الكروماتوغرافي على عمود من نوع (C18) بأبعاد (250×4.6 ملم، 5 ميكروميتر)، استُخدم طور متحرك متماثل التركيز (Isocratic) يتكون من محلول الفوسفات المنظم (الرقم الهيدروجيني 6)، والماء، والأسيتونتريل، والإيثانول بنسبة (70:15:15 حجماً) وبمعدل تدفق بلغ 0.8 مللتر في الدقيقة، مع تبرير ذلك بناءً على أعلى طول موجي للامتصاص والحساسية. ورغم أن ماليات الكلورفينيرامين والباراسيتامول يمتلكان قمم امتصاص قصوى عند 262 نانومتر و249 نانومتر على التوالي، فقد تم اختيار الطول الموجي 228 نانومتر كحل وسط، حيث منح كلا المادتين امتصاصية جيدة واستجابة ممتازة للكاشف مع خصائص ثبات خط الأساس .تم التحقق من صحة الطريقة باستخدام المواد النقية وكذلك المستحضرات الصيدلانية وفقاً للإرشادات القياسية، والتي قيمت الخطية، والدقة، وحد الكشف(LOD) ، وحد التعيين الكمي .(LOQ) وأظهرت العملية قدرة على فصل ماليات الكلورفينيرامين والباراسيتامول بزمن احتجاز بلغ 3.2 دقيقة و5.3 دقيقة على التوالي، وكان إجمالي وقت التحليل أقل من 10 دقائق .أظهرت الطريقة خطية ممتازة في نطاقات التركيز من 10 إلى 120 ميكروغرام/مللتر لماليات الكلورفينيرامين (معامل الارتباط = 1) ومن 10 إلى 110 ميكروغرام/مللتر للباراسيتامول (معامل الارتباط = 0.9999)، كما أبانت عن دقة وموثوقية عاليين. وبلغ حد الكشف 0.06 ميكروغرام/مللتر لماليات الكلورفينيرامين و0.27 ميكروغرام/مللتر للباراسيتامول، في حين كان حد التعيين الكمي 0.18 ميكروغرام/مللتر لماليات الكلورفينيرامين و0.82 ميكروغرام/مللتر للباراسيتامول .تتميز طريقة كروماتوغرافيا السائل عالية الأداء المطورة بأنها سريعة، وحساسة، ودقيقة، واقتصادية. وبناءً على ذلك، فهي مناسبة لتحليلات الرقابة النوعية الروتينية والتقدير الكمي المتزامن لماليات الكلورفينيرامين والباراسيتامول في الأشكال الصيدلانية المركبة.

الكلمات المفتاحية: ماليات الكلورفينيرامين، الباراسيتامول، مستحضر صيدلاني، كروماتوغرافيا السائل عالية الأداء عاكسة الطور.