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HPLC Simultaneous Separation and Micro-determination of Cloperastine fendizoate, Methyl parahydroxybenzoic acid and Propyl parahydroxybenzoic acid in their Anti-cough Suspension Pharmaceutical Formulation

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Abstract

Cloperastine (1-[2-(p-chloro-alpha-phenylbenzyloxy) ethyl] piperidine) is a drug with an antihistaminic effect and central antitussive; it is a well-known anti-cough drug that plays an important rule against respiratory symptoms during the treatment of coronavirus disease 2019 (COVID-19). This study aimed to develop an accurate, simple, and sensitive validated reversed phase high-performance liquid chromatographic (HPLC) method for the determination of cloperastine fendizoate, as active substance simultaneously with the preservation additives methyl parahydroxybenzoic acid (methyl paraben) and propyl parahydroxybenzoic acid (propyl paraben) in its pure and pharmaceutical dosage. Analysis and quantitation were based on drug direct UV detection wavelength of 248 nm using an isocratic mobile phase consisting of acetonitrile as an organic solvent and phosphate buffered solution of pH 3.0. This method was developed on a reversed-phase ZORBAX ECLIPSE plus-C18 (4.6 × 250 mm, 5-µm) analytical column with a flow rate of 1.5 mL min^{-1} at a normal temperature of 25 °C. The separation of cloperastine fendizoate together with additives; methyl paraben and propyl paraben was achieved at 10.8, 3.7, 8.7 minutes, respectively. The developed method validation was performed following previously describe procedures according to the International Conference on Harmonization (ICH) guidelines to confirm its linearity, precision, and accuracy. Finally, using simple, common available analytical tools, separation of analytes achieved within 13 min. Robustness of analytical method confirmed that the proposed method can tolerate small variations on applying different method parameters.

Keywords: HPLC Method; Development; Validation; Micro-determination of anti-cough drug; Cloperastine fendizoate;

1. Introduction

Cloperastine is a commonly well used drug in the treatment of cough of all ages. The World Health Organization (WHO) declared that the coronavirus disease (COVID-19) is a pandemic on March 2020 [1, 2]. Pharmacological and non-pharmacological trials had been done to achieve a rapid action against the spread of the virus and support an effective medical treatment to current infected patients [3, 4]. The related study to coronavirus was performed; where they are pair of proteins known as receptor Sigma-1 (Sig- 1R) and Sigma-2 (Sig-2R) considered as a part of the cell's communication network and support its resistance to stress in its environment. There is a possibility that the virus uses Sigma receptors for producing oily molecules that help in the formation of membranes for new viruses. Cloperastine can act on Sigma receptors and block the virus actions. Cloperastine would be favorable assistant that modulate proteins within the cell by using Sig-1R and Sig-2R, including the antihistamine as antitussive [5]. Cloperastine acts not only as antihistamine, but also it has remarkable effects in modulation of Sig-1R and the inhibition of SGLT1 blocking glucose uptake in lung cells [6]. In the University of Tokyo, cloperastine was studied for the first time and then in Japan in the 70s of the last century [7]. Cloperastine substance was found to be more effective than codeine, which has been commonly used for many years in treating coughs [8,9]. It is widely used in adults and pediatric with a mechanism of dual action at the central bulbar cough center and at the peripheral receptors in the tracheobronchial tree [10].

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Pharmacological works have showed that cloperastine molecule acts on the cough center without any depression of the respiratory center, and without any negative cardiocirculatory effects [11]. In addition, it has a remarkable efficacy against cough caused by tracheal mechanical stimulus and it has 1.9 times greater efficiency than codeine without any accompanied narcotic effects. Cloperastine hydrochloride showed relatively low acute toxicity if it is administered by the intraperitoneal route to mice and rats; whereas when it is orally administered as cloperastine fendizoate, it shows minor toxicity [12].

Some preservation molecules such as, sodium propionate, sodium metabisulfite, sodium salts of hydroxybenzoate are found to be present in tested pharmaceutical formulation of cloperastine [13]. The present tested formulation is a combination of cloperastine fendizoate with methyl paraben and propyl paraben. Cloperastine formulations were determined using scientific techniques such as UV spectroscopy [14], RP- HPLC [15-18] and HPLC tandem mass spectrometry [19].

The examined drug is available in syrup suspension form; it is a combination between cloperastine fendizoate, two preservative molecules of methyl paraben and propyl paraben. The actual structural formulae of the components of suspension form are given by Fig 1.

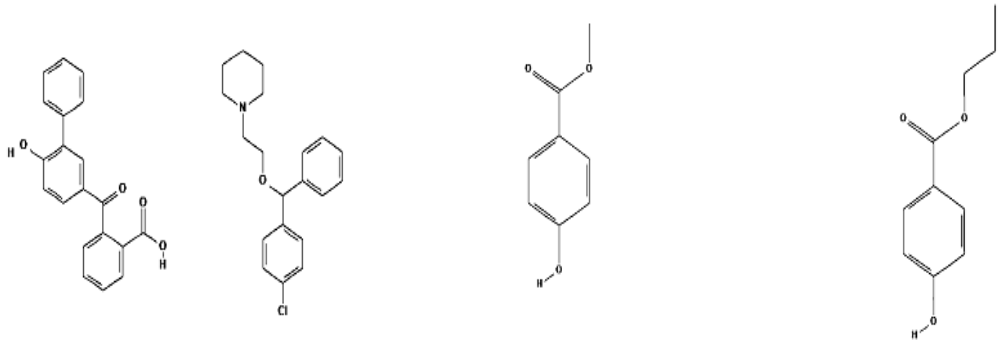


Fig. 1.A. Structure of Cloperastine fendizoate Fig1.B. Structure of Methyl paraben Fig. 1.C. Structure of Propyl paraben

The novelty of the proposed method of analysis is that it is not official in any of the pharmacopoeias and so far, no previously prepared validated published analytical chromatographic method has been reported for the estimation of cloperastine fendizoate in this specific combination in syrup suspension. The aim of the present work is the development of a simple, accurate, sensitive, rapid and valid method of analysis assay by HPLC system for separation and the micro-determination of cloperastine in specific matrix pharmaceutical dosage form such as in syrup suspension.

2. Experimental

2.1 Chemicals and reagents

Cloperastine fendizoate with purity 99.33%, methyl parahydroxybenzoic acid with purity 99.38%, propyl parahydroxybenzoic acid with purity 100.0% and acetonitrile HPLC grade; mono basic potassium phosphate and orthophosphoric acid were purchased from Merck (Germany). The pharmaceutical formulation from the local market analyzed which contain a combination of cloperastine fendizoate as the main active ingredient , methyl paraben and propyl paraben as preservation additives (Suspension syrup). Notussil (708 mg of cloperastine fendizoate per 100 mL suspension produced by Chemipharm pharmaceuticals, Egypt).

2.2 Instrumentation and analysis conditions

Reversed-phase chromatographic analysis was carried out using a liquid chromatography system of Agilent 1200 series equipped with a quaternary pump, vacuum degasser, auto sampler and diode array UV detector of wavelength = 248 nm. The ZORBAX ECLIPSE Plus-C18 (4.6 × 250 mm, 5-µm) analytical column was used as stationary phase. The HPLC mobile phase was a combination between phosphate buffer solution of pH 3.0 and acetonitrile. Injection volume was 30 µL. Chromatographic data processing, acquisition and integration were performed using the Agilent ChemStation CDS software. Standards were weighed using Metler Toledo analytical balance.

Metrohm digital pH- meter was used for pH adjustment.

2.3 Preparation of the solutions for the assay of the pharmaceutical formulations

This formulation was prepared by mixing of 212 µg/mL cloperastine fendizoate solution, 36 µg/mL methyl parahydroxybenzoic acid solution and 5.4 µg/mL propyl parahydroxybenzoic acid. These solutions were prepared by dissolving 36 mg of methyl parahydroxybenzoic acid in acetonitrile and purified water 50:50 v/v and sonication to form methyl paraben stock solution; by dissolving 54 mg of propyl parahydroxybenzoic acid by using the same solvent and sonication to form propyl paraben stock solution and then dissolving 106 mg of cloperastine fendizoate working standard in 100 mL volumetric flask by using the same solvent and sonication to form cloperastine fendizoate stock solution. In a new 100 mL volumetric flask transfer 20 mL from cloperastine fendizoate stock solution, 10 mL from methyl prahydroxybenzoic acid stock solution and 1 mL from propyl parahydroxybenzoic acid stock solution and complete to the volume using the same solvent. Dissolve from pharmaceutical formulation suspension after shaking well what equivalent to 21.2 mg of Cloperastine Fendizoate in 100 ml volumetric flask then add 70 ml of the same solvent used in standard preparation. Sonicate not less than 15 min then complete to 100 ml with solvent. Inject after filtering using 0.45-µm syringe filter.

2.4 Preparation of forced degradation solutions

2.4.1 Acid hydrolysis, alkaline hydrolysis and oxidation degradation solutions

Cloperastine fendizoate, methyl paraben and propyl paraben stock solutions prepared as mentioned in the preparation of the solutions for the assay of the pharmaceutical formulations. In three new 100 mL volumetric flask transfer 20 mL from cloperastine fendizoate stock solution, 10 mL from methyl prahydroxybenzoic acid stock solution and 1 mL from propyl parahydroxybenzoic acid stock solution to each flask. To the acidic hydrolysis flask solution mix with 3 mL of 1 N HCl, with alkaline hydrolysis flask solution mix with 3 mL of 1 N NaOH and for the oxidation degradation flask solution mix with 3 mL of H2O2 (30%). Each solution heated in water bath at 60 °C for 15 minutes, cooled, then neutralized with 1 N NaOH or 1 N HCl in case of acidic and alkaline hydrolysis, respectively, then the volumes were completed with the same solvent.

2.4.2 Photo-degradation and heat degradation solutions

Standard solution was prepared as mentioned in the preparation of the solutions for the assay of the pharmaceutical formulations. The final solution was stored under sun light for 8 hours and degradation was studied. For heat degradation, about 106 mg of cloperastine fendizoate, 36 mg of methyl parahydroxybenzoic acid and 54 mg of propyl parahydroxybenzoic acid working standard was weighed into separated petri dishes, heated in an oven previously adjusted to 100 °C, left for 1 h then cooled. The powder was weighed and dissolved in the same solvent and complete the steps as in preparation of standard solution of assay.

2.5 Preparation of buffer solution, pH 3.0

Buffer solution is obtained by preparing concentration of 0.01M of potassium dihydrogen phosphate via dissolving of 1.36 g in 1000 mL deionized water, sonicated for 5 min and adjust the pH value to 3.0 by using orthophosphoric acid then filtered through 0.45-µm filter.

2.6 Validation procedure

The procedures of the validation study were performed to prove that the method is specific, linear, accurate, robust and precise according the ICH guidelines. The procedure validation was performed by following these validation items: Selectivity, accuracy, precision, ruggedness, robustness and linearity.

3. Results and discussion

3.1 Method development and optimization

3.1.1 Adapting isocratic mobile phase ratios

This developed method of analysis was performed to analyze cloperastine fendizoate suspension pharmaceutical formulations; which containing methyl paraben and propyl paraben as the preservation additives that were added to suspension pharmaceutical formulation during drug manufacturing. The analytical method must be developed to achieve a good separation between the main analyte peak and the preservative components peaks. Fendizoic acid peak used to elute with cloperastine fendizoate active sustenance and also it must be separated to avoid impurity of cloperastine peak. Trials were performed using different percentages of acetonitrile solvent and phosphate buffer solution to achieve the separation process efficiency. Finally, a mobile phase consisting of buffer

solution of pH 3.0 to acetonitrile organic solvent of ratio 62:38% (v/v) by isocratic elution was selected to achieve a combination of good peak shape, significant resolution between analytes' peaks and column performance.

3.1.2 Selection of flow rate

Choice of the suitable flow rate of the mobile phase depends on achieving the best separation, high peak performance and also reducing runtime as possible. The obtained results are shown in Table 1.

Table 1. Relation between flow rate, theoretical plates and retention time of cloperastine fendizoate, methyl paraben and propyl paraben.

	Flow rate (mL min^{-1})	Theoretical plates	Retention time
Cloperastine fendizoate	1.0	12774.3 ±18.5	16.64 ±0.001
	1.2	11749 ±356.9	13.74 ±0.018
	1.5	13470.3 ±477.4	10.83 ±0.001
	1.7	10404.3 ±47.6	9.66 ±0.031
Methyl paraben	1.0	12638.6 ±12.7	5.83 ±0.001
	1.2	11851.6 ±167.2	4.79 ±0.002
	1.5	13247.0 ±17.78	3.75 ±0.002
	1.7	10606 ±169.8	3.32 ±0.002
Propyl paraben	1.0	15163.2 ±5.22	12.9 ±0.02
	1.2	14509.6 ±54.6	11.24 ±0.006
	1.5	15399.3 ±92.9	8.72 ±0.003
	1.7	12936 ±10.6	7.84 ±0.004

From these results it is clear that; the flow rate 1.5 mL min^{-1} is chosen to achieve the more rapid and good column performance. Whereas the flow rate above 1.5 mL min^{-1} is practically non-acceptable taking into consideration to keep running back pressure less than 150 bar.

3.1.3 Optimization of buffer solution pH.

The effect of pH was studied by using three different preparations of buffered solution pH value 2.7, 3.0, 3.3 to check the effect of pH on separation process; theoretical plates and peak shape. The most suitable pH value of the buffered solution was found to be 3.0 in terms of best symmetry and theoretical plates. Another aspect for selecting pH range was chosen to be far enough from pKa value of cloperastine. This is because almost all of pH related fluctuations of retention time occurs for pH values within ±1.5 units of pKa value. Outside this range, the compound retention does not change much with pH and its retention behavior becomes similar to that of a neutral compound [21].

3.1.4 Selection of analytical column.

The analytical method was studied using three analytical columns to choose between them: ZORBAX ECLIPSE Plus-C18 (4.6 × 250 mm, 5-µm), ZORBAX ECLIPSE XDB-C18 (4.6 × 250 mm, 5-µm) and Hypersil BDS-C18 (4.6 × 250 mm, 5-µm). The three columns produced near results in terms of retention times, separation and run time. The first column was selected as it achieved the best output in terms of peak shape, performance and column durability.

3.1.5 Selection of wavelength.

UV spectra of cloperastine fendizoate, methyl paraben and propyl paraben were studied to choose the most suitable running wavelength which could achieve good sensitivity level, away from high interference

low level wavelength range. The obtained results are shown in Fig 2.

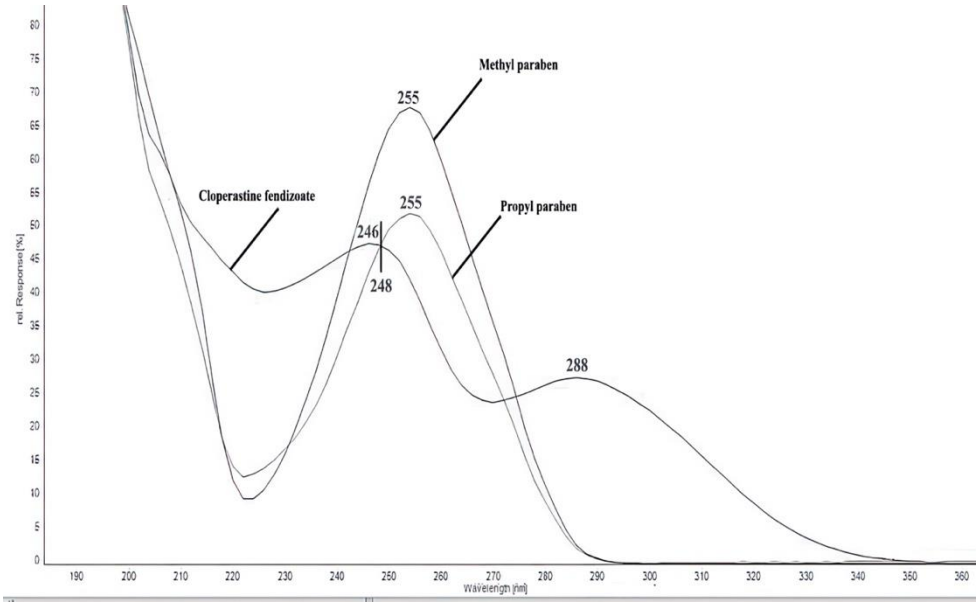


Fig. 2. UV spectra of cloperastine fendizoate, methyl paraben and propyl paraben.

From Fig 2 it is clear that the absorption peak at 248 nm is selected for achieving the most desirable sensitivity for all analysts; especially for propyl paraben which has the lowest concentration in the tested pharmaceutical formulation.

3.1.6 Final chromatographic conditions

Chromatographic conditions were optimized by flowing an isocratic mobile phase consists of acetonitrile and phosphate buffered solution of pH 3.0 run on the instrument with ratio 38 % acetonitrile: 62% buffer from the beginning to the end of the run. It is performed on a 1200 series Agilent HPLC equipped with ZORBAX ECLIPSE Plus-C18 (4.6 × 250 mm, 5-µm) analytical column and the column temperature was adjusted to 25 °C. UV detection was achieved at 248 nm using diode array detector, at a flow rate of 1.5 mL min-1, and injection volume of 30 µL and the obtained results are shown in Fig 3.

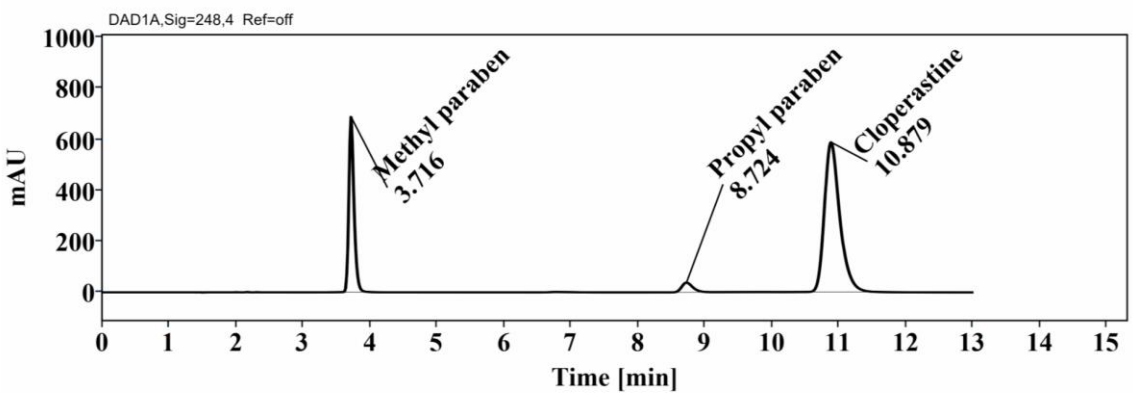


Fig. 3. Chromatographic Separation of cloperastine fendizoate, methyl paraben and propyl paraben preservatives in test sample of anti-cough suspension pharmaceutical formulation

These results refer to that; these parameters will elute cloperastine fendizoate, methyl paraben and propyl paraben after about 10.8, 3.7 and 8.7 minutes retention times, respectively. Isocratic elution of mobile phase is developed to separate cloperastine fendizoate as the active ingredient from the preservation additives methyl paraben and propyl paraben.

3.2 Validation study

Method validation is an important requirement in the practice of chemical analysis confirming that the developed method has performance capabilities consistent with what the application requires. During this work, validation of the analytical method was performed under ICH guidelines [20]. Method validation was performed to study selectivity, sensitivity (in terms of detection (LOD) and quantification limits (LOQ)), accuracy, precision, linearity and analytical method range using different prepared concentrations.

3.2.1 Method selectivity

In this study, not only the detection of cloperastine fendizoate molecules is the method aim but also taking into consecration a good separation from pharmaceutical preservatives; which are added during drug manufacturing. By using isocratic elution of the mobile phase, a good resolution between the active ingredient and preservation molecules was achieved to obtain an accurate detection of the analyte without interference with other excipients (Fig. 3). Fendizoic acid produce an elution peak which is belongs to the cloperastine fendizoate molecule. So, a separation between cloperastine fendizoate and fendizoic acid was one of the aims in this developed method of analysis to achieve cloperastine peak purity. Under this optimized chromatographic conditions, fendizoic acid peak is eluted at 6.7 min with very low intensity.

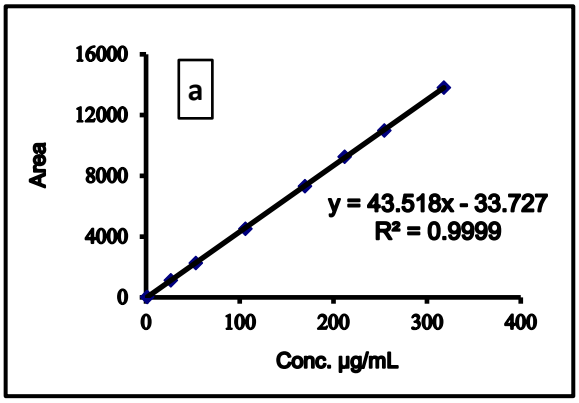
3.2.2 Method sensitivity

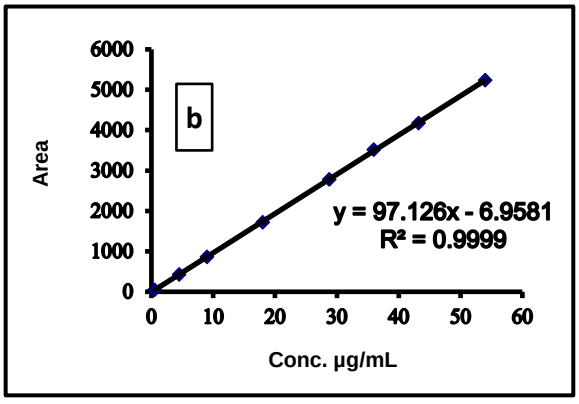
Limit of detection (LOD) has been described as it is the least concentration of an analyte in a test sample that can be measured with a stated probability that the analyte is present at a specific level above that in the blank sample. The limit of quantification (LOQ) has been defined as the minimum concentration of an analyte in a test sample that can be measured with acceptable precision (repeatability) and accuracy under the conditions of the analysis.

Limit of quantitation and limit of detection were calculated from the standard deviation of the blank and slope of the calibration curve. The obtained results are 0.14 µg/mL LOQ and 0.04 µg/mL LOD for cloperastine fendizoate, 0.06 µg/mL LOQ and 0.02 µg/mL LOD for methyl paraben and 0.07 µg/mL LOQ and 0.02 µg/mL LOD for propyl paraben.

3.2.3 Instrument linearity

The linearity of an analytical procedure is a measure of its capability of the method within the range to obtain test results that are directly proportional to the concentration of an analyte in the analyzed sample [20]. A calibration curve was plotted using eight different concentrations ranged from 1.2-318 µg/mL of cloperastine fendizoate, 0.5-54 µg/mL of methyl paraben and 0.67- 8.1 µg/mL of propyl paraben (Fig. 4). Solution for each concentration was injected under the optimized method conditions and calibration curves were constructed between peak area and concentration.





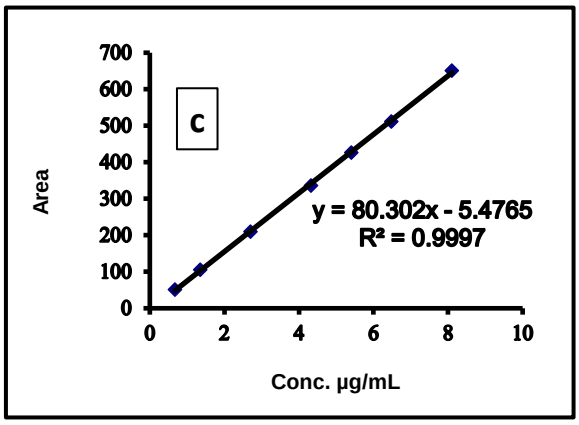


Fig. 4. Linearity curve of: a) Cloperastine fendizoate, b) Methyl paraben, c) Propyl paraben.

It is clear from these results that, the instrument linearity is expressed in terms of the correlation coefficient (r^2) and it is found to be ≥0.999 for cloperastine fendizoate, methyl paraben and propyl paraben which confirms its linearity.

3.2.4 Method accuracy

The analytical method trueness has been expressed as how close the results produced by the method to the true value. Accuracy study of the method was practiced for the drug-matrix and determining the recovery test percentage, which was performed by adding known amounts of cloperastine fendizoate, methyl paraben and propyl paraben to the sample's solutions and the results are depicted in Table 2.

Table 2. Method validation results.

Validation		Chloperastine fendizoate		Methyl paraben		Propyl paraben	
		Recovery %					
Accuracy	Spiking level	120%	99.9±0.25	120%	99.6±0.10	120%	99.9±0.11
		130%	99.8±0.21	130%	99.6±0.20	130%	99.6±0.37
		140%	100.1±0.16	140%	99.7±0.12	140%	99.9±0.18
Repeatability		RSD %=0.11		RSD %=0.09		RSD %=0.11	
Intermediate precision		RSD %=0.13		RSD %=0.09		RSD %=0.50	
Linearity		Range 1.2 - 318 µg/mL		Range 0.5 - 54 µg/mL		Range 0.67 - 8.1 µg/mL	
		R^2= 0.9999		R^2= 0.9999		R^2= 0.9997	
LOQ		0.14 µg/mL		0.06 µg/mL		0.07 µg/mL	
LOD		0.04 µg/mL		0.02 µg/mL		0.02 µg/mL	

The obtained results show that, this test was done on four different solutions, three replicates, corresponding to 100, 120, 130, and 140% and the percent recoveries of each component in drug-matrix form were calculated and presented in Table 2.

3.2.5 Method precision

Repeatability of analytical method is the closeness of agreement between successive results obtained by performing the same method on the same sample, under the same conditions (same operator, same laboratory, same instrument and short intervals of time). Herein, the repeatability was evaluated according to ICH guideline by six independent determinations of test samples of pharmaceutical formulation were injected and the RSD (%) of their recoveries were determined. Relative standard deviation (RSD) was <1.0 as in Table 2.

Method intermediate precision was performed by testing repeating analysis using different instruments and performed by different analysts producing relative standard deviation (RSD) <1.0 for cloperastine fendizoate, methyl paraben and propyl paraben as in Table 2.

3.2.6 Method robustness

The robustness of the method was tested by creating small changes to the method parameters and the results are summarized in Table 3.

Table 3. Method robustness.

Parameter	Modification	Cloperastine fendizoate	Methyl paraben	Propyl paraben
		(Retention time)		
Temeprature (±2°C)	27	10.8±0.025	3.71±0.002	8.71±0.015
	25	10.8±0.025	3.71±0.003	8.71±0.015
	23	10.8±0.009	3.71±0.003	8.70±0.006
pH (±3)	3.3	10.87±0.029	3.72±0.003	8.72±0.017
	3.0	10.84±0.009	3.71±0.004	8.71±0.006
	2.7	10.86±0.025	3.71±0.002	8.71±0.015
		(Mean Area)		
Organic ratio (±2%)	38	9258.96±3.82	3678.57±1.43	440.19±0.84
	36	9232.17±8.54	3661.51±0.61	431.34±0.63
	40	9329.54±1.97	3679.73±1.36	448.79±0.58

These results refer to the fact that, the running temperature was altered by ±2 °C (23,27), the pH of the buffer solution was changed (2.7,3.3), and a small fluctuation of organic solvent ratio in the mobile phase by ±2 % (36,40%). No significant effect was observed and consequently it refers to method robustness.

3.3 Forced degradation study

Different forced degradation products solutions were studied for the purity of analytes and the method was found to be a stability-indicating method of analysis. Appearance of additional peaks in acidic hydrolysis, alkaline hydrolysis, oxidation, heat and photo-degradation conditions.

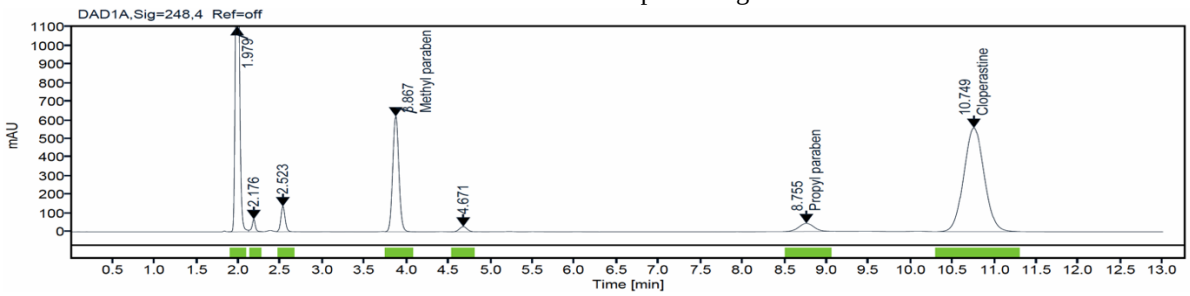


Fig. 5. Chromatogram of forced degradation by oxidation.

Table 4. Forced degradation.

Stress type	Cloperastine fendizoate		Methyl paraben		Propyl paraben	
	Degradation %	Purity	Degradation %	Purity	Degradation %	Purity
Acidic (1N HCl)	30.0	1000	13.8	959.1	12.8	999.4
Alkaline (1N NaOH)	22.2	1000	23.8	999.6	12.1	833.9
Oxidation (30% H_2O_2)	24.2	999.9	25.6	999.8	14.5	817.0
Heat (100 °C)	19.9	1000	16.9	999.7	21.7	954.2
Photolytic (Sun light)	20.1	1000	17.1	955.5	21.6	999.5

The information presented in Table 4 demonstrates the degradation of individual analyte peaks under various stress conditions while ensuring the purity of the peaks, confirming that the analytical method used is stability-indicating.

3.4 Analytical application

The method was tested on actual pharmaceutical drug in the market from the only local drug producer

of this specific combined dosage form of cloperastine fendizoate, methyl paraben and propyl paraben in anti-cough suspension, Chemipharm pharmaceuticals under commercial name Notussil. It was tested and obtained an assay percentage of 3 different samples as shown in Table 5. From the syrup sample an equivalent amount of 21.2 mg of cloperastine fendizoate withdrawn and transferred into 100 mL clean volumetric flask then 70 mL of solvent of acetonitrile and water 1:1 (V/V) was added. After sonication for 15 minutes, cool in room temperature and complete the flask to volume with the same solvent. Solution injected after filtration using 0.45-µ-syringe filter.

Table 5. Assay test results for pharmaceutical dosage form of cloperastine fendizoate anti-cough suspension.

Test solutions	Assay percentage (%) Cloperastine fendizoate	Assay percentage (%) Methyl paraben	Assay percentage (%) Propyl paraben
Test sample 1	97.6 ±0.05	97.5 ±0.02	98.0 ±0.11
Test sample 2	97.0 ±0.16	97.0 ±0.005	97.7 ±0.05
Test sample 3	92.6 ±0.21	92.2 ±0.12	92.6 ±0.19

The data in Table 5 indicate the good applicability of the proposed method for the simultaneous determination of cloperastine fendizoate, methyl paraben and propyl paraben in syrup sample.

4. Conclusion

In this work, an analytical method for the simultaneous micro-determination of one of the widely spread anti-cough suspension such as cloperastine and its preservation additives methyl paraben and propyl paraben in pharmaceutical formulations has been optimized. The optimization of this method was conducted due to the absence of any previously published analysis for this specific combination of cloperastine, methyl and propyl parabens in any pharmacopoeias. Consequently, a validated method had to be developed to address this gap in the existing literature. The method achieved the separation of all analytes in about 13 minutes on a high-performance liquid chromatography (HPLC) system equipped with a UV detector using diode array detector, mobile phase of combination between acetonitrile as organic solvent and phosphate buffered solution of pH 3.0. It has been validated according to ICH guidelines in terms of selectivity, sensitivity, accuracy, range, and robustness and successfully applied on real pharmaceutical formulation available in the market. The developed method is linear with a correlation coefficient (r^2) ≥0.999 for the 3 analytes. Method precision and accuracy were estimated by repeatability test on both analytes expressing a high degree of repeatability with relative standard deviation (RSD) <1.0 and trueness test by spiking known amounts of analyte on the drug-matrix and the percent recoveries were calculated and found in 98-102% limit.

5. Declaration of Competing Interests

Authors declare no conflict of interest

6. Acknowledgement

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7. Role of Authors

Prof. Faten A. Nour El-Dien and **DR. Marwa El-badry**: Supervised the whole work presented in this manuscript, **Prof. Mohamed A. Zayed:** revised its whole content and followed its submission to the journal. The M.Sc. student **Mina G. Morcos:** Did the whole work in lab, tabulate the results and wrote the draft of the whole text.

8. References

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عنوان البحث باللغة العربية

"الفصل الكروماتوجرافي السائل تحت ضغط والتقديرلكل من كلوبراستين فينديذوات وميثيل بارا هيدروكسي حمض البنزويك و بروبيل باراهيدروكسي حمض البنزويك في معلق يستخدم ضد الكحة"

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ملخص البحث باللغة العربية

كلوبراستين فينديذوات هو دواء مشهور بان له تأثير فعال ضد الكحة ويستخدم ضد اي امراض لجهاز التنفس مثال كوفيد -19. وتضمنت تلك الدراسة اقتراح طريقة دقيقة وبسطية وحساسة وغير مكلفة باستخدام الفصل الكروماتوجرافي السائل تحت ضغط العالي لفصل وتقدير كلوبراستين فينديذوات كمادة فعالة في دواء معلق لعلاج الكحة في وجود مضافات اخري بالدواء مثل وميثيل بارا هيدروكسي حمض البنزويك و بروبيل باراهيدروكسي حمض البنزويك. مع استخدام طريقة القياسات الطيفية لكل المكونات بعد الفصل الدقيق لكل مكون علي حدة من خليط الدواء عند الظروف الملائمة للفصل من حرارة ورقم هيدروجين وتراكيز المادة الفعالة وكذلك تراكيز المتداخلات. وقد نجحت الطريقة المقترحة بعد اختبار تكراريتا وثباتها ودقتها في فصل وتقدير كل المكونات بنجاح منقطع النظير مقارنة بالطرق المختلفة المستخدمة في هذا المجال.