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ORIGINAL RESEARCH PAPER

Assessment of the greenness of micellar HPLC method for rapid separation and simultaneous estimation of chlorpheniramine maleate in presence of some co-administered drugs in three pharmaceutical dosage forms using single run

ZEINAB ADEL NASR^{1*}, MARWA M. SOLIMAN¹,
EKRAM H. MOHAMED² and FATMA A. FOUAD¹

¹ Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo 11754, Egypt

² Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, The British University in Egypt, 11837, El sherouk city, Egypt

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ABSTRACT

Sustainable chemistry established one of kind standards to maintain protection of environment through using safer mobile phase composition and/or lower solvent consumption. A fast green micellar HPLC method was developed and applied for the first time aiming at simultaneous determination of chlorpheniramine maleate, one of the most widely used antihistamine in combination with levocloperastine fenodizate or dextromethorphan hydrobromide or dexamethasone, in their pure forms, laboratory prepared mixtures and pharmaceutical dosage forms used in alleviating the symptoms of cough resulting from common colds and allergy. The separation was achieved on Kinetex C₁₈ column (100 mm × 4.6 mm i.d., 2.6-μm particle size) using micellar aqueous mobile phase consisting of (30 mM sodium dodecyl sulfate and 50 mM sodium dihydrogen phosphate, pH 5) and ethanol (85:15) with UV detection at 230 nm. The four drugs were successfully separated using isocratic elution in a single run not exceeding 7 min. According to ICH guidelines, the method was confirmed to be linear, accurate and precise over the concentration ranges of 5–60 μg mL⁻¹ for chlorpheniramine maleate, 10–100 μg mL⁻¹ for levocloperastine fenodizate and dextromethorphan hydrobromide and 5–30 μg mL⁻¹ for dexamethasone. In addition, the greenness of the developed method was assessed using two different tools indicating their least hazardous effect on the environment.

KEYWORDS

green assessment, chlorpheniramine maleate, levocloperastine fenodizate, dextromethorphan hydrobromide, dexamethasone, micellar HPLC

1. INTRODUCTION

Most of analytical techniques lack safety due to hazardous solvents and toxic chemicals used. Thus, green chemistry attracted the attention of analytical chemists to develop analytical approaches that are safe to the environment and mankind. In the last few decades, Liquid chromatography was considered to be unsafe technique due to the use of some toxic and harmful solvents that may result in hazardous effects and adverse impact on the environment and analyst [1]. For these purposes, greening chromatography was investigated and became an urge to substitute hazardous solvents by eco-friendly alternates like water and ethanol [2]. Micellar liquid chromatography (MLC) is a technique supporting the concept of green

*Corresponding author. Tel.: +20
1005620951.
E-mail: zeinabadel@azhar.edu.eg;
z.adel2210@gmail.com

chemistry and represents an excellent alternative to a traditional liquid chromatography. MLC has different advantages such as proficiency of separation of drugs with different nature and probability of improvement the efficiency and solvent strength [3].

Cough could be involuntary as a protective reflex action of the human body to clear and remove foreign substances and particles from the respiratory tract and passage of breathing. It may be acute due to different respiratory tract infections including common cold and flu, or chronic due to smoking and asthma. Cough is generally treated with some drug combinations [4]. Levocloperastine fendizoate, dextromethorphan hydrobromide and dexamethasone alone or in combination with antihistamines as chlorphenamine maleate are effective components used for treatment of cough resulting from common colds and allergy.

Chlorpheniramine maleate (CPM), [(3*RS'*)-3-(4-chlorophenyl)-*N,N*-dimethyl-3-(pyridin-2-yl) propan-1-amine hydrogen (Z)-butenedioate] (Fig. 1), is one of the mostly used drugs for treatment of allergic conditions such as allergic rhinitis, hay fever, cough and the common cold. Levocloperastine fendizoate (LCF), 1-[2-[(*S*)-(4-chlorophenyl)-phenylmethoxy] ethyl] piperidine; 2-(4-hydroxy-3-phenylbenzoyl) benzoic acid (Fig. 1) is centrally acting cough suppressant. It also has some antihistaminic action [5]. It is combined with CPM as it acts on the cough center,

without any depression of respiratory center in brain [6]. Dextromethorphan hydrobromide (DXM), [(+)-3-Methoxy-17-methyl-9, 13, 14-morphinan hydrobromide monohydrate] (Fig. 1), is centrally acting cough suppressant. In cough syrups, it is available mostly in combination with sedating anti-histamine CPM [5]. Dexamethasone (DEX), [9-fluoro-11 β , 17, 21-trihydroxy-16 α -methylpregna-1, 4-diene, 3, 20-dione] (Fig. 1) is a corticosteroid anti-inflammatory agent. CPM and DEX are formulated together in the local Egyptian market in the form of syrups for the relief of asthma [5].

Literature review revealed that several methods have been carried out for the analysis of CPM, LCF, DXM, and DEX in their mixture or in their combination with other drugs. CPM and LCF were determined by HPLC method [6]. CPM and DXM were estimated by spectrophotometric methods [7, 8] and HPLC methods [9–19]. CPM and DEX were determined by spectrophotometric methods [20–23], spectrofluorometric method [24], HPLC methods [25–27], and TLC methods [27].

Moreover, a micellar liquid chromatography was developed for estimation of CPM in various samples including pharmaceutical formulation [28–30].

To the best of our knowledge, no reported micellar HPLC method has been developed previously for assay of these four drugs until now. Hence, the present research was

Fig. 1. Chemical structure of studied drugs

proposed to introduce an eco-friendly simple and rapid micellar HPLC method for the first time to simultaneously estimate CPM in three combined dosage forms with LCF, DXM or DEX using single run in about 7 min. The mobile phase is eco-friendly containing low proportion of ethanol as an organic modifier with the objective of replacement hazardous solvents of the published articles without any sample extraction, pre-treatment filtration or derivatization steps. Moreover, the greenness of the developed method was assessed using analytical Eco-scale and the Green Analytical Procedure Index tools (GAPI).

2. MATERIAL AND METHODOLOGY

2.1. Materials and reagents

- Chlorpheniramine maleate (CPM) with purity of 99.89% was obtained as a gift from Memphis Pharmaceutical Company, Cairo, Egypt.
- Levocloperastine fendizoate (LCF) with purity of 99.92% was kindly supplied by Chemipharm Pharmaceutical Company, Cairo, Egypt.
- Dextromethorphan hydrobromide (DXM) with purity of 99.84% was obtained as a gift from Kahira, Pharmaceutical Company, Cairo, Egypt.
- Dexamethasone (DEX) with 99.86% purity was kindly provided by Amoun, Pharmaceutical Company, Cairo, Egypt.
- Lupituss-CPM[®] syrup (each 5 mL contains 4 mg CPM and 20 mg LCF, B.N.M181259) a product of Lupin Ltd., Mumbai, India.
- Codaphen-N[®] syrup (each 5 mL contains 1 mg CPM and 5 mg DXM, B.N.9226009) a product of Alexandria Pharmaceutical Company, Alexandria, Egypt
- Apidone[®] syrup (each 5 mL contains 2 mg CPM and 0.5 mg DEX, B.N.190095) a product of Amoun Pharmaceutical Company, Cairo, Egypt.
- Acetonitrile, methanol, ethanol, n-propanol, n-butanol, orthophosphoric acid, sodium dodecyl sulfate (SDS), and sodium dihydrogen phosphate were obtained from Sigma-Aldrich, Darmstadt, Germany. High purity distilled water was used in this study.

2.2. Instrumentation

Agilent 1100 HPLC system equipped with binary pump, micro vacuum degasser, thermostatic column compartment, and variable wavelength and UV detector. Sample injection was achieved using Agilent 1100 series autosampler. Data processing and acquisition was done by Agilent Chem-Station software, version A.10.01 (Abbotta, USA). Analysis was performed on a Kinetex C 18 column (100 mm, 4.6 mm i.d., 2.6 μ m); (Phenomenex, USA). Jenway digital pH- meter (Jenway, UK) was necessary for pH adjustment. Ultrasonic bath from BHA-180 T (Abbotta, USA) was used. The mobile phase was filtered through Charles Austen Pumps Ltd.

Filter; model-B100 SE (England, UK) using 0.45 μ m millipore filters (Gelman, Germany).

2.3. Chromatographic conditions

Kinetex C₁₈ Column (100 $\times$ 4.6 mm i.d, 2.6 μ m, 100 Å) (Phenomenex, USA) thermostatted at 25 °C and micellar mobile phase consisted of 30 mM sodium dodecyl sulfate, 50 mM sodium dihydrogen phosphate, adjusted to pH 5 using orthophosphoric acid, and ethanol (85:15). The mobile phase was degassed by sonication for 15 min and filtered through 0.45- μ m Millipore membrane filter before use. The separation was performed at a flow rate of 1.0 mL/min and UV detection at 230 nm.

2.4. Standard solutions

Stock solutions of 500 μ g mL⁻¹ of CPM, LCF, DXM, and DEX were individually prepared in methanol. All solutions were found to be stable for more than 1 week if stored at 4 °C, protected from light.

2.5. Construction of calibration curves

Concentrations ranging from 5–60 μ g mL⁻¹ of CPM, 10–100 μ g mL⁻¹ of LCF, DXM and 5–30 μ g mL⁻¹ of DEX were prepared by serial dilutions of aliquots of the stock solution with the mobile phase. These solutions were then analyzed by injecting into the chromatographic system and eluted with the mobile phase under the above described chromatographic conditions. The peak area was plotted against the corresponding concentrations to construct the calibration graphs; in addition, the linear regression equations were calculated.

2.6. Analysis of laboratory prepared mixtures

Aliquots of CPM in combination with LCF, DXM or DEX in different ratios were transferred into 10 mL volumetric flasks, completed to the volume with mobile phase and injected into the chromatographic system under the above described chromatographic conditions. The percent found for each drug was calculated based on calibration graphs or the derived regression equations.

2.7. Assay of Pharmaceutical dosage forms

Aliquots of Lupituss-CPM[®] Syrup equivalent to 4 mg of CPM and 20 mg of LCF, Codaphen-N[®] syrup equivalent to 1 mg CPM and 5 mg DXM or Apidone[®] syrup equivalent to 2 mg CPM and 0.5 mg DEX were transferred to 100 mL volumetric flasks, completed to the volume with methanol, mixed well and filtered. Different concentrations were analyzed by injecting into the chromatographic system as described before under construction of calibration curves.

3. RESULTS AND DISCUSSION

The main target of this work is to develop a new eco-friendly HPLC method for to determination of CPM in three

combined dosage forms with LCF, DXM and DEX with good resolution and minimum analysis time. Micellar liquid Chromatography (MLC) analysis meets the requirements of green chemistry by using environment-friendly reagents and have lower impact on environmental and less hazardous effect compared to conventional chromatographic separation techniques. Moreover, it can be used to solve problems of separation of drugs which differ in their polarity in presence of low percent of organic modifier. The separation was achieved due to the existence of various types of

interactions of the analytes with micelles in the mobile phase and the column surface altered by the adsorption of surfactant molecules, e.g., electrostatic, hydrophobic and steric interactions, permitting the separation of compounds of different nature [31].

The optimization of chromatographic conditions to allow the optimum separation of the four studied drugs with symmetrical peaks were studied. Figure 2 illustrates a typical chromatogram for the analysis of a synthetic mixture of CPM, LCF, DXM and DEX under the above described optimum chromatographic conditions.

Fig. 2. Typical chromatogram of a synthetic mixture of CPM (30 µg/mL), LCF (50 µg/mL), DXM (50 µg/mL) and DEX (20 µg/mL) using micellar aqueous mobile phase consisting of (30 mM sodium dodecyl sulfate and 50 mM sodium dihydrogen phosphate, pH 5) and ethanol (85:15)

3.1. Method development

3.1.1. Choice of column. Three different columns were studied including: Kinetex C₁₈ Column (100 × 4.6 mm i.d, 2.6 µm), Equisil ODS C₁₈ Column (150 × 4.0 mm i.d, 5 µm) and Shim-pack CLC-C₈ column (150 × 4.6 mm i.d, 5 µm); The first column was the most appropriate one which gave symmetrical, well defined peaks with minimum retention time. While, the use of Equisil ODS C₁₈ Column, only DXM and LCF were separated. On the other hand, usage of Shim-pack CLC-C₈ column; overlapping peaks were observed with longer retention times.

3.1.2. Choice of detection wavelength. Different wavelengths were tried in order to find the most suitable one for the analysis including 210, 220, 230, 240 and 250 nm. The most suitable wavelength was found to be 230 nm showing the best sensitivity detection of the four drugs.

Table 1. Optimization of chromatographic conditions for the determination of studied drugs by the proposed micellar HPLC method

Parameters		No. of theoretical plates (N)				Tailing Factor (T)				Resolution Factor (R _s)			
		CPM	LCF	DXM	DEX	CPM	LCF	DXM	DEX	CPM	LCF	DXM	DEX
Conc. of SDS (mM)	10	6058	5065	3987	2945	1.19	1.33	1.25	1.18	5.11	4.25	4.08	
	30	6238	5414	4663	3569	1.18	1.21	1.14	1.11	5.24	4.68	4.31	
	50	6112	5204	4055	3015	1.24	1.27	1.19	1.28	4.75	4.12	4.11	
	100	5939	5102	3799	2548	1.21	1.23	1.28	1.21	4.56	3.96	3.97	
% of ethanol (% v/v)	5	5984	5219	4365	3211	1.22	1.33	1.23	1.14	5.12	4.39	4.22	
	10	6025	5159	4258	3288	1.20	1.37	1.19	1.19	5.18	4.28	4.18	
	15	6238	5414	4663	3569	1.18	1.21	1.14	1.11	5.24	4.68	4.31	
	20	6099	5239	4291	3321	1.25	1.28	1.28	1.16	5.14	4.51	4.26	
Buffer concentration (mM)	40	5879	5043	4100	3024	1.23	1.26	1.17	1.13	5.13	4.33	4.21	
	50	6238	5414	4663	3569	1.18	1.21	1.14	1.11	5.24	4.68	4.31	
	60	5988	5409	4656	3501	1.14	1.17	1.13	1.13	5.19	4.21	4.32	
pH of the mobile phase	4.0	6087	5023	4235	3052	1.24	1.26	1.20	1.21	4.76	3.86	3.79	
	4.5	6126	5099	4069	3152	1.25	1.24	1.24	1.20	4.96	4.12	3.93	
	5.0	6238	5414	4663	3569	1.18	1.21	1.14	1.11	5.24	4.68	4.31	
	5.5	5991	5121	4205	3055	1.28	1.27	1.18	1.25	5.14	3.96	3.85	
Column Temp (°C)	6.0	6054	4892	4157	2798	1.31	1.35	1.31	1.16	5.09	3.84	3.74	
	25	6238	5414	4663	3569	1.18	1.21	1.14	1.11	5.24	4.68	4.31	
	30	6156	5148	4039	3233	1.22	1.26	1.20	1.17	5.06	4.25	4.12	
	35	6057	5098	4098	3124	1.25	1.28	1.26	1.15	5.10	4.13	4.06	
Flow rate (mL/min)	40	6008	4969	4115	3058	1.27	1.30	1.27	1.14	5.13	4.06	3.92	
	0.8	5967	5213	4215	3187	1.20	1.28	1.19	1.22	5.11	4.13	4.11	
	1.0	6238	5414	4663	3569	1.18	1.21	1.14	1.11	5.24	4.68	4.31	
	1.2	6051	5184	4119	3296	1.19	1.34	1.21	1.18	5.09	4.21	4.08

Table 2. Analytical performance data for the determination of the studied drugs by the proposed micellar HPLC method

Parameters	CPM	LCF	DXM	DEX
Linearity range ($\mu\text{g mL}^{-1}$)	5–60	10–100	10–100	5–30
Regression equation $A = a+bc$				
Slope (b) $\pm$ SD	6.935 ± 0.027	10.871 ± 0.070	9.671 ± 0.053	5.659 ± 0.043
Intercept (a) $\pm$ SD	0.220 ± 0.903	0.439 ± 4.254	0.613 ± 3.200	0.595 ± 0.829
SD of residuals	1.241	5.467	4.126	0.891
Correlation Coefficient (r)	0.9999	0.9998	0.9999	0.9998
LOD ($\mu\text{g mL}^{-1}$)	0.43	1.29	1.09	0.48
LOQ ($\mu\text{g mL}^{-1}$)	1.30	3.91	3.31	1.47

Table 3. Application of the proposed micellar HPLC method for the determination of the studied drugs in pure form

Drug	Proposed method			Comparison methods [6, 19, 27]		
	Conc. added ($\mu\text{g mL}^{-1}$)	Found* ($\mu\text{g mL}^{-1}$)	% Recovery	Taken ($\mu\text{g mL}^{-1}$)	Found* ($\mu\text{g mL}^{-1}$)	% Recovery
CPM	5	4.98	99.64	10	10.04	100.44
	10	9.94	99.49	20	19.91	99.56
	20	20.03	100.18	30	30.04	100.15
	30	29.87	99.57			
	40	40.29	100.74			
	60	59.86	99.77			
Mean% $\pm$ SD			99.90 $\pm$ 0.48			100.05 $\pm$ 0.45
<i>t</i> -test			0.46 (2.36)			
<i>F</i> -test			1.14 (19.29)			
LCF	10	10.02	100.27	10	9.86	98.63
	20	19.81	99.08	20	20.27	101.36
	40	40.61	101.53	30	29.86	99.55
	60	59.66	99.45			
	80	79.44	99.31			
	100	100.43	100.43			
Mean% $\pm$ SD			100.01 $\pm$ 0.92			99.85 $\pm$ 1.39
<i>t</i> -test			0.21(2.36)			
<i>F</i> -test			2.28(19.29)			
DXM	10	9.99	99.91	50	50.31	100.62
	20	19.98	99.93	75	74.38	99.18
	40	40.02	100.05	100	100.31	100.31
	60	59.66	99.45			
	80	80.69	100.87			
	100	99.63	99.64			
Mean% $\pm$ SD			99.98 $\pm$ 0.49			100.04 $\pm$ 0.76
<i>t</i> -test			0.15(2.36)			
<i>F</i> -test			2.41(19.29)			
DEX	5	5.11	102.27	5	5.09	101.80
	10	9.88	98.83	30	29.75	99.15
	15	15.09	100.63	50	50.14	100.28
	20	19.76	98.84			
	25	25.08	100.33			
	30	30.05	100.20			
Mean% $\pm$ SD			100.18 $\pm$ 1.28			100.41 $\pm$ 1.33
<i>t</i> -test			0.25(2.36)			
<i>F</i> -test			1.08(19.29)			

*Average of three separate determinations.

The values between parentheses are the tabulated *t* and *F* values at $P = 0.05$ [32, 33].

Table 4. Precision data for the determination of the studied drugs by the proposed micellar HPLC method

Drug	Taken ($\mu\text{g mL}^{-1}$)	Intraday ^a		Interday ^b	
		Mean $\pm$ S.D.*	Precision (RSD%)	Mean $\pm$ S.D.*	Precision (RSD%)
CPM	10	98.03 $\pm$ 0.81	0.83	98.70 $\pm$ 0.47	0.48
	40	98.41 $\pm$ 0.87	0.88	98.84 $\pm$ 1.07	1.08
	60	97.66 $\pm$ 0.56	0.57	98.68 $\pm$ 0.77	0.78
LCF	10	100.09 $\pm$ 1.33	1.33	100.20 $\pm$ 1.80	1.80
	50	99.76 $\pm$ 1.92	1.93	99.75 $\pm$ 1.55	1.55
	100	101.12 $\pm$ 1.34	1.33	100.59 $\pm$ 1.44	1.43
DXM	10	99.40 $\pm$ 0.88	0.89	99.63 $\pm$ 1.55	1.56
	50	99.69 $\pm$ 1.40	1.40	99.59 $\pm$ 1.61	1.62
	100	100.18 $\pm$ 0.76	0.76	100.30 $\pm$ 1.31	1.31
DEX	10	100.50 $\pm$ 1.44	1.43	99.90 $\pm$ 1.67	1.67
	20	100.38 $\pm$ 0.63	0.63	100.28 $\pm$ 0.83	0.83
	30	101.45 $\pm$ 1.51	1.49	100.91 $\pm$ 1.46	1.45

*Average of three separate determinations.

^a Within the day.^b Three consecutive days.

3.1.3. Effect of mobile phase composition. Various mobile phases were evaluated by using different proportions of SDS and organic modifiers. The effect of changing the concentration of SDS on the retention times and separation of the four drugs was tested over the range 10–100 mM. It was found that 30 mM SDS was the most suitable one giving higher number of theoretical plates and best resolution; Table 1. Different organic modifier was tried in order to achieve the best separation between the four drugs in a short run time such as ethanol, methanol, acetonitrile, n-butanol and n-propanol. In case of using methanol, it failed in separation of four drugs in which CPM wasn't separated. Acetonitrile caused separation of DXM and DEX only with broad peak. N-butanol allowed separation of mixture with very poor sensitivity and longer retention time for CPM, DXM, and DEX. N-propanol caused overlapped peaks of four drugs. It was found that ethanol resulted in better separation and improvement in the peak shape. The effect of the amount of ethanol was examined from 5 to 20%; using 15% ethanol gave good resolution and symmetrical peak in adequate time as shown in Table 1.

3.1.4. Effect of buffer concentration and pH. Three different concentrations of sodium dihydrogen phosphate buffer were tried. The most suitable concentration was found to be 50 mM giving symmetrical, efficiently-separated and well-resolved peaks. Different pH values (4.0–6.0) were tried to investigate their effect on the separation; the number of theoretical plates increased reaching its highest value at pH 5.0. So, pH 5.0 was selected as it led to well-resolved symmetrical peak and better resolution of four analytes (Table 1).

3.1.5. Effect of column temperature and flow rate. The effect of column temperature was studied from 25 to 40 °C. The retention of four drugs were decreased at higher temperature with increased the tailing factor. Thus, the optimal temperature was chosen to be 25 °C to give best resolution

and best peak shape. In addition, the influence of the flow rate on the separation of the drug was also examined. At a flow rate 1.0 mL min⁻¹; good separation with minimum retention time (Table 1).

3.2. Method validation

International conference of Harmonization (ICH) guidelines [32] was followed to validate the proposed method.

3.2.1. Linearity. Under the above suggested experimental conditions, calibration curves relating the obtained peak areas to the corresponding concentrations of the drugs were found to be linear over the ranges of 50–60 $\mu\text{g mL}^{-1}$ for CPM, 10–100 $\mu\text{g mL}^{-1}$ for LCF and DXM and 5–30 $\mu\text{g mL}^{-1}$ for DEX. The regression equations and regression parameters were obtained (Table 2).

3.2.2. Limit of quantification (LOQ) and limit of detection (LOD). Sensitivity of the proposed methods were evaluated and stated based on LOD (3.3 SD/S) and LOQ (10 SD/S), Where SD is the standard deviation of multiple blank samples and S is the slope of calibration curve of the drug (Table 2).

3.2.3. Accuracy and precision. The accuracy of the proposed method was tested by comparing the results with the comparison methods [6, 19, 27]. No significant difference between them regarding the accuracy and precision, respectively, as mentioned by Statistical analysis of the data [33] (Table 3). Repeatability and intermediate precision were evaluated, and the results are summarized in Table 4. The values for the RSD% were less than 2 units, indicating good precision of the proposed method.

3.2.4. Selectivity. The selectivity of the method was calculated by the assay of different laboratory prepared mixtures of CPM and LCF, DXM, and DEX separately within the linearity range and good results were obtained. Acceptable results were obtained and summarized in (Table 5).

Table 5. Determination of CPM in laboratory prepared mixtures with LCF, DXM and DEX by the proposed micellar HPLC method

Laboratory prepared mixtures ($\mu\text{g mL}^{-1}$)		Recovery%*		Recovery%*		Laboratory prepared mixtures ($\mu\text{g mL}^{-1}$)		Recovery%*	
CPM	LCF or DXM	CPM	LCF	CPM	DXM	CPM	DEX	CPM	DEX
50	50	99.78	101.32	101.02	100.06	5	5	99.89	100.22
20	100	102.03	98.22	101.88	102.34	60	15	98.19	101.60
5	10	101.46	98.08	100.16	101.68	20	10	101.83	98.17
40	20	98.82	99.68	100.77	101.98	15	30	101.20	99.28
60	12	98.95	98.89	98.12	100.49	5	20	101.58	100.43
Mean% $\pm$ SD		100.24 $\pm$ 1.46	99.24 $\pm$ 1.33	100.39 $\pm$ 1.41	101.31 $\pm$ 0.99			100.54 $\pm$ 1.51	99.94 $\pm$ 1.29

*Average of three separate determinations.

Table 6. System suitability data for the proposed micellar HPLC method

Parameters	CPM	LCF	DXM	DEX	Reference values
Number of theoretical plates (N)	6238	5414	4663	3569	N>2000
Resolution factor (R_s)		5.24	4.68	4.31	>2
Selectivity factor (α)		1.06	1.07	1.07	1 $\leq$
Capacity factor (K)	3.32	3.12	2.92	3.13	1–10
Tailing factor (T)	1.18	1.21	1.14	1.11	0.9–1.2

Fig. 3. Micellar HPLC determination of CPM (5 $\mu\text{g/mL}$) and LCF (25 $\mu\text{g/mL}$) in Lupituss CPM[®] syrup using micellar aqueous mobile phase consisting of (30 mM sodium dodecyl sulfate and 50 mM sodium dihydrogen phosphate, pH 5) and ethanol (85:15) Fig. 4. Micellar HPLC determination of CPM (5 $\mu\text{g/mL}$) and DXM (25 $\mu\text{g/mL}$) in Codaphen-N[®] syrup using micellar aqueous mobile phase consisting of (30 mM sodium dodecyl sulfate and 50 mM sodium dihydrogen phosphate, pH 5) and ethanol (85:15)

3.2.5. Robustness. The method verified to be robust by testing the effect of slight changes of pH of mobile phase (± 0.1), flow rate (± 0.1) and percent of ethanol ($\pm 1\%$). No significant effect was observed.

3.2.6. System suitability testing. The system suitability parameters were calculated according to guidelines of the United States Pharmacopeia (USP) [34] to ensure system performance during the analysis, where the system was found to be suitable. System suitability parameters were evaluated for the seven peaks (Table 6), and they were found satisfactory and within the reported acceptance criteria.

3.2.7. Application to pharmaceutical dosage forms. The method was utilized successfully for determination of CPM simultaneously in its combined dosage forms with LCF, DXM and DEX. The results were acceptable and revealed high accuracy and good agreement with the comparison methods [6, 19, 27] results as shown in Figs 3–5 and Table 7.

3.2.8. Comparison with other HPLC methods. The proposed green micellar chromatographic method is compared with other previously reported methods for determination of Chlorpheniramine in presence of other drugs used in treatment of cold in terms of composition of mobile phase, linearity

range, LOD, LOQ, mixture, and total run time as shown in Table 8. It is obvious that the proposed method is the first one applied for simultaneous and rapid estimation of chlorpheniramine, levocloperastine fenodizate, dextromethorphan hydrobromide and dexamethasone in a single run which could allow the concurrent analysis of three different dosage forms at the same time using isocratic elution with simple mobile phase, ideal chromatographic peaks with high resolution and high sensitivity. Moreover, it uses a green ecofriendly solvent

presenting no hazards to the environment and personnel replacing classically used organic solvents (i.e., acetonitrile and methanol) in other methods. Also, it displays wider linearity range for the four drugs in comparison to the previously reported methods with low limit of detection and quantification [6, 19, 27], and the total run time for the complete resolution of the four drugs is less than 7 minutes which is less than other reported micellar HPLC methods [28–30].

3.3. Assessment of greenness of the proposed method

The greenness of the methods was evaluated according to the analytical eco-scale [35]. The result of analytical Eco-Scale analysis is the score that is calculated by subtracting penalty points from the basis of 100 points. The penalty points are assigned for high amounts and high hazards connected with utilization of chemicals, high energy consumption, occupational hazards and generation of wastes [36].

The penalty points of the proposed methods were; as shown in Table 9, which proves that the proposed method is an excellent green method of analysis. The green analytical procedure index is a new tool for the assessment of the green character for the entire analytical procedure; from the sample collection and passing through sample preservation, transport, preparation and then the final step of determination [37]. The GAPI evaluates using a pictogram with five colored pentagrams which represent each step of the procedure; there are color-scale with three levels; green for low,

Fig. 5. Micellar HPLC determination of CPM (20 $\mu\text{g/mL}$) and DEX (5 $\mu\text{g/mL}$) in Apidone[®] syrup using micellar aqueous mobile phase consisting of (30 mM sodium dodecyl sulfate and 50 mM sodium dihydrogen phosphate, pH 5) and ethanol (85:15)

Table 7. Assay results for the determination of CPM in combined dosage forms with LCF, DXM and DEX

Dosage form	Proposed method				Comparison methods [6, 19, 27]			
	Conc. taken ($\mu\text{g mL}^{-1}$)		% Found		Conc. taken ($\mu\text{g mL}^{-1}$)		% Found	
	CPM	LCF	CPM	LCF	CPM	LCF	CPM	LCF
Lupituss CPM [®] syrup	5	25	100.42	99.86	2	10	101.38	99.58
	10	50	99.16	98.24	5	25	98.24	99.56
	20	100	98.07	100.59	6	30	99.83	100.78
	Mean $\pm$ S.D.		99.22 $\pm$ 1.18	99.56 $\pm$ 1.20			99.82 $\pm$ 1.57	99.97 $\pm$ 0.71
<i>t</i> -test			0.21(2.776)	0.21(2.776)				
<i>F</i> -test			1.77(19)	2.86(19)				
Codaphen-N [®] syrup	CPM	DXM	CPM	DXM	CPM	DXM	CPM	DXM
	5	25	98.74	100.25	10	50	98.64	100.12
	10	50	102.09	100.22	15	75	98.93	99.67
	20	100	99.20	101.20	20	100	99.69	99.68
Mean $\pm$ S.D.			100.01 $\pm$ 1.82	100.56 $\pm$ 0.55			99.09 $\pm$ 0.54	99.82 $\pm$ 0.26
			0.34(2.776)	0.86 (2.776)				
			11.36(19)	4.47 (19)				
Apidone [®] syrup	CPM	DEX	CPM	DEX	CPM	DEX	CPM	DEX
	20	5	97.89	101.98	20	5	99.63	100.20
	40	10	99.16	100.74	40	10	100.98	99.19
	60	15	98.30	100.11	90	22.5	101.22	98.50
Mean $\pm$ S.D.			98.45 $\pm$ 0.65	100.94 $\pm$ 0.95			100.61 $\pm$ 0.86	99.29 $\pm$ 0.85
			1.42(2.776)	0.92(2.776)				
			1.75(19)	1.25(19)

Table 8. Comparison of the proposed micellar HPLC method with previously reported HPLC methods

Method	Mobile phase	Linearity range ($\mu\text{g mL}^{-1}$)	LOD ($\mu\text{g mL}^{-1}$)	LOQ ($\mu\text{g mL}^{-1}$)	Mixture	Total run time	Ref.
Micellar per aqueous liquid chromatographic method	0.003 mol/L Brij-35 aqueous solution and 0.02 mol/L potassium dihydrogen phosphate: acetonitrile (96:4, v/v), pH 3.0.	1.30–16.00 for CPM 5.22–83.20 for DXM	0.14 $\mu\text{g mL}^{-1}$ for CPM 0.37 for DXM	0.86 for CPM 1.25 for DXM	Chlorpheniramine, pseudoephedrine hydrochloride, caffeine, paracetamol, dextromethorphan and diphenhydramine	30 minutes	28
Micellar electrokinetic chromatography	sodium dihydrogenphosphate: 10 mM sodium tetraborate buffer (pH 9.0) containing 50 mM sodium dodecyl sulfate and acetonitrile (26% v/v)	10–250 for CPM	0.5	4	Chlorpheniramine maleate and paracetamol in presence of other 7 cold remedies	25.5 min	29
Nonionic Micellar Liquid Chromatographic Method	3.0×10^{-3} mol/L Brij-35 and 2.0×10^{-2} mol/L potassium dihydrogen phosphate): methanol (96:4, v/v), pH 3	0.80–1.70 for CPM	0.17	0.57	Chlorpheniramine maleate, paracetamol and pseudoephedrine	Less than 9 minutes	30
RP-HPLC Method	10mM phosphate buffer Buffer (pH 6.5): Acetonitrile (50:50, % v/v)	2.0–6.0 for CPM and 10.0–30.0 for LCF	0.108 for CMP 0.496 for LCF	0.327 for CMP 1.504 for LCF	Chlorpheniramine maleate and Levocloperastine Fendizoate	Less than 7 minutes	6
RP-HPLC Method	Water, pH 2: Acetonitrile (60:40, v/v)	10–30 for CMP 50–100 for DXM	0.04 for CMP and 0.11 for DXM	0.14 for CMP and 0.43 for DXM	Chlorpheniramine maleate and Dextromethorphan	4 minutes	19
RP-HPLC Method	0.1 M ammonium acetate buffer: acetonitrile (60:40, v/v), pH 3	5–50 for DEX 2–90 for CPM	1.03 for DEX 0.29 for CPM	3.14 for DEX 0.88 for CMP	Chlorpheniramine maleate and Dexamethasone in presence of Methyl and Propylparaben	15 minutes	27
Proposed method	30 mM sodium dodecyl sulfate/50 mM sodium dihydrogen phosphate, pH 5 and ethanol (85:15, v/v)	5–60 for CMP, 10–100 for LCF and DXM and 5–30 for DEX	0.43 for CMP 1.29 for LCF 1.09 for DXM 0.48 for DEX	1.30 for CMP 3.91 for LCF 3.31 for DXM 1.47 for DEX	Chlorpheniramine maleate, Levochloperastine Fenodizoate, dextromethorphan hydrobromide and dexamethasone	Less than 7 minutes	

yellow for medium and red for high environmental impact. In general, the greater the number of steps involved in the procedure, the less the greenness as there will be an increased in the energy consumption and the volume of waste produced. For the first step of sample collection; this is the time delay between the sampling and the determination step [38]. The green assessment for the proposed method and other comparison methods using the GAPI tool, are illustrated in Fig. 6 and indicated the highest greenness of the suggested method. So, it can be used for routine analysis of the studied mixtures without harming the environment.

Table 9. The penalty points of the proposed micellar HPLC method according to analytical. Eco-Scale

Reagents/Instruments	Penalty points
SDS	0
Ethanol	0
Sodium dihydrogen phosphate	0
HPLC	0
Occupational hazards	0
Waste	5
Total pps	Σ5
Eco-scale	95
Excellent green analysis	

4. CONCLUSION

The green chemistry has challenged researchers and analysts in all fields to take in consideration the environmental impact of the entirety of their chemical procedures and to assess the greenness of their processes. In this work, we focused on replacing the toxic solvents commonly used, mainly acetonitrile and methanol, with more benign alternative solvents. The most promising green solvent is an ethanol as a green organic solvent in the mobile phase. Consequently, the developed method is the first eco-friendly simple and rapid micellar HPLC method for simultaneous determination of CPM in three combined dosage forms with LCF, DXM, or DEX using isocratic elution with a single run and short time. Moreover, the use of micellar mobile phase has advantages such as non-toxicity, no inflammability, and biodegradability. The proposed method was compared to other previously published methods for assessing their greenness and found more favorable for greener analysis. The ease and availability of the required solvents and instruments in addition to decreasing the organic solvent ratio indicate its reliability and allow its application in quality control laboratories.

Fig. 6. The green assessment profile for the proposed micellar HPLC and the comparison methods, using the GAPI tool

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