



Original article

Green chromatographic method for analysis of some anti-cough drugs and their toxic impurities with comparison to conventional methods

Eglal A. Abdelaleem^a, Nada S. Abdelwahab^{a,*}^a Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Alshaheed Shehata Ahmad Hegazy St., 62514 Beni-Suef, Egypt

ARTICLE INFO

Article history:

Received 21 December 2017

Accepted 19 July 2018

Available online 25 July 2018

Keywords:

Paracetamol
Guaifenesin
Oxomemazine
Sodium benzoate
4-Aminophenol
Guiacol

ABSTRACT

Toxic solvents are widely used in chemical laboratories, which are dangerous on health, safety of workers, and environment. Green chemistry established different principles to keep safety of environment, one of these goals is to replace toxic solvents by greener alternatives or by minimizing the used volumes.

Paracetamol (PAR), Guaifenesin (GUF), Oxomemazine (OX), and Sodium benzoate (SB) combination is a widely used cough preparation. 4-aminophenol (4-AP) is PAR poisonous impurity and related substance. Guiacol (GUC) is GUF impurity and related substance; its presence may lead to rejection of GUF sample.

An eco-friendly HPTLC method was developed to quantify the studied drugs and their impurities. Chromatographic separation was achieved on HPTLC 60F254 plates using ethylacetate: methanol: 0.05 M ammonium chloride buffer (100: 2: 5, by volume) as a mobile phase and scanning at 225 nm. The linear ranges were 0.25–3.50, 0.50–8.00, 0.25–4.00, 0.20–8.00, 0.05–4.00, and 0.25–4.00 µg/band for PAR, GUF, OX and SB, 4-AP, and GUC. Method was successfully applied to available syrup and suppositories. It compared well with the reported method. It can be considered as an alternative green method for previously developed TLC method. Greenness profile of the method proved that it is greener than the reported methods being time and solvents saving.

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1. Introduction

Many cough and cold pharmaceutical preparations contain different combinations of expectorants, analgesics, antipyretics, antihistaminics, and mucolytics. The combination under study contains Paracetamol (PAR), Guaifenesin (GUF), Oxomemazine (OX), and Sodium benzoate (SB).

Paracetamol (PAR), Fig. 1 is a derivative of para-aminophenol with analgesic, antipyretic and mild anti-inflammatory activities. It is commonly used in pain and fever relief preparations such as common cold and other viral infection preparations (Ivković et al., 2014). Guaifenesin (GUF), Fig. 1 is an effective expectorant drug, it promotes the lining of the airway bronchial glands and produces a thin secretion which lubricates thick mucus and thus mucus becomes easy to be expelled with coughing (Porel et al.,

2011). On the other hand, Oxomemazine (OX), Fig. 1 is a derivative of phenothiazine. It has anticholinergic, antihistaminic, and anti-tussive characters. So, it is widely used in cough and common cold preparations (Pujet et al., 2002; Amin et al., 2008). Finally, Sodium benzoate (SB), Fig. 1 is a potent bacteriostatic and fungicidal component of wide applications as a preservative in several pharmaceutical formulations (Ivković et al., 2014). In cough preparations, it is used as antibacterial and antifungal agent. It is used as bronchial secretions stimulator (Farid et al., 2014).

Paracetamol, GUF, and SB are official drugs in both British (BP) (The British Pharmacopoeia, 2007) and United States (USP) (The United States Pharmacopoeia, 2009) pharmacopoeias but OX is a non-official one. In both BP and USP, 4- amino phenol (4-AP), Fig. 1 is considered to be PAR impurity (K). Similarly, it is reported to be its hydrolytic degradate and related substance (Abdelaleem and Abdelwahab, 2013). Moreover, 4-AP is a toxic impurity that has a nephrotoxic and teratogenic (Abdelaleem and Abdelwahab, 2013) effects. In the same manner, Guaiacol (GUC), Fig. 1 is reported to be GUF impurity and related substance in both BP (The British Pharmacopoeia, 2007) and USP (The United States Pharmacopoeia, 2009). GUF samples containing GUC or GUF β-isomer should be discarded according to USP (The United States Pharmacopoeia, 2009) impurity limits of GUF.

* Corresponding author.

E-mail address: nada.abdelwahab@pharm.bs.u.edu.eg (N.S. Abdelwahab).

Peer review under responsibility of King Saud University.



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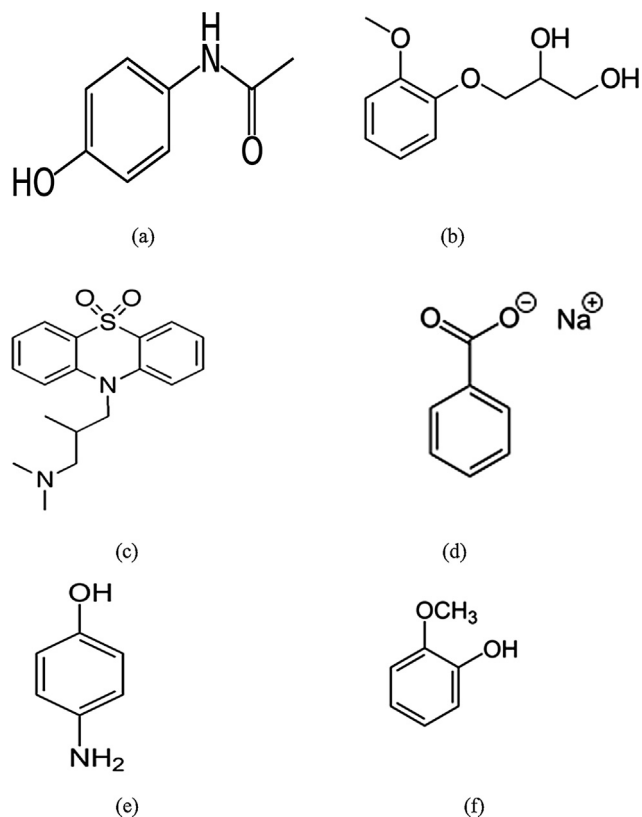


Fig. 1. Chemical structure of (a) paracetamol, (b) guaifenesine, (c) oxememazine, (d) sodium benzoate, (e) 4-aminophenol, and (f) guaiacol.

An overview of the literature, some analytical methods were found for analysis of the studied drugs. Most of these published methods were for analysis of some of them either with each other or in combination with other drugs. PAR and GUF were analyzed together by using HPLC (Satyanarayana et al., 2014; Rekulapally and Rao, 2015; Kolhal et al., 2014; Nalini et al., 2014; Yehia and Mohamed, 2016; Yehia and Essam, 2016) and UPLC (Siddareddy et al., 2016) methods, PAR with SB were determined by HPLC methods (Ivković et al., 2014; El-Gindy et al., 2013) while binary mixture of GUF and SB was estimated by the same analytical method (Adjekum and Kebede, 2011). On the other hand, ternary mixture of PAR, GUF and SB was resolved in their formulation (Mallu et al., 2011) or in presence of 4-AP (El-Shabrawy et al., 2014) by different HPLC methods.

Only two methods were published for resolving the proposed quaternary mixture. In the first one, the mixture was analyzed using HPLC and TLC- densitometric methods (Farid et al., 2014). In the reported TLC method, a developing system of methylene chloride- methanol- acetic acid- 33% ammonia was used and this method was suitable only for separation of the main drugs without the presence of their impurities. While the second method depended on separation of the quaternary mixture, 4-AP, and GUC by HPLC method (Hewala, 1994) using a mobile phase system of methanol- water, pH 3.9 in gradient elution mode.

From the pharmaceutical importance view point, the proposed combination is considered as one of the most commonly used cough preparations. As previously mentioned, the chosen impurities are very harmful. Furthermore, the published TLC method (Farid et al., 2014) has low selectivity since it was applied only for resolving the parent drugs in absence of their toxic impurities. Also it depended on using methylene chloride which is environmentally hazard solvent (Keith et al., 2007). On the other hand, the reported HPLC methods (Farid et al., 2014; Hewala, 1994) were

considered as money and time consuming. Additionally, they needed higher amounts of the consumed solvents since their run times were more than 10 min. Additionally, it was observed that none of those reported methods considered the harmful effects and bad environmental impacts of the used solvents.

It was necessary to develop a sensitive, selective, and eco-friendly analytical method for analysis of the quaternary mixture of PAR, GUF, OX and SB and their toxic impurities, 4-AP and GUC. The developed method has advantages of higher selectivity and lower environmental harmful effects comparing to the published TLC and HPLC methods (Farid et al., 2014). Likewise, it was more economic and lower time consuming comparing to the reported HPLC methods (Farid et al., 2014; Hewala, 1994).

2. Experimental

2.1. Instruments

In the developed HPTLC method, UV scanning was performed by using TLC scanner 3 densitometer (Camag, Muttenz, Switzerland). Samples were applied using TLC Linomat IV applicator equipped with 100 μ L syringe (Camag, Muttenz, Switzerland). The instrument parameters were adjusted as follow: the slit dimensions were (6 \times 0.3 mm), scanning speed was 20 mm/s, data resolution was 100 μ m/ step, the used band width was 6 mm, and finally, chromatogram and integrated peak area were the result output.

The used stationary phase was (20 \times 20 cm) HPTLC aluminum plates, coated with silica gel 60 F₂₅₄ (Merck, Germany) with 0.25 mm thickness.

During method optimization, UV lamp (with wavelengths of 254 and 365 nm) (Vilber Lourmat, Marne La Vallee, Cedex, France) was used.

For dissolving the prepared samples, Sonix TV ss-series ultrasonicator (USA) was used.

3. Materials and reagents

3.1. Pure standard

Pure samples (PAR, GUF, OX, and SB) were given as a gift from AMRIA FOR PHARMACEUTICAL INDUSTRIES, ALEXANDRIA-EGYPT. Their purity was found to be 100.06, 100.21, 99.68, and 99.73%, respectively according to the reported method (Farid et al., 2014). On the other hand, standard 4-AP and GUC were purchased from SIGMA-ALDRICH Co., Cairo, Egypt and their purity was labeled to be 99.56 and 99.30%, respectively.

3.2. Pharmaceutical formulations

Oplex[®] syrup with a batch number of 3521524, was marked to contain 0.666 g PAR, 0.666 g GUF, 0.033 g OX, and 0.666 g SB per 100 mL. This dosage form was formulated by AMRIA FOR PHARMACEUTICAL INDUSTRIES, ALEXANDRIA-EGYPT. Under license of PHONE POULENC RORER, PARIS- FRANCE.

Rectoplexil[®] suppositories, batch number 3791201, were manufactured by AMRIA FOR PHARMACEUTICAL INDUSTRIES, ALEXANDRIA-EGYPT. Under license of PHONE POULENC RORER, PARIS- FRANCE. Each suppository was labeled to contain 66.6 mg PAR, 66.6 mg GUF, 3.3 mg OX, and 66.6 mg SB.

3.3. Chemicals

Chemicals and solvents (analytical grades) were used without further purification.

Ethylacetate, methanol, and ammonium chloride buffer (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt).

3.4. Solutions

3.4.1. Stock standard solutions of PAR, GUF, OX, SB, 4-AP, and GUC (1 mg mL^{-1})

All stock solution were prepared in methanol by accurately transferring 100 mg of each component into six separate 100-mL calibrated flasks.

3.5. Procedure

3.5.1. Calibration curves construction

Accurate volumes of PAR, GUF, OX, SB, 4-AP, and GUC stock solutions ($1000 \mu\text{g/mL}$) were separately transferred to six sets of 10-mL measuring flasks in order to prepare 25–350, 50–800, 25–400, 20–800, 5–400, and 25–400 $\mu\text{g/mL}$ of each, respectively. Triplicate 10 μL injection was performed from each solution using the previously mentioned applicator. Bands were spaced by 5 mm from each other and 15 mm from the lower edge of the used HPTLC plate. Development was done in a glass chamber previously saturated for 15 min with the mobile phase mixture of ethylacetate: methanol: 0.05 M ammonium chloride buffer (100: 2: 5, by volume). The developing system was left to transfer for a distance of 15 cm. After that, scanning was done at 225 nm following the previously illustrated instrumental parameters. Peak areas were then recorded and used to calculate the calibration curves and the regression equations.

3.5.2. Application to pharmaceutical formulations

For *Oplex*[®] syrup: the bottle was shaken well and then 5 mL syrup was accurately taken and diluted to 25 mL with methanol to obtain a stock solution containing $1332 \mu\text{g/mL}$ each of PAR, GUF, and SB and $66 \mu\text{g/mL}$ OX. Further dilutions were made to prepare different concentrations within the linearity ranges of the method. Steps given under construction of calibration curves were then followed and each sample was analyzed three times. The area under the peak for each component was recorded and used along with the previously computed regression equation for calculating the concentrations of PAR, GUF, SB, and OX. Standard addition technique was carried out on three concentration levels to assess the validity of the method.

For *Rectoplexil*[®] suppositories: five suppositories were transferred to a beaker and then 40 mL methanol was added. Sample was then ultrasonicated for 15 min in an ultrasonic containing hot water. The mixture was cooled and filtered in a 100-mL calibrated flask using a filter paper, then the solid fatty residue was returned back to the beaker. Again, 20 mL methanol was added and the sample was ultrasonicated. This process was repeated twice, in each time the filtrate was added to the content of the volumetric flask. Finally, the volume was adjusted with methanol to obtain stock solution containing $3330 \mu\text{g/mL}$ PAR, GUF, and SB and $165 \mu\text{g/mL}$ OX. Further dilutions were carried out to obtain samples within the linearity ranges of the suggested method. Chromatographic conditions mentioned before were then followed.

4. Results and discussion

Recently, the environmental pollution becomes a massive problem especially after the wide use of hazardous chemicals and solvents. Hence, all social sectors should co-operate together and concentrate their efforts to minimize this risk. One of the goals of green chemistry is to develop new safe methods or to modify the present methods by replacing the used toxic solvents and reagents

by eco-friendly ones or minimizing the volume of the used solvents (Rojanarata, 2012). On the other hand, detection and quantitation of impurities, especially toxic ones, in newly discovered drugs is obligatory since monitoring of impurities gives a complete picture about the safety and quality of these drugs.

After careful search in the literature, three methods were published for simultaneous analysis of the four drugs. The reported HPLC methods (Farid et al., 2014; Hewala, 1994) were time and money consuming while the published TLC method has limited selectivity since it was not concerned with the presence of drug toxic impurities. From the environmental saving point of view, large amounts of organic solvents were consumed during the analysis (in the published HPLC methods) and all the used solvents are environmentally harmful (Milojkovic-Opsenica and Andric, 2014; Elzanfaly et al., 2015) (orthophosphoric acid, acetonitrile and methylene chloride). All these drawbacks motivated us to develop an analytical method which was more selective, time and cost saving using small amounts of non-harmful solvents. The proposed HPTLC method was applied for simultaneous quantitation of PAR, GUF, OX, and SB along with their toxic impurities, 4-AP and GUC. Additionally, the method was applied for estimation of the drugs in available syrup and suppositories dosage forms.

4.1. Method optimization

In carrying out different trials to optimize the developed HPTLC method, our basic goal was to test green solvents only in order to decrease their hazardous effects. Initial trials started with methanol: ethylacetate, ethanol: ethylacetate, butanol: ethylacetate, and isopropanol: ethylacetate. All these mixtures were prepared in different ratios (10:90, 10:100, 5:100, and 2:100, v/v). In all these previous trials, bad resolution was obtained among all components. After that, methanol: ethylacetate mixture was chosen to continue method optimization because methanol was cheaper than other tested solvents. pH of the mobile phase was then tested by using either ammonia solution or acetic acid. It was noticed that addition of ammonia solution resulted in bad separation between GUF, OX, 4-AP, and GUC while SB was retained on the stationary phase. In contrast, acetic acid gave insufficient resolution between PAR, GUF, 4-AP, and GUC while retained OX on the base line. Finally, 0.05 M ammonium chloride buffer was checked by addition of different volumes to the mobile phase mixture. Good and complete resolution was obtained on using a mobile phase mixture of ethylacetate: methanol: 0.05 M ammonium chloride buffer (100:2:5, by volume). In trials to obtain the maximum sensitivity with the lowest possible noise, different scanning wavelengths were tested (215, 225, and 254 nm). Detection of all separated bands at 225 nm gave high sensitivity and symmetrical peaks. Six mm was chosen as the optimum band width while bands were interspaced by 5 mm and the slit dimensions were selected to be (6 mm \times 0.3 mm). The resulted densitogram is given in Fig. 2.

4.2. Method validation

ICH (ICH, 2005) and USP (The United States Pharmacopeia, 2009) guidelines were used to carry out method validation.

4.2.1. Linearity

When the response (integrated peak area) was plotted against the corresponding concentration, linearity was proved in different concentration ranges; 0.25–3.50, 0.50–8.00, 0.25–4.00, 0.20–8.00, 0.05–4.00 and 0.25–4.00 $\mu\text{g}/\text{band}$ for PAR, GUF, OX, SB, 4-AP, and GUC, respectively. Also, it was observed that the sensitivity of the method and the regression parameters have been significantly affected by the type of the regression equation used where

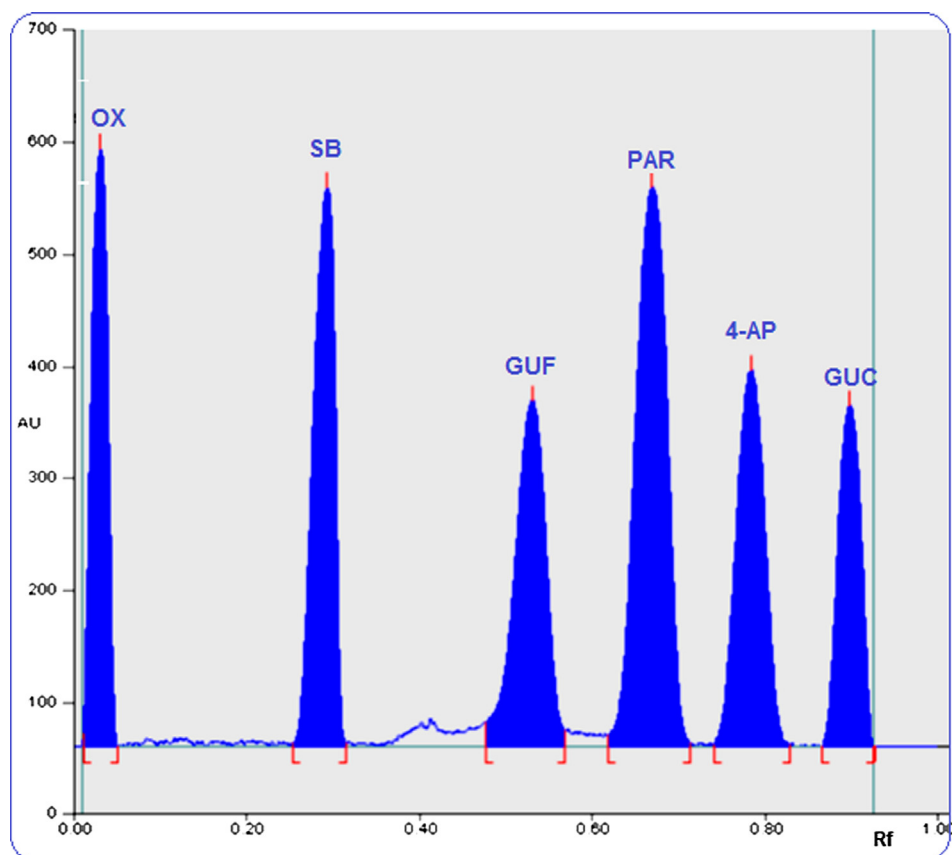


Fig. 2. HPTLC densitogram of a mixture of the studied components.

polynomial regression resulted in acceptable results with good correlation coefficients, Table 1.

4.2.2. Accuracy

It was expressed as mean % recoveries and it was confirmed by analysis of several pure samples of each of the studied compounds following the instructions of the developed method. The obtained results are shown in Table 1. Additionally, the standard addition technique was used to assure the accuracy of the method. It was performed by analysis of separate mixtures of Oplex[®] syrup spiked with pure PAR, GUF, OX and SB on three different concentration

levels. The concentrations of the added drugs were then calculated and the mean recoveries were computed and found to be 96.49 ± 0.608 , 102.49 ± 1.527 , 99.98 ± 2.277 , and 101.70 ± 2.127 for PAR, GUF, OX, and SB, respectively. As shown in Table 2.

4.2.3. Precision

Repeatability was evaluated by analysis of three samples of each pure component three times a day. While intermediate precision was tested by analysis of the previous samples on three consecutive days using the developed method. Relative standard deviation (% RSD) was then computed for each component. The

Table 1
Assay parameters and method validation for the determination of pure samples of paracetamol, guaifenesin, oxomemazine, sodium benzoate, 4-aminophenol and guaiacol by the proposed HPTLC method.

Parameters	Paracetamol	Guaifenesin	Oxomemazine	Sodium benzoate	4-aminophenol	Guaiacol
Range ($\mu\text{g}/\text{band}$)	0.25–3.50	0.50–8.00	0.25–4.00	0.20–8.00	0.05–4.00	0.25–4.00
Slope						
Coefficient 1 ^a	−784.4100	−9.9916	−113.2700	−163.3500	−196.1900	−350.5300
Coefficient 2 ^b	8527.70	1819.00	3681.30	3622.70	5818.20	2746.50
Intercept	2955.10	1397.00	1422.60	596.36	474.36	70.641
Correlation coefficient (r)	0.9997	0.9995	0.9996	0.9999	0.9998	0.9998
Accuracy (mean \pm SD)	100.31 ± 1.311	100.97 ± 1.849	100.93 ± 1.599	99.40 ± 1.203	100.47 ± 1.580	99.69 ± 1.269
Precision (%RSD)						
Repeatability ^a	1.117	0.663	1.195	1.306	1.595	2.135
Intermediate precision ^b	2.680	1.696	2.008	1.535	2.358	2.971
LOD	0.05	0.16	0.05	0.07	0.02	0.08
LOQ	0.15	0.45	0.18	0.20	0.05	0.25

Following the polynomial regression: $A = aX^2 + bX + C$, where (A) is the integrated peak area, (X) is the concentration in $\mu\text{g}/\text{band}$, (a and b) are the coefficients 1 and 2, respectively, and C is the intercept.

^a The intraday (n = 3), average of three different concentrations (1, 2 and 3 $\mu\text{g}/\text{band}$) repeated three times within day.

^b The interday (n = 3), average of three different concentrations (1, 2 and 3 $\mu\text{g}/\text{band}$) repeated three times in three successive days.

Table 2
Determination of the studied drugs in pharmaceutical formulations by the proposed HPTLC method.

Pharmaceutical formulation	Paracetamol			Guaiphenesine			Oxomemazine			Sodium benzoate		
	Taken (µg/band)	Found ^a % ± %RSD	Standard addition Mean ± SD ^b	Taken (µg/band)	Found ^a % ± %RSD	Standard addition Mean ± SD ^b	Taken (µg/band)	Found ^a % ± %RSD	Standard addition Mean ± SD ^b	Taken (µg/band)	Found ^a % ± %RSD	Standard addition Mean ± SD ^b
Oplex [®] syrup Batch number 3,521,524	3.33	99.15±2.581 Found ^a % ± %RSD	96.49±0.608	3.33	103.36±0.862 Found ^a % ± %RSD	102.49±1.527	0.30	101.21 ± 2.318 Found ^a % ± %RSD	99.98 ± 2.277	3.33	101.07±1.882 Found ^a % ± %RSD	101.70±2.127
Rectoplexil [®] suppositories Batch number 3,791,201	3.996	101.40±2.183 Found ^a % ± %RSD		3.996	101.85±1.812 Found ^a % ± %RSD		0.36	97.19 ± 2.343 Found ^a % ± %RSD		3.996	101.61±1.371 Found ^a % ± %RSD	

^a Average of 6 determinations.

^b Average of 3 determinations.

values given in Table 1 confirmed that the method has good precision.

4.2.4. Specificity

Densitogram given in Fig. 2 verified the specificity of the method where complete separation between the six components was resulted; likewise, symmetrical peaks were obtained. Similarly, no interference from syrup and suppositories excipients was detected and the results obtained upon applying the method to pharmaceutical formulations confirmed the high selectivity of the method, Table 2.

4.2.5. Robustness

It was ascertained by making deliberate small changes in amount of the used buffer (± 0.05 mL) and saturation time (± 5 min). Variation in these parameters had no significant effect on R_f values of all the separated components which assured the robustness of the introduced method, Table 3.

4.2.6. Ruggedness

Ruggedness of the method was confirmed by performing the analysis by two different analysts. No significant changes in R_f values of the studied components were resulted, indicating that the method is rugged, Table 3.

4.2.7. System suitability

It was tested by computing different chromatographic parameters like, resolution factor (R_s), selectivity (α) factor and peak asymmetry. The values given in Table 4 showed that the value of R_s factor is ≥ 1.5 , (α) factor > 1 and peak symmetry values are close to 1 for all the studied components. All these values assessed the specificity of the method and confirmed the good efficiency of the chromatographic separation.

4.3. Application of the method

After optimization of the suggested method, it was successfully applied to available dosage forms; Oplex[®] syrup and Rectoplexil[®] suppositories. The results obtained were satisfactory, confirming that syrup and suppositories additives did not interfere with the separation and quantitation of the studied components, Table 2. Furthermore, standard addition technique was carried out by preparing different mixtures of Oplex[®] syrup with different concentrations of PAR, GUF, OX, and SB and then the instructions of the developed method have been followed. The obtained results agreed with the acceptance criteria, confirming accuracy of the method, Table 2.

4.4. Statistical comparison

When the developed method was statistically compared to the reported HPLC one (Farid et al., 2014) using student's t and F tests and at a probability of 95%, no significant difference between the two methods with respect to accuracy and precision, Table 5.

4.5. Greenness profile of the proposed method

The greenness profile of the suggested method was considered. It is used to evaluate the greenness of any analytical method and to compare between the greenness of different methods. It depends on evaluating of four criteria for the used solvents; any of the used solvents should not be one of PBT solvents (persistent, bio-accumulative, and toxic), is not a hazardous one, is not corrosive (pH during the analysis is < 2 or > 12), and the amount of waste generated should not be > 50 g. In the proposed method, methanol and

Table 3
Robustness and ruggedness studies of the developed method.

Robustness (SD) [*]						
Factor	Guaiacol	4-Aminophenol	Paracetamol	Guaifenesine	Sodium benzoate	Oxomemazine
1- amount of the used buffer (± 0.05 mL)	0.45	1.03	1.10	0.89	1.34	1.15
2- saturation time (± 5 min)	0.62	0.98	0.35	0.25	1.25	0.94
Ruggedness (SD) [*]						
1-Two analysts	0.12	0.09	0.46	0.29	0.19	0.15

^{*} SD: SD of the change in Rf value of each component.

Table 4
Parameters of system suitability of the developed HPTLC method for the determination of the studied components.

Parameters	Oxomemazine	Sodium benzoate	Guaifenesine	Paracetamol	4-Aminophenol	Guaiacol
R _f	0.05	0.26	0.52	0.64	0.76	0.87
Symmetry factor	1.07	0.90	1.03	1.00	0.92	0.93
Resolution (R _s)	6.06		3.31	1.5	1.62	1.95
Selectivity (α)	6.67		3.10	1.64	1.75	2.13

Table 5
Statistical comparison of the results obtained by analysis of pure drugs using the developed method and the reference one.

Method	Paracetamol		Guaifenesine		Oxomemazine		Sodium benzoate	
	HPTLC	Reference method Farid et al. (2014)	HPTLC	Reference method Farid et al. (2014)	HPTLC	Reference method Farid et al. (2014)	HPTLC	Reference method Farid et al. (2014)
Mean \pm SD	100.33 \pm 1.324	100.06 \pm 1.264	100.49 \pm 1.811	100.21 \pm 1.307	100.95 \pm 1.539	99.68 \pm 1.070	99.41 \pm 1.227	99.73 \pm 1.446
Variance	1.753	1.598	3.279	1.708	2.369	1.145	1.506	2.091
n	6	6	6	6	6	6	6	6
Student's t test (2.228)	0.356		0.306		1.350		0.408	
F-test (5.05)	1.097		1.919		1.225		1.389	





Reference method: HPLC method using C18 column and acetonitrile: methanol: 35 mM KH₂PO₄ (20: 5:75; by volume, pH was adjusted to 2.9 \pm 0.1) as the mobile phase. The flow rate was 1.5 mL/min with UV scanning at 220 nm.

*The values between parenthesis are corresponding to the theoretical values of *t* and *F* (*P* = 0.05).

ethylacetate were used and they were not listed as PBT or hazardous solvents by the EPA's Toxic Release Inventory (Emergency Planning and Community Right-to-Know Act, 2004; Code of Federal Regulations, 2014). Additionally, pH of the used developing system was about 5 and so it was not considered as a corrosive one. Besides, the waste generated from the method was

less than 50 g per sample. As a result, the proposed method agreed with the four criteria of the greenness profile. Moreover, comparison between the developed HPTLC method and the reported ones was carried out. Results in Table 6 proved that the suggested method was greener than the reported ones with little time and solvents consumption.

Table 6
Comparison of greenness profile between the developed method and reported ones for determination of the studied drugs and their impurities.

Methods	Mobile phase	Run time (min)	Flow rate (mL/min)	Waste [*]	Greenness profile ^{**}
The proposed method	Ethylacetate: methanol: 0.05 M ammonium chloride buffer (100: 2: 5, by volume)	–	–	5 g/sample	
TLC densitometric method Farid et al. (2014)	Methylene chloride: methanol: acetic acid: 33% ammonia (89: 8.4: 2: 0.6, by volume)	–	–	1 g/sample	
HPLC method Farid et al. (2014)	Acetonitrile: methanol: 35 mM KH ₂ PO ₄ (20: 5:75; by volume, pH 2.9 \pm 0.1)	11	1.5	16.5 g/run	
HPLC method Mallu et al. (2011)	Methanol: water, pH 3.9 in gradient elution mode	22	1.5	33 g/run	

^{*} (run time \times flow rate) [for HPLC methods], (number of samples on TLC plate/volume of mobile phase per run) [for TLC methods].

^{**} The profile criteria are summarized by four key terms PBT (persistent, bio-accumulative, and toxic), Hazardous, Corrosive, and Waste.

5. Conclusion

A green simple HPTLC method was developed for resolution and estimation of PAR, GUF, OX, and SB along with their harmful impurities, 4-AP and GUC. The method succeeded to determine the six components with high specificity and sensitivity. It was further used to quantify the four co-formulated drugs in different pharmaceutical formulations without any interference from formulations additives. Comparing to the previous reported TLC-densitometric method, it was superior in using non-harmful solvents being able to separate and estimate the studied drugs and the impurities. In the same way, the developed method is time and money saving relative to the reported HPLC methods. Comparison of the greenness profiles of the developed method and the reported chromatographic methods confirmed the superiority of this method over the published methods in being greener one. All these advantages promote the usage of the developed method for analysis and evaluation of impurity profiles of similar drugs.

Conflicts of interest

Authors A and B declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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