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ORIGINAL ARTICLE

Stability indicating RP-HPLC method for simultaneous determination of guaifenesin and pseudoephedrine hydrochloride in the presence of syrup excepients

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Abstract The present work concerns with the development of stability indicating the RP-HPLC method for simultaneous determination of guaifenesin (GUF) and pseudoephedrine hydrochloride (PSH) in the presence of guaifenesin related substance (Guaiacol). GUC, and in the presence of syrup excepients with minimum sample pre-treatment. In the developed RP-HPLC method efficient chromatographic separation was achieved for GUF, PSH, GUC and syrup excepients using ODS column as a stationary phase and methanol: water (50:50, v/v, pH = 4 with orthophosphoric acid) as a mobile phase with a flow rate of 1 mL min^{-1} and UV detection at 210 nm. The chromatographic run time was approximately 10 min. Calibration curves were drawn relating the integrated area under peak to the corresponding concentrations of PSH, GUF and GUC in the range of 1–8, 1–20, 0.4–8 μg mL^{-1}, respectively. The developed method has been validated and met the requirements delineated by ICH guidelines with respect to linearity, accuracy, precision, specificity and robustness. The validated method was successfully applied for determination of the studied drugs in triaminic chest congestion® syrup; moreover its results were statistically compared with those obtained by the official method and no significant difference was found between them.

1. Introduction

Pseudoephedrine HCl (PSH) is (1*S*,2*S*)-2-(methylamino)-1-phenylpropan-1-ol hydrochloride (The British Pharmacopoeia, 2007). It is a stereoisomer of ephedrine and has similar action. PSH and its salts are orally used for the symptomatic relief of nasal congestion and are commonly used in combination with other ingredients in preparations intended for the relief of cough and cold symptoms (Martindale, 2005). Guaifenesin (GUF) is (2*RS*)-3-(2-methoxyphenoxy)propane-1,2-diol (The

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Table 1 Comparison between sensitivity of some of the reported methods and that of the developed RP-HPLC method.

The reported methods	PSH Range ($\mu g\ mL^{-1}$)	GUF Range ($\mu g\ mL^{-1}$)
USP (2009)	100	100
Shuhan et al. (2005)	3–15	20–100
Xiaohui and Stewart (2000)	15–150	100–1000
Wilcox and Stewart (2000)	7.5–30	50–200
Rahul et al. (2011)	300–1500	30–150
Siavash et al. (2011)	5–30	5–33
The developed HPLC method	1–8	1–20

British Pharmacopoeia, 2007), it is reported to increase the volume and reduce the viscosity of tenacious sputum and is used as an expectorant for productive cough. Also it has been given to patients with altered nasal mucociliary clearance associated with HIV infection (Martindale, 2005). Guaiacol (GUC) is 2-methoxyphenol (The British Pharmacopoeia, 2007), in BP and USP (The British Pharmacopoeia, 2007; USP, 2009), it is considered as GUF impurity and related substance. Also it is reported in USP (2009) that the GUF sample must be excluded when Guaiacol and Guaifenesin$^{\beta}$-isomer is detected.

The literature survey revealed that some techniques have been published for the determination of PSH and GUF either in their combined form or in combinations with other drugs. RP-HPLC methods have been used for the determination of PSH and GUF in their ternary mixture with dextromethorphan hydrogen bromide (USP, 2009; Shuhan et al., 2005; Yongqing et al., 2000; Xiaohui and Stewart, 2000; Leroy et al., 1998; Shervington, 1997; Louhaichi et al., 2009). Moreover, combinations of the studied drugs with other cough and cold medicines have been analyzed by different RP-HPLC (Louhaichi et al., 2009; Lei and Nan, 2004; Histochi et al., 2005; Vaidya et al., 2001; Wilcox and Stewart, 2000) and GC (Thresiana et al., 2005) methods. On the other hand, USP (2009) determined PSH and GU binary mixtures by a RP-HPLC method but with using two mobile phases. Also, PSH and GUF combination has been determined by different spectrophotometric methods such as simultaneous equation (Rahul et al., 2011), Q-analysis spectrophotometry (Rahul et al., 2011) and multivariate calibration method (Siavash et al., 2011).

Due to the low sensitivity, Table 1, and selectivity of the reported methods and also due to the importance of detection of the lowest concentration of GUC in GUF samples (USP, 2009), our target was to develop and validate a sensitive, selective and simple stability indicating RP-HPLC method for the determination of PSH, GUF and GUC with good accuracy and precision without interference from syrup excepients. The developed method has an advantage of using a single mobile phase with a simple composition for good chromatographic separation of all the studied components and excepients within short analysis time.

2. Experimental

2.1. Samples

2.1.1. Pure standards

Pure *PSH and GUF* samples with claimed purity of 98.5% and 99.0%, respectively according to the manufacturer's certificate were kindly supplied by Novartis Pharma S.A.E Cairo, Egypt.

Pure *GUC* sample with claimed purity of 99.3% was purchased from Sigma–Aldrich Co., Cairo, Egypt.

2.1.2. Pharmaceutical formulation

Triaminic chest congestion® syrupbatch No. Y0005, labeled to contain 15 mg PSH and 50 mg GUF per 5 mL syrup, manufactured by NOVARTIS PHARMA S. A. E Cairo, EGYPT under license from Novartis Consumer Health Switzerland and its affiliate in USA.

2.2. Chemicals and solvents

Methanol and orthophosphoric acid were of HPLC grade (Sigma–Aldrich Chemie GmbH, Germany), de-ionized water was from SEDICO Pharmaceuticals Co., Egypt.

2.3. Solutions

Stock standard solutions of PSH, GUF and GUC were prepared in methanol in the concentration of 1 mg mL^{-1} by accurately weighting 0.1 g each of PSH, GUF and GUC into three separate 100-mL volumetric flasks, dissolving in and diluting to the volume with methanol.

Working standard solutions of PSH, GUf and GUC were prepared in methanol in the concentration of 0.1 mg mL^{-1} by transferring 10 mL each of PSH, GUF and GUC stock standard solutions into three separate 100-mL volumetric flasks and completing to the volume with methanol.

Pharmaceutical formulation solution: 5 mL of triaminic chest congestion® syrup was accurately transferred (after vigorous shaking) into a 50 mL measuring flask, sonicated in 25 mL methanol for 5 min and the volume was then made up to the mark with methanol in order to prepare the stock solution (1 mg mL^{-1}) of GUF and the corresponding concentration of PSH from which sample working solution (0.1 mg mL^{-1} GUF) was prepared.

2.4. Instruments

HPLC (Shimadzu, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP controller, DGU-12 A degasser and SPD-10 AVP UV–VIS detector. Separation and quantitation were made at room temperature on a 250 mm × 4.6 mm (i.d.) Hidrosorb RP- C18 column (5 μm particle size) purchased from Merck (Dalmstaad, Germany). The detector was set at 210 nm.

3. Procedure

3.1. Chromatographic conditions

Chromatographic separation was carried out using isocratic elution and de ionized water: methanol (50:50, by volume pH 4 with orthophosphoric acid) as a mobile phase delivered at a flow rate 1 mL min^{-1}. Injection volume was 20 μL and detection has been carried out at 210 nm at room temperature. The run time was 10 min and the total peak height was used to quantify each of the studied components.

3.2. Linearity and construction of calibration curves

Accurate aliquots in the range of 1–8, 1–20 and 0.4–8 μg mL^{-1} each of PSH, GUF and GUC, respectively were separately prepared from their respective working standard solutions (100 μg mL^{-1}) in the mobile phase into three separate series of 10-mL volumetric flasks. Triplicate injections were made for each concentration; the peak height was used to construct the calibration curve for each component from which its regression equation was constructed.

3.3. Application to pharmaceutical formulation

Different concentrations (2.4, 3 and 6 μg mL^{-1} PSH, 8, 10 and 20 μg mL^{-1} GUF) were prepared from the previously prepared pharmaceutical formulation working solution (0.1 mg mL^{-1}) and the procedure mentioned under linearity and construction of calibration curves was followed. Concentrations of PSH and GUF have been calculated from their respective regression equations and the percentage recoveries were then calculated.

3.4. Recovery studies

Recovery studies were carried out by applying the developed method to the drug sample to which known amounts of pure PSH and GUF corresponding to 80%, 100% and 120% (of concentration 3 μg mL^{-1} PSH and 8 μg mL^{-1} GUF) were added. At each level three determinations were performed and the percentage recoveries of the added pure drugs were calculated.

4. Results and discussion

HPLC has become a widely used tool for the routine analysis and separation of drugs in pure form or in pharmaceutical formulations (Rao et al., 2010) either alone or in the presence of degradation products and excepients (Patel and Rao, 2011; Moussa et al., 2011; Hassib et al., 2011).

In USP (2009) GUC is reported to be GUF impurity and related substance which should be absent in the accepted GUF sample. All the reported methods concerned with the analysis of GUF and PSH without taking into consideration the presence of GUC or syrup excipients. Hence the aim of this work was to develop selective stability indicating RP- HPLC method for good chromatographic separation of the studied drugs from syrup excepients and to determine the lowest amounts of GUF impurity.

4.1. Method development and optimization

The first step in method development is to test all the published RP-HPLC mobile phases. None of these mobile phases was able to achieve good resolution among GUF, PSH, GUC and syrup excepients. Different parameters were manipulated to obtain an acceptable resolution between the three studied components and syrup excepients, reduce the analysis time, enhance LOD and LOQ of the method and to satisfy HPLC system suitability parameters.

The chromatographic separation was started with ODS column as a stationary phase and acetonitrile: de ionized H_2O with different ratios (30:70–70:30 v/v) as a mobile phase

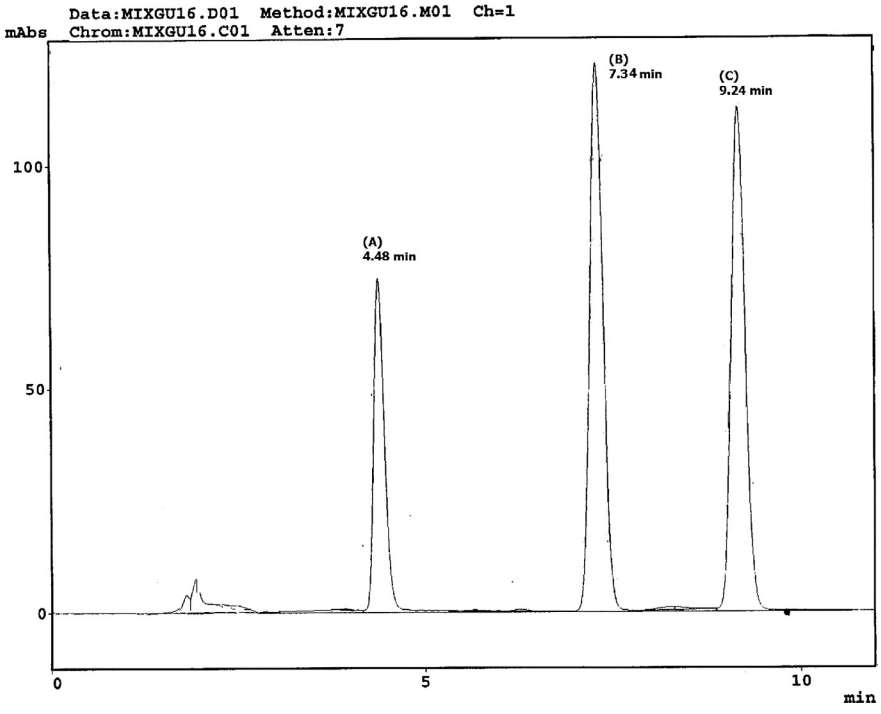

Figure 1A RP-HPLC chromatogram of a resolved mixture of standard PSH (A) (R_t = 4.48 min), GUF (B) (R_t = 7.34 min) and GUC (C) (R_t = 9.24 min) using de ionized water: methanol (50:50, v/v pH = 4 with phosphoric acid).

maintaining the flow rate at 1 mL min^{-1}, where bad resolution among the studied components was obtained. Replacing acetonitrile with methanol in the mobile phase slightly enhanced the resolution and the ratio (50:50 v/v) gave the best resolution among GUF, PSH and GUC but with bad resolution between GUF and syrup excepients. Different pH values (3–6) were tested where pH = 4 gave the best chromatographic resolution between PSH, GUF, GUC and excepients. On the other hand, different scanning wavelengths were tried (210, 225, 278 and 254 nm) in order to enhance the sensitivity of the method where scanning at 210 nm gave considerable sensitivity for all the studied components.

After method optimization, the chromatographic separation has been carried out using ODS column (250 mm × 4.6 mm, 5 μm particle size) and mobile phase of methanol: de ionized water (50:50 v/v, pH = 4 with orthophosphoric acid), flow rate of 1 mL min^{-1} and UV detection at 210 nm for all components. Typical HPLC chromatograms, Fig. 1A, showed good separation between PSH (4.48 min), excepients (6.7 min), GUF (7.34 min) and GUC (9.24 min).

Table 2 Regression and analytical parameters of the proposed RP-HPLC for the determination of Pseudoephedrine HCl, Guaifenesin and Guaiacol.

Parameters	PSH	GUF	GUC
Calibration range	1–8 μg mL^{-1}	1–20 μg mL^{-1}	0.4–8 μg mL^{-1}
Slope	0.7151	0.6063	1.3595
Intercept	0.1461	0.0803	0.2753
Correlation coefficient	0.9996	0.9997	0.9995
Accuracy	99.42	99.81	101.20
Precision			
Repeatability	0.966	1.662	0.957
Intermediate precision	1.466	0.928	1.137
LOD	0.30 μg mL^{-1}	0.20 μg mL^{-1}	0.10 μg mL^{-1}
LOQ	1 μg mL^{-1}	1 μg mL^{-1}	0.4 μg mL^{-1}

4.2. Methods validation

ICH guidelines (ICH, 2005) for analytical method validation have been followed during method validation.

4.2.1. Linearity

Under optimum chromatographic conditions, linear relationships were obtained between the mean integrated peaks height and the corresponding concentrations for each of PSH, GUF and GUC in the ranges of 1–8, 1–20 and 0.4–8 μg mL^{-1}, respectively. The evaluation parameters like slope, intercept and the correlation coefficients were calculated and presented in Table 2.

4.2.2. Accuracy

Accuracy was calculated as the percentage recoveries of blind pure PSH, GUF and GUC, it was further assured by performing recovery studies at three levels (80%, 100% and 120% addition) and the average percent recovery was then calculated. Good percentage recoveries were obtained as shown in Table 2.

4.2.3. Precision

It was studied with respect to both repeatability and intermediate precision. *Repeatability* was calculated by the analysis

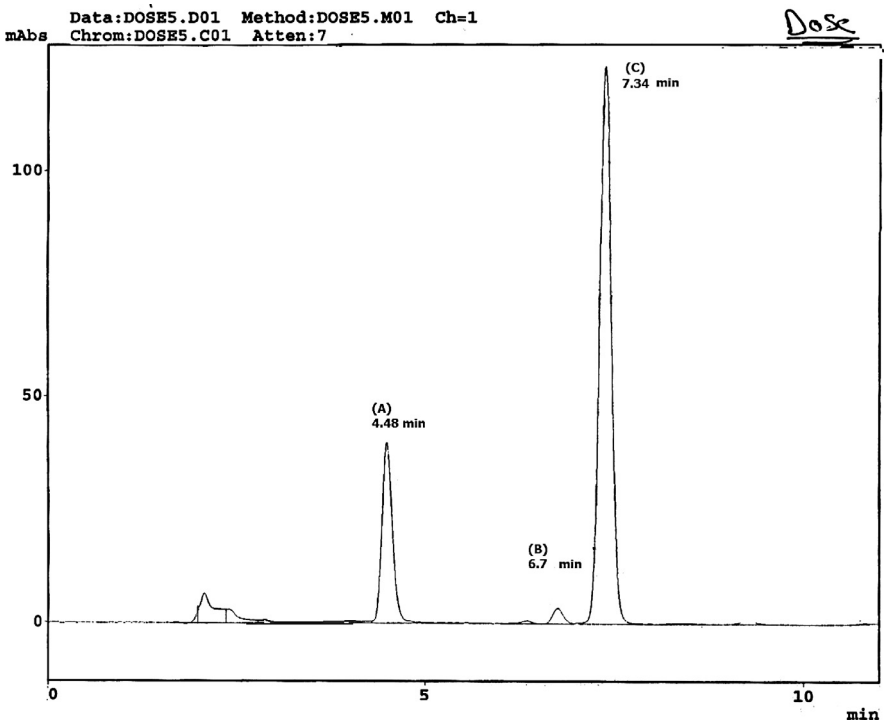

Figure 1B RP-HPLC chromatogram of Triaminic chest congestion® syrup containing PSH (A) (R_t = 4.48 min), excepient (B) (R_t = 6.7 min) and GUF (C) (R_t = 7.34 min) using de ionized water: methanol (50:50, v/v pH = 4 with phosphoric acid).

of three different concentrations of pure components (3, 5 and 7 μg mL^{-1} for PSH and GUC), (4, 10 and 15 μg mL^{-1} for GUF) in triplicate on the same day. The experiment was repeated on the same concentrations seven times on four consecutive days to determine *the intermediate precision*. Good results and acceptable RSD%, Table 2, were obtained.

4.2.4. Specificity

Specificity of the method was tested by how accurately and specifically the analytes of interest are determined in the presence of other components (impurities, degradates or excepients) (US FDA, 2000). It was verified from the HPLC chromatogram, Fig. 1B. Furthermore, good results obtained on applying the method to triaminic chest congestion® syrup, Table 3 proved that syrup additives did not interfere with any of the three separated components.

4.2.5. Limits of detection and quantitation (LOD and LOQ)

ICH recommendations (ICH, 2005) using visual non instrumental method were followed to calculate the values of LOD and LOQ. The low values of LOD and LOQ indicate the high sensitivity of method, Table 2.

4.2.6. Robustness

Deliberate small changes in the method parameters (e.g. changing in% methanol in the mobile phase ±1%, changing in mobile phase pH ± 0.2, changing in flow rate ± 0.05 mL min^{-1} and change in scanning wavelength ± 1 nm) did not lead to significant changes in R_t value, peak height or symmetry of the peaks.

Table 3 Determination of the studied drugs in triaminic chest congestion®
syrup by the proposed RP-HPLC method and statistical comparison with the reported RP-HPLC method.

Pharmaceutical formulation			
Triaminic chest congestion® syrup [a] (B. No. Y0005)	PSH		
	% Recovery [a]		
	98.36±1.402		
	Standard Addition Technique [b]		
	Pure added (μg mL^{-1})	Found (μg mL^{-1})	% Recovery
	2.40	2.36	98.33
	3.00	3.03	101
	3.60	3.57	99.17
	Mean ± RSD%		99.50±1.372
	F-test (5.050) [c]		
	1.437		
	Student's –t test (2.228) [c]		
	1.612		
	GUF		
	% Recovery [a]		
	96.66±1.505		
	Standard Addition Technique [b]		
	Pure added (μg mL^{-1})	Found (μg mL^{-1})	% Recovery
	6.40	6.51	101.72
	8.00	7.94	99.25
	9.60	9.68	100.83
	Mean+ RSD%		100.60± 1.244
	F-test (5.050) [c]		
	1.133		
	Student's –t test (2.228) [c]		
	1.976		

[a]Average of 6 determinations.
[b]Average of 3 determinations.
[c]The values in the parenthesis are the corresponding theoretical values at p = 0.05.

Table 4 System suitability testing parameters of the developed RP-HPLC method.

Parameters	PSH	Excepient	GUF	GUC
R_t	4.48 min	6.7 min	7.34 min	9.24 min
Peak a symmetry	1	1.06	1.07	1
Resolution (R_s)	3.7	1	2.4	
Capacity factor (k')	1.08	2.12	2.41	3.3
Selectivity (α)	1.96	1.14	1.37	

4.2.7. System suitability

System suitability parameters were carried out to prove that the overall system performed well, it was checked by calculating different parameters, e.g. capacity, selectivity, resolution and peak asymmetry. The obtained values were in the acceptable ranges as shown in Table 4.

4.3. Application of the method

After method optimization and validation, the developed RP-HPLC method has been successfully applied for the determination of PSH, and GUF in triaminic chest congestion® syrup. The agreement between the obtained results and the labeled amounts confirmed the specificity of the developed method, Table 3. The accuracy of the method was further assessed by application of standard addition technique where good results were obtained and given in Table 3.

The results of the developed method were statistically compared with those obtained by the reported.

RP-HPLC method (USP, 2009) at 95% confidence level using the student's *t* test and variance ratio F-test. No significant difference was found between the two methods, Table 3.

5. Conclusion

The present work concerns with the development and validation of stability indicating the RP-HPLC method for the simultaneous determination of PSH, GUF and GUC without sample pretreatment and without interference from syrup excepients. The developed method has an advantage over any reported method in being able to determine the studied drug along with GUF impurity with high sensitivity, selectivity and short analysis time using the isocratic mobile phase for all components. Moreover, it has been successfully applied to triaminic chest congestion® syrup and no interference from excepients has been found. The developed method can be easily applied for quality analysis of the studied drugs.

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