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Short communication

Simultaneous determination of diclofenac potassium and methocarbamol in ternary mixture with guaifenesin by reversed phase liquid chromatography

Ehab F. Elkady

Pharmaceutical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., Cairo 11562, Egypt

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ABSTRACT

New, simple, rapid and precise reversed phase liquid chromatographic (RP-LC) method has been developed for the simultaneous determination of diclofenac potassium (DP) and methocarbamol (MT) in ternary mixture with guaifenesin (GF), degradation product of methocarbamol. Chromatographic separation was achieved on a Symmetry® Waters C18 column (150 mm × 4.6 mm, 5 μm). Gradient elution based on phosphate buffer pH (8)-acetonitrile at a flow rate of 1 mL min^{-1} was applied. The UV detector was operated at 282 nm for DP and 274 nm for MT and GF. Linearity, accuracy and precision were found to be acceptable over the concentration ranges of 0.05–16, 0.5–160 and 0.5–160 μg mL^{-1} for DP, MT and GF, respectively. The optimized method proved to be specific, robust and accurate for the quality control of the cited drugs in pharmaceutical preparation.

1. Introduction

Diclofenac [2-(2,6-dichlorophenylamino) phenyl)] acetic acid (Fig. 1A) is a non-steroidal drug with anti-inflammatory, anti-pyretic and analgesic properties. It is usually found as sodium or potassium salts [1]. Methocarbamol, 2-hydroxy-3-(2-methoxyphenoxy) propyl carbamate, the carbamate of guaifenesin (Fig. 1 B) is a central muscle relaxant used to treat skeletal muscle spasms. The combination of diclofenac potassium (DP) and methocarbamol (MT) is frequently prescribed to alleviate pain associated with muscle spasm. Guaifenesin, 3-(2-methoxyphenoxy)-propane 1,2-diol (Fig. 1C) is considered as a related substance of methocarbamol [2]. It is the start compound of methocarbamol synthesis [3]. Moreover, it may be produced by hydrolysis of methocarbamol especially in alkaline medium [4]. Guaifenesin is clinically used as an expectorant.

Several analytical methods have been described in the literature for the determination of diclofenac in pharmaceutical and biological samples. These methods include potentiometry [5–7], spectrofluorimetry [8–10], HPLC [11–13], gravimetry [14], UV spectrophotometry and partial least-squares (PLS) [15–18]; and densitometry [19]. However, fewer methods have been reported for the quantitative determination of methocarbamol, the most commonly used being GC [20] and HPLC [21–24]. Guaifenesin has been determined by HPLC [25–30], UV spectrophotometry [31–33] and voltammetry [34]. Besides, some methods have been adopted for the simultaneous determination of methocarbamol and paracetamol in combined dosage forms based on spectrophotometry [35], derivative spectrophotometry [36], GC [37,38] and HPLC [39].

In comparison with other analytical techniques, HPLC greatly reduces the analysis time and allows for the determination of many individual components in a mixture using one single procedure [40]. Moreover, HPLC with UV detection is often preferred in ordinary laboratories because of its wide availability and suitability. For these considerations and also for lack of LC methods, this paper describes simple, sensitive and selective gradient RP-LC method with UV detection for the simultaneous determination of DP and MT in ternary mixture with GF. The proposed method was designed to be suitable for the quality assessment of these compounds in pharmaceutical preparations.

2. Experimental

2.1. Instrumentation

The HPLC system consisted of a Schimadzu LC-20 AT Liquid Chromatograph (Japan) using a Symmetry® C18 column (150 mm × 4.6 mm, 5 μm) Waters (Ireland). The system was equipped with an UV–visible detector (SPD-20A, Japan) and an autosampler (SIL-20A, Schimadzu, Japan). An Elma S100 ultrasonic processor model KBK 4200 (Germany) was used.

E-mail address: ehabelkady75@yahoo.com.

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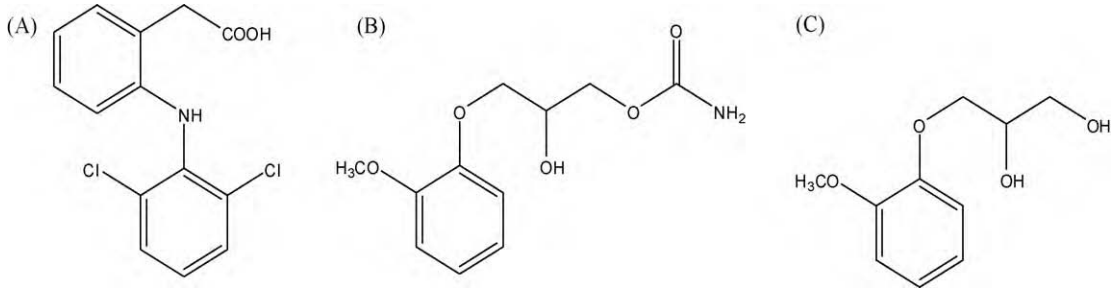

Fig. 1. Chemical structures of diclofenac (A), methocarbamol (B) and guaifenesin (C).

2.2. Reagents and reference samples

Pharmaceutical grade DP and MT were kindly supplied by October Pharma Company (Cairo, Egypt) and certified to contain 99.68 and 99.77%, respectively. Pharmaceutical grade GF was kindly supplied by El Goumhoria Co. and certified to contain 99.78%. Dimra® tablets (batch number B0070110) nominally containing DP (50 mg) and MT (500 mg), were manufactured and supplied by October Pharma Co. (Cairo, Egypt). Inactive ingredients of the tablets include colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycolate and cornstarch. HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Loughborough, Leicestershire, UK). Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Membrane filters 0.45 μm from Teknokroma (Barcelona, Spain) were used. All other chemicals and reagents used were of analytical grade unless indicated otherwise.

2.3. Chromatographic conditions

Chromatographic separation of the ternary mixture was performed using a gradient elution based on a mobile phase consisting of (A) phosphate buffer (pH 8) and (B) acetonitrile. The gradient elution was carried out by varying mobile phase (A) from 75 to 35% (0–3 min) and then from 35 to 75% (3–8 min). The buffer solution was filtered through 0.45 μm membrane filter and degassed for 30 min in an ultrasonic bath prior to its use. The mobile phase was pumped through the column at a flow rate of 1 mL min^{-1}. Analyses were performed at ambient temperature and detection was carried out at 274 and 282 nm. The injection volume was 25 μL.

2.4. Standard solutions

2.4.1. DP standard solution

An accurately weighed 40 mg DP was transferred into a 100-mL volumetric flask, then dissolved in and completed to volume with methanol. Then, 10 mL aliquot of this solution was transferred into another 100 mL volumetric flask and completed to volume with methanol (0.04 mg mL^{-1}).

2.4.2. MT standard solution

An accurately weighed 40 mg MT was transferred into a 100-mL volumetric flask, then dissolved in and completed to volume with methanol (0.4 mg mL^{-1}).

2.4.3. GF standard solution

An accurately weighed 40 mg GF were transferred into a 100-mL volumetric flask, then dissolved in and completed to volume with methanol (0.4 mg mL^{-1}).

2.5. Sample preparation

Twenty tablets were accurately weighed and powdered in a mortar. A quantity of the powdered tablets equivalent to (4 mg) DP and (40 mg) MT was extracted with methanol (3 × 20 mL) and filtered into a 100-mL volumetric flask. The volume was completed with methanol to obtain a sample solution of concentration equivalent to DP 0.04 mg mL^{-1} and MT 0.4 mg mL^{-1}.

2.6. Procedure

2.6.1. Linearity and repeatability

Accurately measured aliquots of working standard solutions equivalent to 0.5–160 μg DP, 5.0–1600 μg MT and 5.0–1600 μg GF were transferred into three series of 10 mL volumetric flasks. The volumes were completed with methanol. A volume of 25 μL of each solution was injected into the chromatograph. The conditions including the mobile phase at a flow rate 1 mL min^{-1}, detection at 274 nm for MT and GF and at 282 nm for DP and run time program for 8 min were adjusted. A calibration curve for each compound was obtained by plotting area under the peak (AUP) against concentration (C).

The repeatability of the method was assessed by analyzing a mixture containing 8, 80 and 24 μg mL^{-1} of DP, MT and GF, respectively ($n = 6$). This mixture was considered as the working standard solution. The precision (R.S.D.) for each compound was calculated.

2.6.2. Assay of laboratory prepared mixtures and Dimra® tablets

The procedure mentioned in Section 2.6.1 was repeated using laboratory prepared mixtures equivalent to 6–14 μg mL^{-1} DP, 60–140 μg mL^{-1} MT and 12–112 μg mL^{-1} GF. The concentration ratio of GF to MT in the mixtures ranged from 20 to 80% (w/w).

For the determination of the examined drugs in Dimra® tablets, the sample solution prepared in Section 2.5 was diluted to prepare a solution equivalent to 8, 80 μg mL^{-1} of DP and MT, respectively; and then injected in triplicates. The concentrations of the examined drugs were calculated in reference to the working standard solution.

3. Results and discussion

No previous method was adopted for the LC determination of DP, MT and GF in mixture. Thus, the aim of this work was to develop a new, simple, accurate, reproducible and sensitive LC method for the simultaneous determination of DP, MT and GF, the degradation product of MT (a stability-indicating assay for MT).

3.1. Method development

For the separation of the examined drugs, various reversed-phase columns, isocratic mobile phase systems were attempted. But, due to the similar physicochemical properties of MT and GF,

Table 1
System suitability results of the proposed method.

Compound	*N*	*R*	*T*	% R.S.D. of	
				t_R	Peak area
GF	2067	–	1.0	0.06	0.41
MT	3062	2.5	1.01	0.29	0.31
DP	6591	4.6	1.0	0.46	0.27

N, number of theoretical plates; *R*, resolution factor; *T*, tailing factor; t_R, retention time.

isocratic elution could not separate them. So, gradient elution was chosen for the separation of the three compounds. The mobile phase consisted of (A) phosphate buffer (pH 8) and (B) acetonitrile with a linear gradient by varying mobile phase (A) from 75 to 35% (0–3 min) and then from 35 to 75% (3–8 min). Good separation of MT from its degradation product (GF) and DP with minimum retention times (<8 min) was obtained at a flow rate 1 mL min^{-1}. Detection was carried out at 274 nm for MT and GF and at 282 nm for DP, where high detector sensitivity was achieved at these two wavelengths. The retention times were 3.97, 4.83 and 5.91 min for GF, MT and DP, respectively; as presented in Fig. 2.

In the course of optimizing the conditions of the proposed method, different parameters affecting the chromatographic separation were studied. The system suitability tests were used to verify that the conditions of the chromatographic system were adequate for the resolution and hence for the analysis [2], Table 1. Satisfactory results were obtained for *N* (number of theoretical plates), *R* (resolution factor), *T* (tailing factor) and R.S.D. of retention times (t_R) and peak areas.

3.2. Method validation

3.2.1. Linearity

Linearity was studied for MT, DP and GF. A linear relationship between area under the peak (AUP) and component concentration (C) was obtained. The regression equation $y = bC \pm a$ for each drug was also computed. In this study, 10 concentrations for each compound were used. The linearity of the calibration curves was validated by the high value of correlation coefficients. The analytical data of the calibration curves including standard deviations for the slope and intercept (S_b, S_a) are summarized in Table 2.

3.2.2. Range

The calibration range was established within protocol range necessary, according to the intact drugs' concentrations in pharmaceutical product to give accurate and precise results. In addition, a wide calibration range of GF was obtained. The calibration ranges of the proposed procedure are given in Table 2.

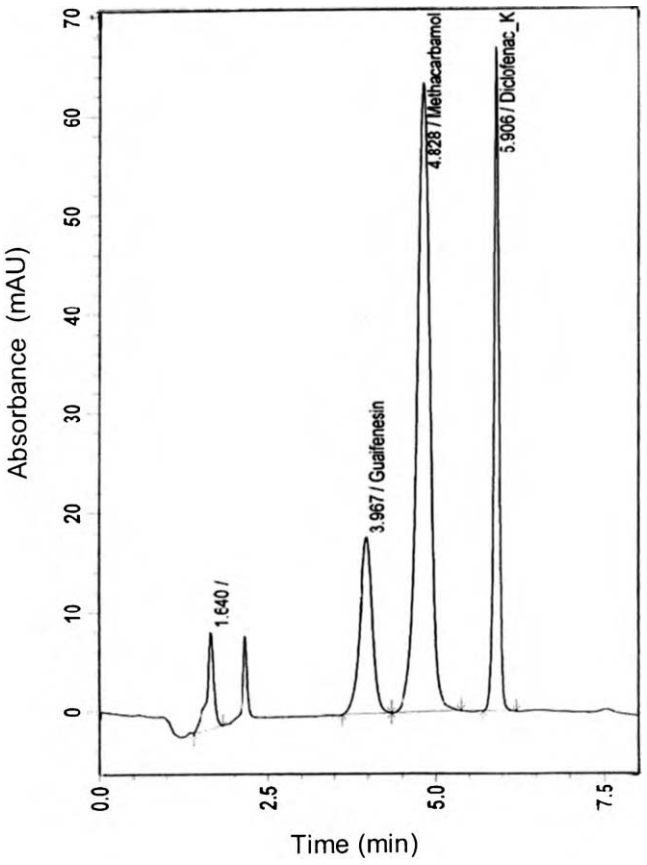

Fig. 2. A typical LC chromatogram of 25 μL injector of synthetic ternary mixture of diclofenac potassium (8 μg mL^{-1}), methocarbamol (80 μg mL^{-1}) and guaifenesin (24 μg mL^{-1}).

3.2.3. Accuracy

Accuracy of the results was calculated by % recovery of five different concentrations of the three drugs analyzed by the proposed method. The concentration of samples employed to perform accuracy were from 6 to 14 μg mL^{-1} DP, 60 to 140 μg mL^{-1} MT and from 12 to 112 μg mL^{-1} DP. The results obtained including the mean of the recovery, standard deviation, relative standard deviation, inter-

Table 2
Results obtained by the RP-LC method for the simultaneous determination of diclofenac potassium, methocarbamol and guaifenesin in ternary mixture.

Item	Diclofenac potassium	Methocarbamol	Guaifenesin
Retention time	5.91 min	4.83 min	3.97 min
Wavelength of detection	282 nm	274 nm	274 nm
LOD	0.29	2.14	3.95
LOQ	0.88	6.48	11.98
Range of linearity	0.05–16 μg mL^{-1}	0.5–160 μg mL^{-1}	0.5–160 μg mL^{-1}
Regression equation	$y = 58774x + 1733.6$	$y = 14195x - 1630.2$	$y = 17801x - 5623.6$
Correlation coefficient (r)	0.9998	0.9999	0.9996
S_b	392.80	53.57	1622.51
S_a	2883.06	3730.22	112.978×10^3
Confidence limit of the slope	58774.0 ± 927.00	14195.0 ± 123.75	17801 ± 3748.0
Confidence limit of the intercept	1733.6 ± 6804.02	1630.2 ± 8616.81	$5623.6 \pm 2.61 \times 10^5$
Standard error of the estimation	6514.86	9204.12	278.766×10^3
Intra-day			
%R.S.D.	0.09–0.69	0.16–0.50	0.29–0.42
Inter-day			
%R.S.D.	0.79–1.55	0.27–1.36	0.65–1.76
Drug in laboratory made mixture	99.13 ± 1.94	99.69 ± 0.96	99.53 ± 1.52
Drug in dosage form	96.59 ± 1.27	–	97.79 ± 0.66

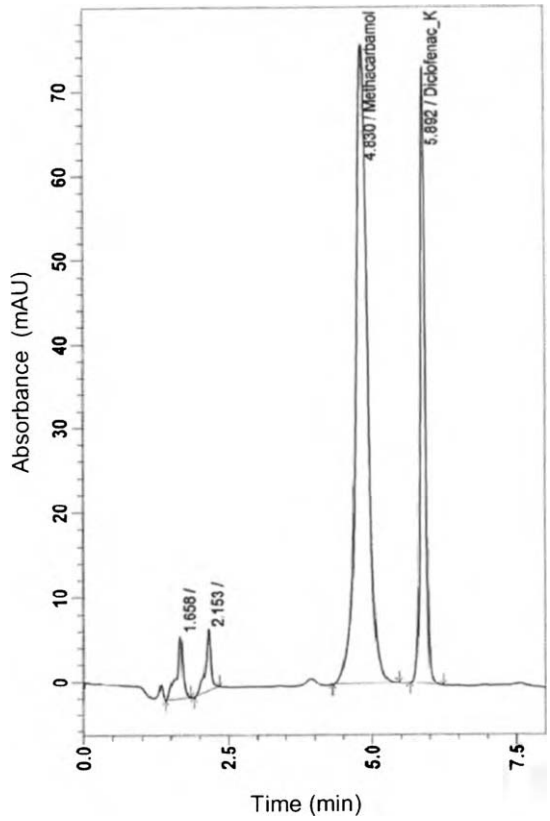

Fig. 3. A typical LC chromatogram of 25 μL injector of Dimra® sample solution.

day and intra-day precision (using three different concentrations in triplicates for 3 days) are displayed in Table 2.

3.2.4. Precision

Precision was estimated by repeatability. The repeatability of the method was assessed by analyzing a mixture containing 8, 80 and 24 μg mL^{-1} of DP, MT and GF, respectively (n = 6). The values of the precision (R.S.D.) for DP, MT and GF peaks were 0.27, 0.31 and 0.41%, respectively.

3.2.5. Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences including degradation products and related substances. In the present work, the degradation product under study is GF, degradation product of MT. So, specificity was checked by analyzing DP and MT in laboratory prepared mixtures with GF. Good resolution and absence of interference between drugs being analyzed are shown in Fig. 2. Moreover, the proposed method was applied to a pharmaceutical formulation containing DP and MT, Fig. 3. Satisfactory results were obtained indicating the high specificity of the proposed method for the determination of DP and MT in ternary mixture with GF and also in pharmaceutical formulations, Table 2. It is worth noting that a small peak corresponding to GF was obtained in the pharmaceutical preparation but it was below its limit of detection (LOD).

3.2.6. Robustness

Robustness was performed by deliberately changing the chromatographic conditions. The flow rate of the mobile phase was changed from 1.0 to 0.8 mL min^{-1} and 1.2 mL min^{-1}. The organic strength was varied by ±2%, while pH was varied by ±0.1 units. These variations did not have significant effect on chromatographic resolution by the proposed LC method for DP, MT and GF.

3.2.7. Limit of detection and limit of quantification

Limit of quantification (LOQ) which represents the concentration of analyte at S/N ratio of 10 and limit of detection (LOD) at which S/N is three were determined for the proposed method and results are given in Table 2. LOD and LOQ were computed based on the standard deviation of the response and the slope.

4. Conclusion

The proposed LC method has the advantages of simplicity, precision, accuracy and convenience for the separation and quantitation of DP, MT drug combination and can be employed for the assay of their respective dosage form. Moreover, the proposed LC method is a stability indicating assay that is capable of determining MT and its degradation product (GF) simultaneously. Hence, the proposed LC method can be used for the quality control of the cited drugs in ordinary laboratories.

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