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PAPER



# An eco-friendly micellar HPLC method for the simultaneous determination of triamterene and xipamide in active pharmaceutical ingredients and marketed tablet dosage form

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## ABSTRACT

In the last few years, the use of surfactants as mobile phase additives in reversed phase liquid chromatography (RPLC) has been steadily developing and improving. Surfactants modify the polarity of the stationary phase which in turn decreases the amount of organic solvent required for elution of the analytes rendering the methodologies linked to them greener and more eco-friendly. Brij-35 is a fatty alcohol ethoxylates non ionic surfactant, which is less widely used as mobile phase additive. Brij-35 can decrease stationary phase polarity while remaining neutral. In this research, Brij-35 was studied in the separation and determination of marketed antihypertensive combination therapy composed of triamterene (TRM) and xipamide (XIP). TRM and XIP are diuretics used for treatment of essential hypertension and associated edema conditions. Chromatographic separation was achieved on RP-C18 column (Kinetix®, 5 µm, 15 cm × 4.6 mm) at flow rate 1 mL min<sup>-1</sup> and UV-detection at 254 nm. Isocratic elution was performed using mobile phase composed of 0.1 M Brij-35: methanol (MeOH) (60:40, v/v). The analytes were well separated and quantified within linearity ranges of 5–50 µg mL<sup>-1</sup> for both drugs in short retention time (2.6 and 5.3 min. for TRM and XIP, respectively). Since claiming greenness is not enough, Green Analytical Procedure Index (GAPI) was used to demonstrate the superiority of the proposed method over the previously reported methods. GAPI is a new metric for evaluation of the ecological impact of analytical procedures. The proposed method was validated according to ICH guidelines and applied successfully for simultaneous determination of the drugs in their co-formulated tablets.

## KEYWORDS

micellar liquid chromatography, triamterene, xipamide, Brij-35, green chromatography

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## 1. INTRODUCTION

The use of surfactants as mobile phase additives in reversed phase liquid chromatography (RPLC) has been growing fast over the last decade. Retention behavior in RPLC depends on partitioning between the hydrophobic stationary phase and the aqueous mobile phase. That is why the mobile phase must contain an organic solvent to enhance its elution power. Brij-35

is a non-ionic polyoxyethylene-23 lauryl ether surfactant. It has high molecular weight ( $\approx 1200$ ) and large cloud point ( $\approx 100$  °C) [1]. This ethoxylated fatty alcohol surfactant can decrease the stationary phase polarity while remaining neutral. This in turn leads to decreasing the amount of the hazardous organic solvents and the required mobile phase elution power to elute the hydrophobic analytes retained onto the stationary phase. Acetonitrile (ACN) and methanol (MeOH) are the most widely used organic modifiers in LC. Although ACN proved to produce higher elution strength and lower viscosity than MeOH, it has higher price and higher ecological impact than MeOH [2]. Thus, Brij-35 modified mobile phases enable the use of the cheaper and lower elution strength MeOH without affecting the speed or efficiency of analysis. Moreover, Brij-35 is biodegradable and even can be used for enhancing biodegradation of some environmental contaminants [3, 4], hence it is more environmentally safe.

Xipamide (XIP) is a diuretic drug used for treatment of hypertension. It has actions and uses similar to thiazide diuretics including its hypokalemic effects [5]. Triamterene (TRM) is another weak potassium-sparing diuretic used for treatment of hypertension. It is used as adjunctive therapy with thiazide diuretics to compensate for their hypokalemic effects. It is also used to conserve potassium in those at risk from hypokalemia during treatment of edema associated with hepatic cirrhosis and nephritic syndromes [5]. The combination of TRM and XIP (Chemical structures shown in Fig. 1) in diuretic regimens for treatment of hypertension results in increasing sodium excretion, but with significant decrease in potassium elimination. Such combination can be also misused for forcing diuresis to mask the intake of some prohibited substances or even to achieve loss of weight especially in sports based on weight classification. That is why such diuretics are prohibited by the World Anti-Doping Agency (WADA).

Several methods had been described for the quantitative simultaneous determination of XIP and TRM. Most of these are chromatographic methods focusing on identification of the drugs under study together with other diuretics and CNS stimulants in urine for purpose of doping control using ultra-high performance liquid chromatography (UHPLC) with MS/MS detection [6–8] or high performance liquid chromatography (HPLC) coupled with MS/MS detector [9–13]. Such reported methods are expensive due to the high cost of mass detectors especially when coupled with UHPLC due to the added working and maintenance cost and hence not favored by users in pharmaceutical economic facilities who require more simple and cheap methodologies for the

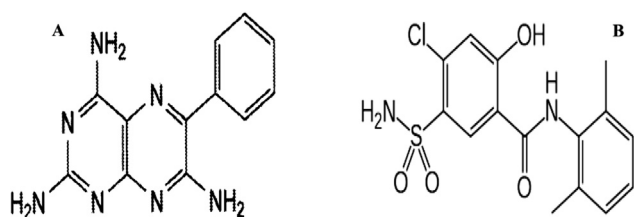


Figure 1. Structures of drugs under study; (A) TRM, (B) XIP

analysis purposes [14]. Other methods were reported using HPLC with UV/VIS detection [15, 16]. Although such methods used the advantage of micellar mobile phases by adding sedum lauryl sulfate, however they focused mainly on the determination of a large group of diuretics. So, the elution time of our drugs under study was quite longer. Only three methods were reported focusing on determination of the drugs under study using HPLC-UV detection [17–19]. Two of those methods have the disadvantage of using gradient technique with run time exceeding 12 min. besides the re-equilibration time required for base line adjustment. All of the three methods use high percentage of ACN (above 50%) as elution solvent. ACN is not favored by green chemistry concepts due to its responsibility of acid rains and the high energy required for its production [2]. Not to mention it is 5 times higher in cost than MeOH. Only two methods were reported for determination of XIP and TRM using UV spectrophotometry [20, 21].

The purpose of our study is to develop a new methodology for determination of XIP and TRM that's both economic and eco-friendly using the advantages of micellar mobile phases containing Brij-35 in reducing the organic solvent required for fast elution. This in turn will encourage economic pharmaceutical research and quality control laboratories to use in their millions of analyses performed in routine daily work.

## 2. MATERIALS AND METHODOLOGY

### 2.1. Materials

TRM and XIP were analytical standards that were supplied along with their certificates from EIPICo., Egypt. MeOH, HPLC grade was purchased from Merck, Germany. Brij-35®, analytical grade, was purchased from LOBA Chemie, India.

Epitens® tablets (containing 30 mg TRM and 10 mg XIP per tablet) were purchased from the Egyptian market (Lot no. 54881).

Millipore water purification system was used for fresh preparation of de-ionized water.

### 2.2. Instrumentation

The chromatographic system used was Young line HPLC system (model-9100, Korea) equipped with vacuum degasser (model-YL9101), quaternary Pump (model-YL9110), and UV/Vis detector (model-YL9120). Samples were injected using 20  $\mu$ L loop injector.

HPLC superficially porous particulate RP-C18 column; Kinetix® (5  $\mu$ m, 150\*4.6 mm) was purchased from Phenomenex, USA.

### 2.3. Chromatographic conditions

Elution was done using isocratic mobile phase consisting of 0.1 M aqueous solution of Brij-35® and MeOH at ratio (60:40, v/v) at flow rate 1 mL min<sup>-1</sup>. Column temperature

was kept at 30 °C and using UV-detector which was set at wavelength 254 nm. Sampler injection volume was 20 µL.

#### 2.4. Working standards and quality control samples

Stock solutions were prepared individually for TRM and XIP in MeOH at concentration (100 µg mL<sup>-1</sup>). Working solutions were then prepared by mixing suitable aliquots of the stock solutions and dilution using the mobile phase. Linearity was tested using six working solutions within the concentration range of 5–50 µg mL<sup>-1</sup> for both TRM and XIP. Accuracy was tested using six quality control working samples prepared at concentrations (5, 10, 15, 20, 30 and 40 µg mL<sup>-1</sup>) of both drugs by dilution of stock solution using a placebo solution. Three quality control samples prepared from the stock solution at low, medium and high concentration levels of both drugs within the specified range (10, 30 and 50 µg mL<sup>-1</sup>) by using a placebo solution. The quality control samples were used to test precision of the proposed method. Placebo solution was prepared by mixing the excipients labeled in Epitens® tablets; magnesium stearate, titanium dioxide, starch, methocel and spray dried lactose, in mixture of equal volumes of MeOH and water.

#### 2.5. Analysis of dosage forms

The accurate weights of ten tablets from Epitens® dosage form were determined, and then they were finely powdered and mixed well. The average weight of one powdered tablet was dissolved in 100 mL MeOH with the aid of a sonicator for 5 min., and then the solution was filtered. Five milliliter of this solution was diluted to 100 mL using the mobile phase and 20 µL of the final solution was injected on the chromatographic system.

#### 2.6. Method validation

Validation of the proposed analytical procedure in terms of, linearity, accuracy, precision, specificity, reproducibility

and robustness, was carried out according to ICH guidelines [22].

### 3. RESULTS AND DISCUSSION

#### 3.1. Method development

During method development stage, the effect of using Brij-35 as mobile phase additive was tested on the separation performance in terms of number of theoretical plates, resolution between peaks and selectivity. Other variables were also studied such as percentage of MeOH and flow rate which can affect the chromatographic performance. The effect of changing the concentration of Brij-35 on selectivity of the method was studied within concentration range of 0.06–0.15 M at constant flow rate 1 mL min<sup>-1</sup>. It was found that at concentration 0.1 M Brij-35®, separation efficiency in terms of largest number of theoretical plates (*N*), resolution and selectivity. MeOH percentage in the mobile phase was changed between (50, 45, 40, 35 and 30%) at constant flow rate 1 mL min<sup>-1</sup>. The lowest amount of MeOH that enhanced base line separation was achieved at (40% MeOH, v/v). Lowering MeOH% lead to increase retention time, while higher MeOH% lead to overlapping of peaks. The effect of changing flow rate on the chromatographic separation was tested over the range of 0.8–1.2 mL min<sup>-1</sup>. A flow rate 1 mL min<sup>-1</sup> was found to be optimum. Results are summarized in Table 1.

#### 3.2. Method validation

**3.2.1. Selectivity.** Separation of the two drugs was successfully obtained at the chosen chromatographic conditions with high resolution between their peaks. Chromatograms shown in Fig. 2 demonstrate the separation of the two drugs

Table 1. Effect of experimental parameters on separation efficiency of drugs under study

Parameter		Number of theoretical plates ( <i>N</i> ) per 15 cm stationary phase		Resolution ( <i>R<sub>s</sub></i> )	Selectivity ( <i>α</i> )
		TRM	XIP		
Concentration of Brij-35 (M)	0.06	422	670	4.5	2.4
	0.08	324	1023	4.17	2.5
	0.1	936	1119	5.42	2.7
	0.12	1000	770	3.2	1.4
	0.15	471	600	2.5	2.1
Flow rate (mL min <sup>-1</sup> )	0.8	520	780	1.02	1.81
	1.0	900	1000	5.10	2.57
	1.2	500	580	1.917	2.01
Methanol %	50	350	1100	7.5	10
	45	460	850	5.3	7
	40	320	1118	2.23	1.56
	35	230	640	1.21	1.1
	30	250	600	0.98	1.2



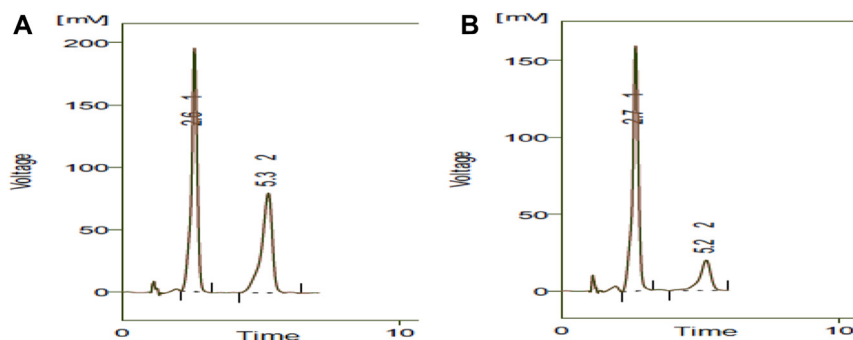


Figure 2. HPLC chromatograms of TRM and XIP; (A)  $40 \mu\text{g mL}^{-1}$  laboratory prepared mixture, (B) Epitens® tablets

in laboratory mixture prepared by spiking both drugs in placebo solution at concentration  $40 \mu\text{g mL}^{-1}$  and in Epitens® tablet dosage form. The excipients in dosage form or in the placebo solution did not show any interference.

**3.2.2. Calibration curve and linearity.** Linearity was tested across the range specified using working standard solutions injected in triplicates. The calibration plots were constructed for the peak areas corresponding to TRM and XIP as a function of the corresponding concentration. Good linearity results were obtained as indicated by the regression data demonstrated in Table 2.

Calculation of the detection limits (LOD) and the quantification limit (LOQ) were done according to ICH guidelines. LOD was defined as the injected quantity giving S/N of three and LOQ equals S/N of 10 (in terms of peak area). LOD and LOQ for the proposed method for TRM and XIP are shown in Table 2. The results indicate that the validated method is sensitive for low concentrations of the two drugs.

**3.2.3. Accuracy and precision.** Working samples at six different concentrations within the range specified were used to establish accuracy of the proposed method. Correctness of the analytical procedure for its purpose was demonstrated by accuracy results showed in Table 2. Assay precisions in the same day and in three successive days were determined

Table 2. Analytical parameters for the analysis of TRM and XIP obtained by the proposed method

Parameter	TRM	XIP
Range ( $\mu\text{g mL}^{-1}$ )	5–50	5–50
Determination coefficient ( $r^2$ )	0.9999	0.9999
Regression equation	$69.65X + 52.55$	$49.16X + 53.56$
LOD ( $\mu\text{g mL}^{-1}$ )	0.58	0.67
LOQ ( $\mu\text{g mL}^{-1}$ )	1.78	2.03
Selectivity ( $\alpha$ )	–	2.6
Resolution ( $R_s$ )	–	5.4
Accuracy ( $n = 6$ ) <sup>*</sup>	$100.1 \pm 0.59$	$100.0 \pm 0.906$
Intra-day precision <sup>*</sup>	$99.4 \pm 0.77$	$100.32 \pm 0.69$
Inter-day precision <sup>*</sup>	$99.9 \pm 1.55$	$100.7 \pm 0.62$

LOD: limit of detection, LOQ: limit of quantitation.

<sup>\*</sup> Results = % Recovery  $\pm$  SD: standard deviation.

using three quality control samples injected in triplicates. Table 2 shows Inter- and intra-day results which indicated high repeatability and intermediate precisions.

**3.2.4. Robustness.** Validated chromatographic conditions of the proposed method were tested for minor variations. The percentage of organic modifier (MeOH) in mobile phase was changed between (38, 40 and 42%). Flow rate was also changed between (0.8, 1.0 and 1.2  $\text{mL min}^{-1}$ ). The recovery percentage for the quality control samples were calculated and compared to those obtained using the optimum conditions. Results (Table 3) showed that such deliberate variations did not produce worthy effect on neither separation efficiency nor recovery percentage.

**3.2.5. Analytical application.** Simultaneous determination of TRM and XIP in their tablet dosage form was applied by the proposed method, Epitens®, and results obtained were compared to results obtained from a previously reported method [18]. Student *t*-test and *F*-values (Table 4) were calculated and compared. Results showed that the methods were both favorable and accurate.

### 3.3. Evaluation of the analytical procedure

The philosophical approach of green chemistry incorporates concepts based on minimizing the ecological impact of any chemical procedure. Metrics evaluating the greenness of such procedures have been developing in the past few years. Green chemistry metrics must consider the ecological

Table 3. Robustness study of the proposed HPLC method for determination TRM and XIP

Variation	TRM % Recovery <sup>a</sup> $\pm$ SD	XIP % Recovery $\pm$ SD
Optimum condition	$99.3 \pm 1.1$	$100.7 \pm 0.4$
MeOH %		
42%	$99.2 \pm 0.2$	$98.5 \pm 0.4$
38%	$101.9 \pm 0.9$	$99.7 \pm 1.2$
Flow rate		
0.8 $\text{mL min}^{-1}$	$100.0 \pm 0.5$	$99.6 \pm 0.4$
1.2 $\text{mL min}^{-1}$	$100.8 \pm 0.2$	$98.1 \pm 0.6$

SD: standard deviation.

<sup>a</sup>Mean of three replicate measurement.



Table 4. Application of the proposed method in determination of drugs under study in their combined dosage forms

Epitens® tablet	% Recovery $\pm$ SD <sup>a</sup>		Student <i>t</i> -test <sup>b</sup>	<i>F</i> -values <sup>b</sup>
	Proposed	Reported [19]		
TRM	100.70 $\pm$ 0.35	100.2 $\pm$ 0.70	1.56	4.00
XIP	99.76 $\pm$ 1.32	100.7 $\pm$ 1.11	1.34	1.41

<sup>a</sup>The values are the mean of five determinations. <sup>b</sup>The tabulated *t*- and *F*-values at 95% confidence limit are 2.23 and 5.05, respectively.

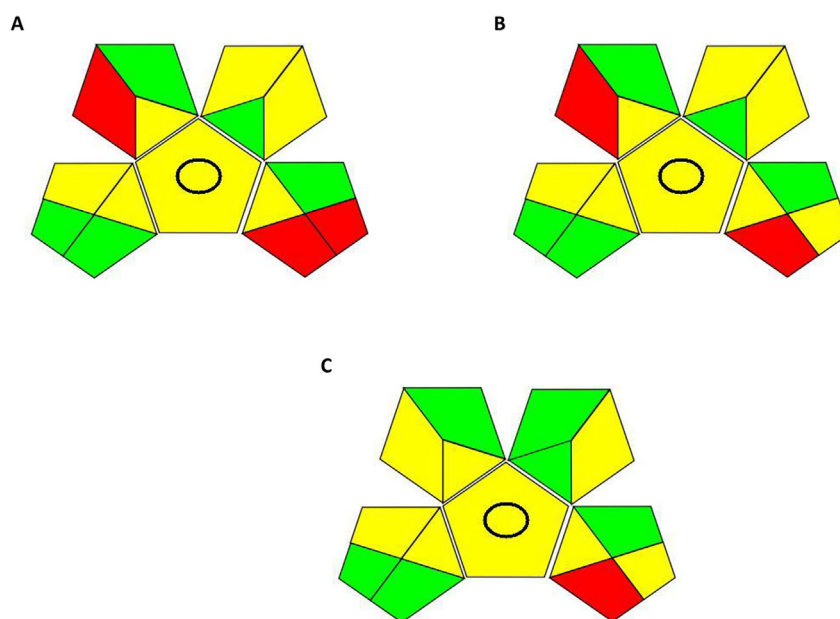


Figure 3. GAPI pictograms for previously reported methods; A: [18], B: [19], and C: the proposed method for determination of XIP and TRM. The red zones represent high ecological impact, yellow zones represent lower impact, and green zones represent safe effect to environment

impact of solvents and reagents used, disposal of waste and energy requirements. Several metrics are being used for evaluation of analytical procedures. The first introduced metric was the National Environmental Methods Index (NEMI) [14]. The analytical eco-scale reported in 2012 [23] calculates penalty points for any step in the analytical procedure that doesn't match green chemistry ideas. Green analytical procedure index (GAPI) is a recent metric consisting of five pentagrams comprising 15 zones covering the whole analytical procedure [24]. GAPI covers the steps of sample collection, transportation, preservation, storage as well as its preparation and extraction steps. It also counts for type, volume and hazards of organic solvents used as well as amount of generated waste and its treatment proposed by the analytical procedure. GAPI uses a color code for evaluating each step in the proposed analytical procedures. Green color symbolizes low ecological impact, while yellow and red colors represent higher impacts. GAPI was used to compare the proposed method to the other reported methods [18, 19] (Fig. 3). Both reported methods brought for comparison utilized ACN as organic modifier at percentage 50% and higher. Abd-Elhay [19] et al., proposed a superior isocratic

technique over El-Kimary [18] who proposed a gradient elution technique. However, for both methods, the amount of ACN used and amount of generated waste taken along with its biohazards and high price may be less favorable than MeOH in the proposed study. Figure 3 shows that the proposed method has the largest number of green and yellow zones (six green & eight yellow zones) and the lowest number of red zones (only one zone) as compared to the previously reported methods.

#### 4. CONCLUSION

In the proposed study, a green HPLC method was developed and validated for the simultaneous determination and quantification of TRM and XIP in active pharmaceutical ingredients and co-formulated pharmaceutical dosage forms. High accuracies and precision results were obtained. The validated method had lower solvent consumption, replaced dangerous solvents by safer one and considered economic selection criteria for industrial establishments. The proposed method is eligible for daily routine simple use in different



research and pharmaceutical quality control laboratories for the simultaneous quantification of the drugs under study.

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