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Spectrophotometric and spectrodensitometric determination of triamterene and xipamide in pure form and in pharmaceutical formulation

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Sensitive and validated UV-spectrophotometric, chemometric and TLC-densitometric methods were developed for determination of triamterene (TRM) and xipamide (XIP) in their binary mixture, formulated for use as a diuretic, without previous separation. Method A is the isoabsorptive point spectrophotometry, in which TRM concentration alone can be determined at its λ_{max} while XIP concentration can be determined by measuring total concentration of TRM and XIP at their isoabsorptive point followed by subtraction. Method B is the ratio subtraction spectrophotometry, where XIP can be determined by dividing the spectrum of the mixture by the spectrum of TRM (as a divisor) followed by subtracting the constant absorbance value of the plateau region, then finally multiplying the produced spectrum by the spectrum of the divisor, while TRM concentration can be determined at its λ_{max}. Method C is a chemometric-assisted spectrophotometry where classical least squares, principal component regression, and partial least squares were applied. Method D is a TLC-densitometry; this method depends on quantitative densitometric separation of thin layer chromatogram of TRM and XIP using silica gel plates at 254 nm. The proposed methods were successfully applied for the analysis of TRM and XIP in their pharmaceutical formulation and the results were statistically compared with the established HPLC method. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords: triamterene; xipamide; isoabsorptive point; ratio subtraction; chemometric; TLC-densitometry

Introduction

Triamterene (TRM) is 6-phenyl-2,4,7-triaminopteridine.[1] It is a diuretic drug belonging to the potassium-sparing diuretic family.[2] It is generally administered together with other, more powerful, diuretics such as derivatives of anthranilic acid and thiazide, with the purpose of reducing their potassium-wasting effects.[2] TRM has a limited diuretic efficacy. It acts on the collecting tubules and collecting ducts, inhibiting sodium reabsorption and decreasing potassium excretion. After oral administration, TRM is absorbed and metabolized by hydroxylation and subsequent immediate conjugation rendering its main metabolite hydroxytriamterene sulfate.[3]

Xipamide (XIP) is 4-chloro-2′,6′-dimethyl-5-sulfamoylsalicylanilide.[1] it has a moderately powerful diuretic action. It decreases active reabsorption of sodium and accompanying chloride by binding to the chloride site of the electroneutral Na^+/Cl^- co-transport system and inhibiting its action.[3] The structural formula of TRM and XIP are shown in Figure 1.

The literature survey reveals several analytical methods for the determination of TRM including spectrophotometric,[4–6] fluorimetric[7–9] and chromatographic[10–13] methods, while XIP was determined by different HPLC methods.[14–17]

Few methods for the determination of TRM and XIP in their binary mixture have been reported in literature, including HPLC methods[18–22] and GC method.[23]

The aim of this work is to develop simple, sensitive and selective analytical methods for the determination of TRM and XIP in their binary mixture and in pharmaceutical formulation without previous separation.

Experimental

Instruments

A double beam UV-Visible spectrophotometer (SHIMADZU, Japan) model UV-1601 PC with quartz cell of 1 cm pathlength, connected to an IBM compatible computer. The software was UVPC personal spectroscopy software v. 3.7. The spectral bandwidth was 2 nm and wavelength-scanning speed 2800 nm/min. All data analysis was performed using PLS-Toolbox 2.0 running under MATLAB®, v. 6.5.[24] A UV lamp with short wavelength 254 nm UV Lamp (Viber Lourmat, MARNE LA VALLEE Cedex 1, France). A TLC scanner 3 densitometer (Camag, Muttenz, Switzerland). The following requirements are taken into consideration: slit dimensions: 5 × 0.2 mm; scanning speed: 20 mm/s; spraying rate: 10 s μL^{-1}; and data resolution: 100 µm/step. TLC plates (20 × 20 cm) coated with silica gel $60F_{254}$ (Fluka, Sigma-Aldrich Chemie GmbH, Germany). A sample applicator for TLC Linomat IV with 100 µL syringe (Camag, Muttenz, Switzerland).

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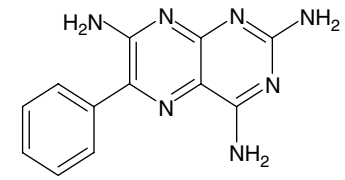
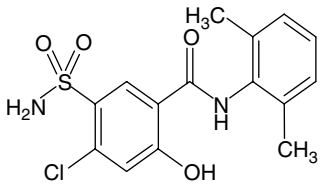


(a)

Triamterene
Mol. Formula $C_{12}H_{11}N_7$
Mol. Wt. 253.27



(b)

Xipamide
Mol. Formula $C_{15}H_{15}ClN_2O_4S$
Mol. Wt. 354.81

Figure 1. Chemical Structure of Triamterene (a) and Xipamide (b).

Materials

Pure standards

Triamterene and xipamide were kindly supplied by Egyptian Int. Pharmaceutical Industries Co. E.I.P.I. Co. (10th of Ramadan City, Egypt). Their purity was found to be 100.21% and 100.07%, respectively, according to the reported high performance liquid chromatography (HPLC) method.[20]

Pharmaceutical dosage form

Epitens® tablets (Batches No. 078 125, 088 004, 076 677) labelled to contain 30 mg of triamterene and 10 mg of xipamide, manufactured by Egyptian Int. Pharmaceutical Industries Co. E.I.P.I. Co. (10th of Ramadan City, Egypt).

Chemicals and reagents

All chemicals used throughout this work were of analytical grade, and the solvents were of spectroscopic grade. Chloroform and 33% ammonia solution (El-Nasr Pharmaceutical Chemicals Co., Abu-Zabaal, Cairo, Egypt); methanol of HPLC grade (CHROMASOLV®, Sigma-Aldrich Chemie GmbH, Germany).

Standard solutions

Stock standard solutions of TRM and XIP (1 mg mL^{-1}): 0.1 gm of TRM and XIP were accurately weighed into two separate 100-mL volumetric flasks; 50 mL of methanol was added to each flask, shaken to dissolve and then the volume was completed to the mark with methanol. Working standard solutions of TRM and XIP (100 µg mL^{-1}): 10 mL of each of TRM and XIP stock standard solutions (1 mg mL^{-1}) was transferred accurately into two separate 100-mL volumetric flasks; then the volume was completed to the mark with methanol.

Laboratory prepared mixtures

Mixtures containing different ratios of TRM and XIP were prepared using their respective working solutions (100 µg mL^{-1} in methanol).

Procedures

Isoabsorptive point spectrophotometric method

Into two separate sets of 10-mL volumetric flasks, different aliquots containing 20–120 µg and 20–100 µg of TRM and XIP, respectively, were accurately transferred from their working solutions; the volume was then completed with methanol. The zero order absorbance of each set was recorded and the absorbencies at $\lambda_{225.2}$ nm (Aiso) for XIP and at λ_{max} 367.8 nm for TRM were measured. The calibration curves relating the absorbance of each curve at the selected wavelength to the corresponding drug concentrations were constructed and the regression equation corresponding to each calibration curve was calculated.

The absorbance of mixtures containing different ratios of TRM and XIP were measured at λ_{max} 367.8 nm corresponding to the concentration of TRM alone and at $\lambda_{225.2}$ nm (Aiso) corresponding to total concentration of TRM and XIP in the mixture. Both the total concentration of TRM and XIP in the mixture and the concentration of TRM alone were calculated using respective regression equations. Then, XIP concentration in the mixture was calculated by subtracting TRM concentration from the total concentration.

Ratio subtraction spectrophotometric method

Different aliquots of XIP containing 20–100 µg were accurately transferred into a set of 10-mL volumetric flasks from its working solution and the volume was completed with methanol. The spectra were divided by the spectrum of 10 µg mL^{-1} of TRM (as a divisor). The absorbencies in the plateau at λ above 367 nm were subtracted from the corresponding spectra of the mixture and then the produced spectra were multiplied by the spectrum of the divisor. XIP concentration was determined at its λ_{max} 230.8 nm using its corresponding regression equation while TRM was measured at its λ_{max} 367.8 nm in the zero-order spectrum of the mixture and its concentration determined from its corresponding regression equation.

Chemometric methods

Construction of the training set

Multilevel multifactor design was used for the construction of the calibration and validation sets.[25] A five-level, five-factor calibration design was used. Different mixtures of TRM and XIP in different ratios were prepared (Table 1). The absorption spectra of the prepared mixtures were recorded and transferred to Matlab® for subsequent data manipulation.

Fifteen mixtures were used for building the calibration model, while ten mixtures were chosen to be used as an external validation set. Several multivariate calibration models (CLS, PCR, and PLS) were constructed using the data obtained. Initial developed models were found to have high spectral residuals in the region below 230 and above 285 nm; as a result, this region was rejected. For CLS method, construct CLS model with non-zero intercept.

Selection of the optimum number of factors to build the PCR and PLS models

The cross validation method was used, leaving out one sample at a time, to select the optimum number of factors.[26] Given a set of 15 calibration samples, the PCR and PLS calibrations were performed, and using this calibration, the concentration of the sample left out

Table 1. The concentration of mixtures of xipamide and triamterene used in the training and validation sets

Mixture No.	Xipamide (μg mL^{-1})	Triamterene (μg mL^{-1})
1	4	4
2	6	4
3	4	3
4	3	3
5	3	5
6	5	6
7	6	5
8	5	4
9	4	6
10	6	6
11	6	2
12	2	5
13	5	2
14	2	4
15	4	5
16	5	5
17	5	3
18	3	2
19	2	3
20	3	4
21	4	2
22	2	2
23	2	6
24	6	3
25	3	6

- The concentrations of mixtures used in the validation set are highlighted.

was predicted. The predicted concentrations were then compared with the actual concentrations and the root mean square error of cross validation (RMSECV) was calculated. The RMSECV was calculated in the same manner each time a new factor was added to the model. The maximum number of factors used to calculate the optimum RMSECV was selected to be 8 (half the number of samples + 1).[27] Visual inspection was used for selecting the optimum number of factors. Upon building the models autoscale, the data gave better results for both PCR and PLS.

Construction of the validation set

Ten different mixtures of TRM and XIP were prepared by transferring different volumes of their working standard solutions their concentrations given in Table 1. The developed models were applied to predict the concentration of TRM and XIP in each mixture.

Spectrodensitometric method

Into a set of 10-mL volumetric flasks, different aliquots of TRM and XIP were accurately transferred from their working solutions; the volume was then completed with methanol. 10 µL of each solution was spotted as bands of 6 mm width on TLC plates (20 × 10 cm with 250 µm thickness) using a Camag Linomat IV applicator. The bands were applied at 5 mm intervals and 10 mm from the bottom and sides. Linear ascending chromatogram developing to a distance of 8 cm was performed in a chromatographic tank previously saturated for 1 h with the developing mobile phase consisted of chloroform–methanol–ammonia solution (8 : 2 : 0.2, by volume) at room temperature. The peak areas were recorded using scanning wavelength at 254 nm and the calibration curves were constructed by plotting the integrated peak area versus the corresponding concentrations of each drug and the regression equations were computed.

Application to pharmaceutical formulation (Epitens® tablets)

The contents of 20 Epitens® tablets were powdered and mixed well. An accurately weighed portion of the powdered tablet equivalent to 75 mg of TRM and 15 mg of XIP was transferred into 100-mL volumetric flask; 75 mL methanol was added and sonicated for 30 min, filtered, and then completed to volume with methanol. The solution was diluted to obtain 100 µg mL^{-1} working solution using methanol as a solvent. The procedure of each method was followed and the concentration of TRM and XIP was calculated from the corresponding regression equation.

Results and Discussion

The main task of this work was to develop simple, sensitive and accurate analytical methods for the determination of TRM and XIP in their binary mixture either in bulk powder and pharmaceutical formulation with satisfactory precision for good analytical practice (GAP).

Isoabsorptive point spectrophotometric method

In this work, the so-called 'isoabsorptive spectrophotometry', developed by Erram and Tipnis,[28–30] is applied for simultaneous determination of TRM and XIP in their binary mixture.

The theory of this method could be confirmed experimentally by recording the absorbance spectra of 8 µg mL^{-1} of TRM and XIP separately, and that of a mixture containing equal concentration of TRM and XIP (4 µg mL^{-1} of each of TRM and XIP), as shown in Figure 2.

In Figure 2, it can be seen that the mixture and the pure drugs have different absorbance spectra; meanwhile they possess the same absorbance at their isoabsorptive point. These data permits us to conclude, according to the isoabsorptive spectrophotometry theory, that the mixture of both drugs acts as a single component and gives the same absorbance value as pure drugs at their isoabsorptive points. Thus, by measuring the absorbance value at the chosen isoabsorptive point, the total concentration of the mixture could be calculated as explained by the theory.[28–30] By applying the suggested procedure the absorbance at $\lambda_{225.2}$ nm (Aiso) for XIP was obtained over different ranges, while the concentration of TRM in TRM and XIP mixture could be calculated at its λ_{max} 367.8 nm without any interference from XIP. Thus the concentration of XIP could be calculated by subtraction.

Linear correlations were obtained between absorbance at 367.8 nm for TRM and its concentration in the range of 2–12 µg mL^{-1} and at 225.2 nm for XIP and its concentration in the range of 2–10 µg mL^{-1} from which the regression equations were calculated and found to be:-

For TRM at 367.8 nm, $A_1 = 0.0782\,C_1 + 0.0051$ $r_1 = 0.9998$

For XIP at 225.2 nm, $A_2 = 0.1175\,C_2 + 0.0282$ $r_2 = 0.9999$

Where A_1 and A_2 are the absorbance of TRM and XIP, respectively, C_1 and C_2 are the concentration of TRM and XIP in

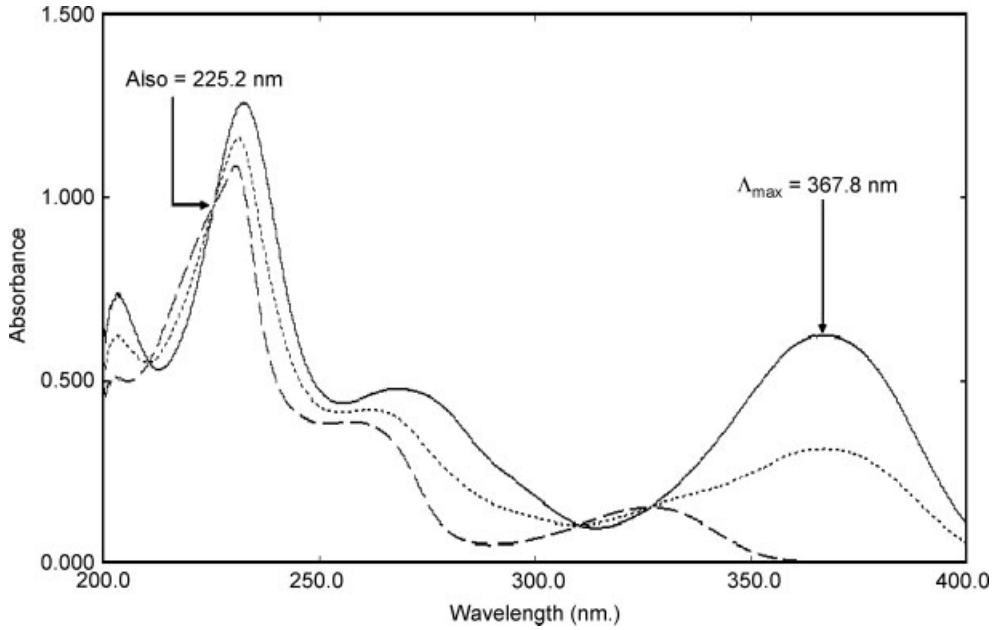


Figure 2. Zero order absorption spectra of 8 μg mL^{-1} of Triamterene (———), 8 μg mL^{-1} of Xipamide (- - - -) and a (1 : 1) mixture containing 4 μg mL^{-1} of each of Triamterene and Xipamide (· · · ·) using methanol as a blank.

Table 2. Determination of triamterene and xipamide in laboratory prepared mixtures by the proposed isoabsorptive and ratio subtraction spectrophotometric methods

		Recovery % *		
			XIP by	
Mixture ratio TRM : XIP	Concentration (μg mL^{-1})	TRM at 367.8 nm	Isoabsorptive at 225.2 nm	Ratio subtraction at 230.8 nm
3 : 1**	9 : 3	100.1	102.0	100.0
2 : 1	8 : 4	99.5	98.5	99.0
1 : 3	2 : 6	99.5	101.5	101.5
1 : 2	4 : 8	101.5	100.0	100.3
1 : 1	5 : 5	100.4	100.2	100.3
4 : 1	8 : 2	100.5	99.5	99.8
Mean ± SD		100.3 ± 0.75	100.3 ± 1.29	100.2 ± 0.82

* Average of 3 determinations.
** The ratio of pharmaceutical formulation.

μg mL^{-1}, respectively, and r_1 and r_2 are the correlation coefficients of TRM and XIP, respectively.

Results given in Table 2 show that the method is valid for determination of TRM and XIP in different laboratory prepared mixtures.

Ratio subtraction spectrophotometric method

First, the linearity of XIP was determined in the concentration range 2–10 μg mL^{-1} at 230.8 nm in the zero order spectra. Different divisor concentrations (2, 5, 7 and 10 μg mL^{-1}) were tried. The divisor concentration 10 μg mL^{-1} of TRM was found to be the best regarding accuracy and precision when the method was used for calculation of XIP concentration in its laboratory prepared mixtures.

Second, the spectrum of the mixture of XIP and TRM in methanol was divided by the spectrum of the divisor (10 μg mL^{-1} of TRM). The value of the absorbance in the plateau region at λ above 367 nm was subtracted from the spectrum of the divided mixture; the obtained spectrum was then multiplied by the spectrum of the divisor as shown in Figure 3. Finally, XIP concentration was measured from the last spectrum obtained at 230.8 nm, while TRM concentration was determined from zero-order spectrum at its λ_{max} 367.8 nm.

Linear correlation was obtained between absorbance of XIP at 230.8 nm and its concentration in the range 2–10 μg mL^{-1} from which the regression equation was calculated and found to be:

$$\text{For XIP at 230.8 nm,} \quad A = 0.1281\,C + 0.0573 \quad r = 0.9998$$

where A is the absorbance, C is the concentration in μg mL^{-1}, and r is the correlation coefficient.

The proposed method was successfully applied for the determination of XIP in laboratory prepared mixtures containing different ratios of XIP and TRM as given in Table 2.

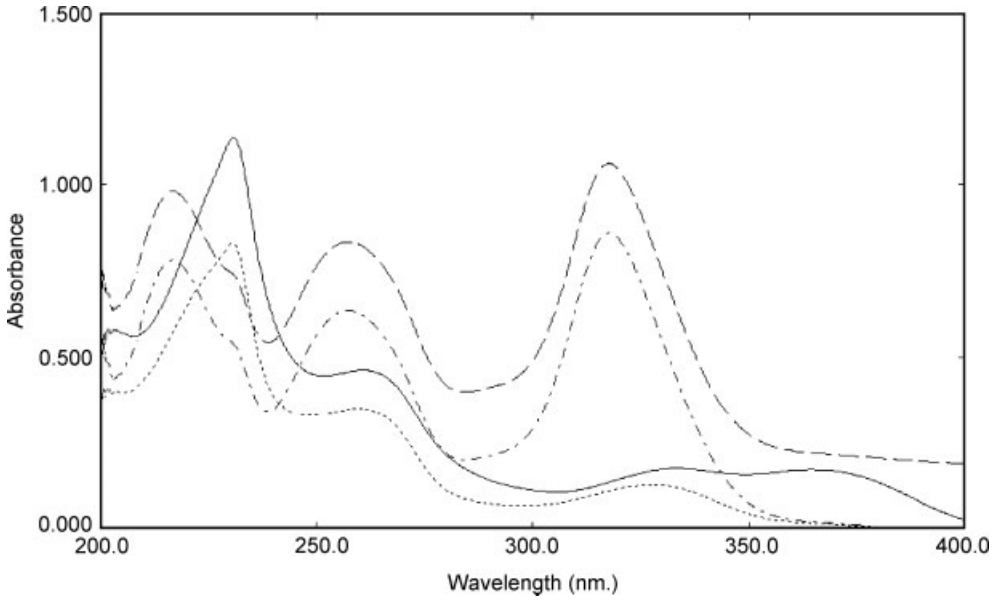


Figure 3. Absorption spectra of Xipamide and Triamterene mixture (———), mixture after division by the divisor (- - - -), mixture after subtraction of the constant (·¯·¯·¯·) and mixture after multiplication by the divisor (· · · ·) using methanol as a blank.

Table 3. Results of determination of xipamide and triamterene in the validation set using the proposed multivariate method

	Recovery % *					
	Xipamide			Triamterene		
Mixture No.	CLS	PCR	PLS	CLS	PCR	PLS
1	99.5	100.0	100.0	99.0	98.3	98.3
2	99.3	99.7	99.7	100.0	99.7	99.7
3	100.4	100.2	100.2	99.2	99.8	99.8
4	100.3	100.5	100.5	98.7	99.8	99.8
5	100.8	101.3	101.3	98.8	99.6	99.6
6	101.8	99.8	99.8	97.4	97.6	97.6
7	100.2	100.0	100.0	98.3	99.0	99.0
8	100.3	99.7	99.7	103.0	100.5	100.5
9	101.3	100.7	100.7	98.5	99.8	99.8
10	100.5	100.5	100.5	101.0	99.5	99.5
Mean ± SD	100.4 ± 0.75	100.2 ± 0.51	100.2 ± 0.51	99.4 ± 1.60	99.4 ± 0.84	99.4 ± 0.84

* Average of 3 determinations.

Chemometric methods

In this method, different chemometric approaches were applied for the determination of XIP and TRM, including CLS, PCR and PLS. These multivariate calibrations were useful in spectral analysis because the simultaneous inclusion of many spectral wavelengths instead of a single wavelength greatly improved the precision and predictive ability.[31]

The first step in the simultaneous determination of the components by multivariate calibration methods involves constructing the calibration matrix for binary mixture. The calibration set was obtained by using the absorption spectra of a set of 15 mixtures of XIP and TRM with different ratios of each component as given in Table 1. Better results were obtained upon rejecting the spectral region above 285 nm and below 230 nm.

In this study, the 'leave one out' cross validation method was used and the RMSECV values of different developed models were compared. Four factors were found suitable for both PCR and PLS models. To validate the prediction ability of the suggested models, the validation set given in Table 1 was used to predict the concentration of TRM and XIP, where satisfactory results were obtained as shown in Table 3.

The predicted concentrations of the validation samples were plotted against the true concentration values. This was used to determine whether the model accounted for the concentration variation in the validation set. All plots had a slope of nearly one and an intercept close to zero.

The RMSEP was another diagnostic tool for examining the errors in the predicted concentrations; it indicates both the precision and accuracy.[26] The RMSEP values were 0.26, 0.20 and 0.21 for TRM and 0.21, 0.33 and 0.35 for XIP indicating the high predictive abilities of the three models.

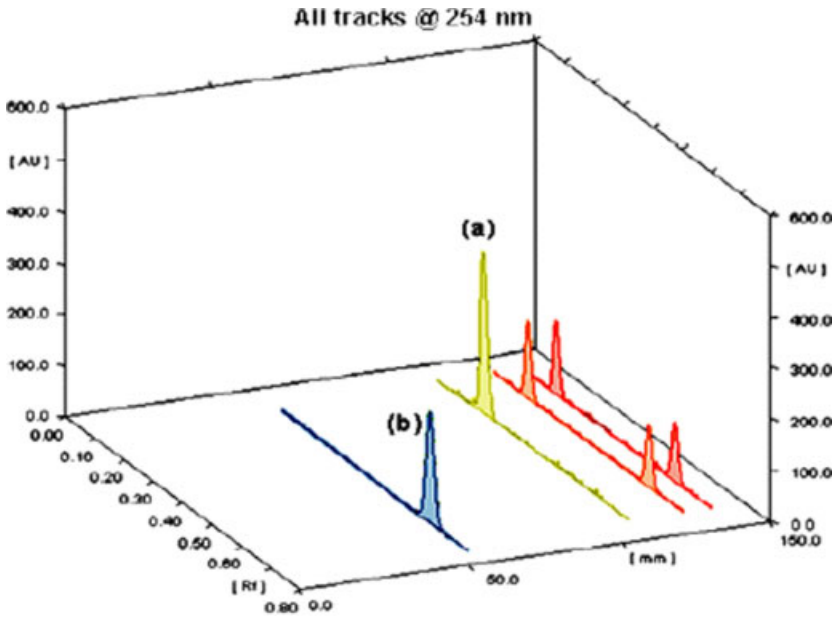


Figure 4. Thin layer chromatogram of separated peaks of Xipamide (a) and Triamterene (b) using chloroform: methanol: ammonia (8: 2: 0.2, by volume) as a mobile phase.

Table 4. Determination of xipamide and triamterene in pharmaceutical formulation by the proposed methods

Pharmaceutical formulation	Batch No.	Component	Found %* ± SD					
			Isoabsorptive	Ratio subtraction	Multivariate CLS	Multivariate PCR	Multivariate PLS	Spectrodensitometry
Epitens® tablets claimed to contain 30 mg TRM and 10 mg XIP/tablet	078125	XIP	100.5 ± 1.23	100.8 ± 1.32	99.5 ± 1.26	100.3 ± 1.23	100.2 ± 1.23	100.6 ± 1.53
		TRM	100.8 ± 1.20		100.5 ± 1.29	100.78 ± 1.21	100.8 ± 1.22	100.9 ± 1.45
	088004	XIP	102.3 ± 1.18	101.9 ± 1.23	102.3 ± 1.31	101.9 ± 1.28	101.9 ± 1.29	101.5 ± 1.35
		TRM	101.1 ± 1.02		100.9 ± 1.31	100.8 ± 1.28	100.8 ± 1.28	100.9 ± 1.41
	076677	XIP	101.8 ± 1.31	102.1 ± 1.21	100.9 ± 1.29	101.1 ± 1.21	101.1 ± 1.22	100.8 ± 1.41
		TRM	99.9 ± 1.28		99.50 ± 1.31	99.7 ± 1.28	99.7 ± 1.28	100.1 ± 1.39

* Average of 6 determinations.

TLC-densitometric method

TLC-densitometry is a useful technique for the resolution and, in turn, for the determination of drug mixtures. This technique offers a simple way to quantify directly on TLC plate by measuring the optical density of the separated bands. The amounts of compounds are determined by comparing to a standard curve from reference materials chromatographed simultaneously under the same condition.[32]

To improve separation of bands, it was necessary to investigate the effect of different variables. Studying the optimum parameters for maximum separation was carried out as following:

Mobile phase

Different developing systems of different composition and ratios were tried for separation, e.g., chloroform–ethyl acetate (7 : 3, v/v), chloroform–acetone (8 : 2, v/v), chloroform–methanol (8 : 2, v/v) and chloroform–methanol–acetic acid (8 : 2:0.2, by volume). The best mobile phase was chloroform–methanol–ammonia (8 : 2:0.2, by volume). This selected mobile phase allows good separation between the binary mixture with good R_f values without tailing of the separated bands (Figure 4).

Band dimensions

Different band dimensions were tested in order to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 6 mm and the inter-space between bands was 5 mm.

Scanning wavelength

Different scanning wavelengths were tried, where 254 nm was the best wavelength for both drugs at which peaks were sharper and symmetrical and minimum noise was obtained; at this wavelength maximum sensitivity for both drugs was obtained.

Slit dimensions of scanning light beam

The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track

Table 5. Application of standard addition technique for the determination of xipamide and triamterene in pharmaceutical formulation

Pharmaceutical formulation	Method			Standard addition technique	
				Dosage taken (μg mL^{-1})	Mean ± SD
Epitens® tablets claimed to contain 30 mg TRM and 10 mg XIP/tablet (Batch No. 078125)	Isoabsorptive method		XIP	3	99.5 ± 0.57
			TRM	3	99.7 ± 0.91
	Ratio subtraction method		XIP	3	99.4 ± 1.03
			TRM	3	99.7 ± 0.91
	Multivariate analysis	CLS	XIP	3	100.0 ± 1.32
			TRM	2	99.2 ± 1.79
		PCR	XIP	3	100.7 ± 1.34
			TRM	2	99.6 ± 0.63
		PLS	XIP	3	101.0 ± 0.87
			TRM	2	99.4 ± 1.38
	Spectrodensitometric method		XIP	0.4	100.2 ± 0.74
			TRM	0.3	100.5 ± 1.94

* Average of 3 determinations.

Table 6. Results of assay validation parameters of the proposed methods for the determination of xipamide and triamterene

Parameters	TRM at 367.8 nm	XIP		TLC-Densitometry	
		Isoabsorptive at 225.2 nm	Ratio subtraction at 230.8 nm	XIP	TRM
Range	2–12 μg mL^{-1}	2–10 μg mL^{-1}		0.3–1 μg band^{-1}	0.2–1 μg band^{-1}
Linearity					
Slope	0.0782	0.1175	0.1284	6880.4110	3405.4390
Intercept	0.0051	0.0282	0.0560	1540.1640	373.1579
Correlation coefficient (r)	0.9998	0.9999	0.9998	0.9997	0.9999
Standard error of the slope	0.0006	0.0006	0.0009	73.5590	23.6737
Confidence limit of the slope	0.0769–0.0795	0.1161–0.1189	0.1261–0.1306	6691.3220–7069.5001	3347.5110–3463.3660
Standard error of the intercept	0.0044	0.0039	0.0061	50.5826	16.2083
Confidence limit of the intercept	−0.0049–0.0150	0.0191–0.0373	0.0415–0.0705	1410.1380–1670.1910	333.4977–412.8181
Accuracy (mean ± SD)	100.1 ± 1.09	100.0 ± 0.91	99.9 ± 1.12	99.8 ± 1.26	99.9 ± 0.81
Selectivity	100.3 ± 0.75	100.3 ± 1.29	100.2 ± 0.82	–	–
Precision (RSD %)					
Repeatability*	0.754	0.588	0.509	0.882	0.865
Intermediate precision*	0.812	0.671	0.705	0.975	1.089
LOD**	0.81 μg mL^{-1}	0.76 μg mL^{-1}	0.77 μg mL^{-1}	0. μg band^{-1}	0.08 μg band^{-1}
LOQ**	1.52 μg mL^{-1}	1.73 μg mL^{-1}	1.80 μg mL^{-1}	0.21 μg band^{-1}	0.16 μg band^{-1}

* The intra-day precision (n = 3), average of three different concentrations repeated three times within day. The inter-day precision (n = 3), average of three different concentrations repeated three times in three successive days.
** Limits of detection and quantitation are determined experimentally for TLC-densitometric method and via calculations[33] for other methods. LOD = (SD of the response/slope) × 3.3; LOQ = (SD of the response/slope) × 10.

without interference of adjacent bands. Different slit dimensions were tried, where 5 mm × 0.2 mm proved to be the slit dimension of choice which provides highest sensitivity.

System suitability

System suitability testing of TLC-densitometric method gave good resolution (R_s = 11), selectivity factor (α = 3.2), capacity factor (K′ for XIP = 0.78, for TRM = 0.36) and symmetry factor of 1 for XIP and 1.04 for TRM.

This method is based on the difference in the R_f values of TRM (R_f = 0.64) and XIP (R_f = 0.22), as shown in Figure 4.

The calibration curves were constructed by plotting the integrated peak area versus the corresponding concentrations in the range of 0.2–1 μg band^{-1} for TRM, and in the range 0.3–1 μg band^{-1} for XIP. The concentration of TRM and XIP were calculated from the following regression equations.

$$\text{For TRM,} \quad Y_1 = 3405.4390\,C_1 + 373.1579 \quad r_1 = 0.9999 \quad (1)$$

$$\text{For XIP,} \quad Y_2 = 6880.4110\,C_2 + 1540.1640 \quad r_2 = 0.9997 \quad (2)$$

Where Y_1 and Y_2 are the integrated peak area of TRM and XIP, respectively, C_1 and C_2 are the concentration of TRM and XIP in μg

Table 7. Statistical comparison of the results obtained by the proposed methods and the established method for the determination of pure xipamide and triamterene

	Xipamide						Established method*[20]		Triamterene				
			Multivariate methods							Multivariate methods			
Items	Isoabsorptive method	Ratio subtraction method	CLS	PCR	PLS	TLC-Densito-metric method	XIP	TRM	Spectrophotometric method at 367.8 nm	CLS	PCR	PLS	TLC-Densito-metric method
Mean	100.0	99.9	100.4	100.4	100.4	99.8	100.1	100.2	100.1	99.4	99.4	99.4	99.9
SD	0.91	1.12	0.74	0.73	0.73	1.26	1.04	0.98	1.09	1.60	0.84	0.86	0.81
RSD %	0.91	1.12	0.74	0.73	0.73	1.26	1.04	0.98	1.09	1.60	0.85	0.86	0.81
n	9	9	10	10	10	7	6	6	11	10	10	10	8
Variance	0.82	1.25	0.55	0.53	0.53	1.58	1.09	0.96	1.18	2.55	0.71	0.73	0.65
Student's t-test	0.180 (2.160)	0.298 (2.160)	0.829 (2.145)	0.632 (2.145)	0.632 (2.145)	0.360 (2.201)	–		0.214 (2.131)	1.137 (2.145)	1.848 (2.145)	1.774 (2.145)	0.631 (2.179)
F-value	1.32 (3.69)	1.15 (4.82)	1.97 (3.48)	2.03 (3.48)	2.03 (3.48)	1.45 (4.95)	–		1.23 (4.74)	2.65 (4.77)	1.36 (3.48)	1.31 (3.48)	1.48(3.97)

* RP-HPLC method using 0.2 mM ammonium acetate: acetonitrile (95 : 5, v/v) and flow rate 1 mL min^{-1} and detection at 254 nm.
- Figures between parenthesis represent the corresponding tabulated values of t and F at P = 0.05.

band^{-1}, respectively, and r_1 and r_2 are the correlation coefficients of TRM and XIP, respectively.

Results given in Table 4 show that the suggested methods are valid and applicable for the analysis of TRM and XIP in their pharmaceutical formulation (Epitens® tablets) with an acceptable percentage recovery. Furthermore, the validity of the proposed method was assessed by applying the standard addition technique, which showed accurate results and there was no interference from tablet excipients as shown in Table 5.

Method Validation

Method validation was performed according to USP guidelines[33] for the proposed methods. Table 6 shows results of accuracy, repeatability and intermediate precision of the methods. Other regression equation parameters in Table 6 show good linear relationship for the method as revealed by the correlation coefficient. Descriptive statistics of the regression showed low values of standard error of intercept and slope which revealed high accuracy with minimum deviations and low scattering of the calibration points.[34]

Table 7 shows statistical comparison of the results obtained by the proposed methods and the established HPLC method[20] for the determination of XIP and TRM. The calculated *t* and F-values are less than the theoretical ones indicating that there is no significant difference between the proposed methods and the reported method with respect to accuracy and precision.

Conclusion

The present work provides new, different and selective analytical techniques for the determination of TRM and XIP in their binary mixture either in bulk powder or in pharmaceutical formulation. Reviewing the literature in hand, the other published HPLC and GC methods are expensive, time-consuming and require complicated instruments, while this work has the advantage of being simpler and easier as in isoabsorptive and ratio subtraction methods and more sensitive as in spectrodensitometric method.

The spectrophotometric methods can be regarded as a useful alternative to chromatographic techniques in the routine quality control analysis of pharmaceutical formulations, allowing qualitative and quantitative measurements to be simultaneous and rapid at relatively low costs. The disadvantage of spectrophotometric methods is its low sensitivity.

The advantages of TLC-densitometric method is that several samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowering analysis time and cost per analysis and providing high sensitivity and selectivity. The disadvantage of TLC-densitometric method is that it requires a complicated instrument.

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