International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 5, 2016

Original Article

DEVELOPMENT AND VALIDATION OF A STABILITY-INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF XIPAMIDE IN PURE AND DOSAGE FORMS

HEBA M. EL-SAYED*[a], SOAD S. ABD EL-HAY[a,b]

[a]Analytical Chemistry Department, Faculty of Pharmacy, Zagazig University, Zagazig 44519,Egypt, [b]Pharmaceutical Chemistry Department, Faculty of Pharmacy, King Abdul-Aziz University, Saudi Arabia
Email: heba328@yahoo.com

Received: 25 Feb 2016 Revised and Accepted: 08 Apr 2016

ABSTRACT

Objective: A simple, selective, precise and stability-indicating RP-HPLC-method was developed and validated for the determination of xipamide (XIP).

Methods: Stability tests were done through exposure of the analyte solution to thermal, photolytic, hydrolytic and oxidative stress conditions. The chromatographic separation was carried out in less than five min on a RP stainless-steel C-18 analytical column (150 mm ×4.6 mm ID, 5 µm) with an isocratic elution system of 0.023 M orthophosphoric acid of pH 2.6 and acetonitrile as the mobile phase in the ratio of 60: 40 at 1.5 ml/min flow rate at room temperature. A diode array UV was used at 220 nm for detection.

Results: The degradation products were well separated from the pure drug. The elution time of XIP was found to be 4.561±0.024 min. The method was validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness. Good linearity was found in the concentration range of 1–100 µg/ml with a correlation coefficient of 0.9999. Intraday and interday precision were within 1.4%. LOD and LOQ were 0.088 µg/ml and 0.267 µg/ml, respectively and percentage recovery of XIP was found to be 99.92±1.02 %.

Conclusion: The proposed method was successfully applied to the determination of XIP in pure form and in its pharmaceutical preparation without interference from its degradation products.

Keywords: Xipamide, Stability indicating RP-HPLC, Stress degradation, Pure form, Dosage forms

INTRODUCTION

XIP is 4-chloro-2, 6-dimethyl-5-sulfamoylsalicylanilide [1]. It has a moderately powerful diuretic action and is used for the treatment of high blood pressure and edema [2]. 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 XIP is shown in fig. 1.

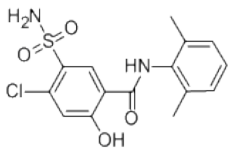

Fig. 1: Chemical structure of XIP

Despite its wide use, few procedures have been developed for its determination. These include spectrophotometry [4-6], spectro-fluorimetry [6], TLC-densitometry [5] and HPLC [7-12]. However, there is no reported LC method for stability determination of XIP, so the development of a simple, reproducible and selective analytical HPLC method would greatly aid in XIP stability determination either in pure or dosage forms.

The present manuscript describes the degradation behavior of XIP under acidic, basic, oxidative and photolytic conditions. Optimization of LC conditions to separate the drug from its degradation products on a RP C18 column in less than 5 min and method validation were also achieved.

MATERIALS AND METHODS

Chemicals and reagents

Pharmaceutical grade XIP (99.09%) was kindly provided by EIPICO Pharmaceutical Industries Company, Cairo, Egypt. Analytical reagent grade chemicals were used in all experiments. HPLC grade methanol, acetonitrile (TEDEA, Fairfield, USA). HCl, NaOH and H_2O_2 (Merck, USA), orthophosphoric acid 85% (Burdick & Jackson, USA) were used. Fresh double distilled water was used throughout the analysis. The pharmaceutical preparation used was Epitens® (EIPICO, Egypt, 10 mg and 30 mg triamterene/tablet).

Instrumentation and chromatographic conditions

HPLC apparatus (Agilent 1100) consisted of quaternary pump G1310A with solvent cabinet quaternary, vacuum degasser G1322A and a four-channel gradient pump; autosampler G1313A, variable wavelength detector (VWD) G1314A with the standard flow cell 10 mm path length, 14 µl volume, 40 bar maximum pressure. LC separations were performed on an Agilent 5 µm Hypersil BDS C18 column (150 mm x 4.6 mm ID). pH meter (Metrohm, USA) was used.

For the determination of XIP and its degradation products, an isocratic mobile phase consisting of 0.023 M orthophosphoric acid of pH 2.6 and acetonitrile as the mobile phase in the ratio of 60: 40 was used, 1.5 ml/min flow rate at room temperature. pH is adjusted using H_3PO_4 and NaOH. 10 µl of sample was injected each run. Detection was achieved at 220 nm.

Preparation of stock solutions

Standard stock solution of XIP (0.1 mg/ml) was prepared by dissolving 10 mg of the drug in methanol, sonicated and completed to 100 ml with methanol in a 100 ml volumetric flask. The standard working solutions were prepared by diluting aliquots of the stock solution with methanol to obtain concentrations ranging from 1–140 µg/ml.

Construction of calibration curves

The calibration graph was constructed by plotting the peak areas obtained at wavelength 220 nm against the corresponding injected concentrations of XIP.

Assay of tablets

A total of 10 tablets of Epitens® were finely powdered. An accurately weighed quantity of the powder equivalent to 10 mg XIP was

transferred into 100-ml volumetric flask and extracted using 25 ml methanol and then completed to 100 ml with methanol. The flask was shaken for 15 min using an ultrasonic shaker. The solution was then filtered into a dry conical flask. It was serially diluted and then injected. The concentrations of XIP were calculated using calibration equation.

Forced degradation studies of xipamide

The stock solution was used for the forced degradation study to provide an indication of the stability indicating property and specificity of proposed method.

Acid and base-induced degradation

A 1 ml of methanolic stock solution was transferred into 10 ml volumetric flask and the volume was completed with 2 N HCl. Alkaline degradation study was carried out in a similar manner using 0.1 N NaOH. These experiments were left at 80 °C for 2 h.

Hydrogen peroxide-induced degradation

To a 1 ml of methanolic stock solution, 2 ml of 30 % H_2O_2 was added, and the mixture was diluted with water to 10 ml. It was left at 80 °C for 2 h.

Sunlight degradation

The photochemical stability of the drug was also studied by exposing the standard methanolic solution of XIP to sunlight for 4 h.

10 µl of all the above resultant stressed solutions were injected onto the column, and the chromatograms were run as described.

RESULTS AND DISCUSSION

Optimization of HPLC conditions

In order to maximize the resolution and sensitivity of the proposed HPLC method for the separation of XIP and its degradation products, different experimental conditions were studied and optimized. This was performed at a detection wavelength of 220 nm which provided the best resolution.

Type of organic modifier

Isocratic elution using different proportions of acetonitrile in the mobile phase was tried.

Detection was carried out at 220 nm to obtain good peak intensity for both drug and degradation products. Optimum resolution with good separation of the drug and its degradation products was achieved within reasonable run time (Less than 5 min) using isocratic elution system of 0.023 M orthophosphoric acid of pH 2.6 and acetonitrile as the mobile phase in the ratio of 60:40. The retention time of XIP was found to be 4.561±0.024 min (fig. 2). At lower concentration of acetonitrile (35%), XIP peak with its degradation products co-eluted together resulting in tailing and distortion of the peak shape as in the case of alkaline degradation (fig. 3) (A).

pH of the aqueous phase

Various pH values ranging between 2.4 and 5.0 (adjusted using H_3PO_4 and NaOH) were used to study the influence of pH of the aqueous phase on the separation of XIP and its degradation products. It was observed that as the pH value increases, retention time (t_R) of XIP decreases (t_R was 3.587 min at pH 4 and 2.534 min at pH 5). In addition, XIP and its degradation products co-eluted as in case of alkaline degradation at pH 5 (fig. 3) (B). Thus, pH 2.6 was chosen as working pH providing maximum peak resolution and symmetry.

Effect of flow rate

It was noticed that the increase in flow rate does not have a significant effect on the separation, but it clearly shortens the analysis time. Decreasing flow rate to 1.0 ml/min increases the retention time for XIP and its degradation products as for sunlight degradation (fig. 3) (C).

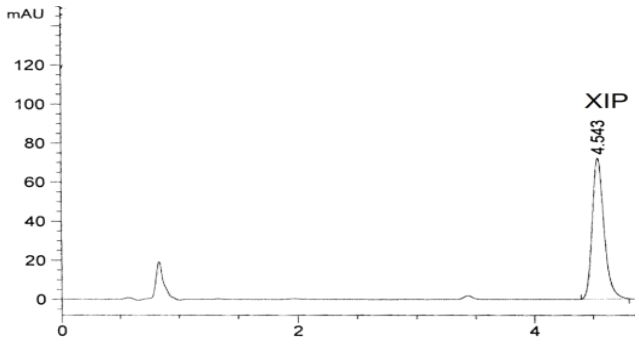

Fig. 2:A typical chromatogram of a 10 µl injection of a standard solution of XIP (90 µg/ml) using 0.023 M orthophosphoric acid (pH 2.6) and acetonitrile as the mobile phase (60: 40) at a flow rate 1.5 ml/min and detection at 220 nm

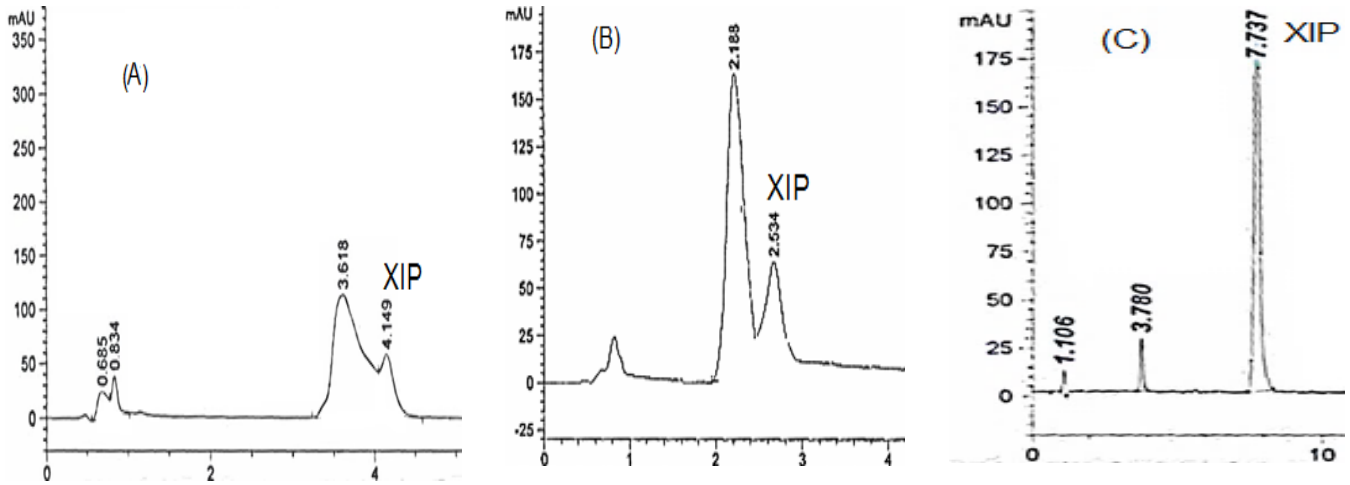

Fig. 3: A typical chromatogram of a 10 µl injection of a standard mixture of XIP (100 µg/ml) using (A) 35% acetonitrile (B) Alkaline degradation at pH 5.0 (C) Sunlight degradation using 35% ACN, flow rate = 1.0 ml/min and the other chromatographic conditions as optimized

Degradation behavior

Acid and base-induced degradation

A previous study [13] confirmed that XIP was stable in acidic as well as in basic media at room temperature, so at a lower temperature no degradation was observed. When XIP was treated with strong acid (2 N HCl) at 80 °C for 2 h, a significant degradation of XIP was obtained with the appearance of an additional peak at 0.690 min (fig. 4) (A). The effect of heat in acidic degradation is weaker than that in alkaline degradation. For alkaline degradation, when XIP was treated with 0.1 N NaOH at 80 °C for 2 h, the nearly complete disappearance of the intact drug occurred with the appearance of additional peaks (fig. 4) (B) and (C).

Hydrogen peroxide-induced degradation

Weak degradation of XIP was observed under oxidative conditions. It was found that treating XIP with 30 % H_2O_2 for 2 h at 80 °C resulted in the appearance of an additional peak at 1.760 min (fig. 4) (D). The oxidative degradation pathway of XIP was expected to be through the oxidation of the phenolic hydroxyl group with the formation of the quinonoid structure as in the case of ethamsylate drug previously reported [14].

Photolytic degradation

A methanolic solution of XIP was somewhat stable to sunlight. After exposure to sunlight for about 4h, a small peak appeared at 0.819 min, so it must be stored in brown and air tight containers (fig. 4) (E).

Although there were several degradation products, there was no interference with the peak of the intact drug, indicating that the method is stability-indicating. In addition, no reported LC method was developed for the determination of XIP stability until now.

Method validation

The developed analytical method was validated by means of linearity, accuracy, precision, LOD, LOQ and robustness according to the International Conference on Harmonization (ICH) guidelines (ICH 2005) [15].

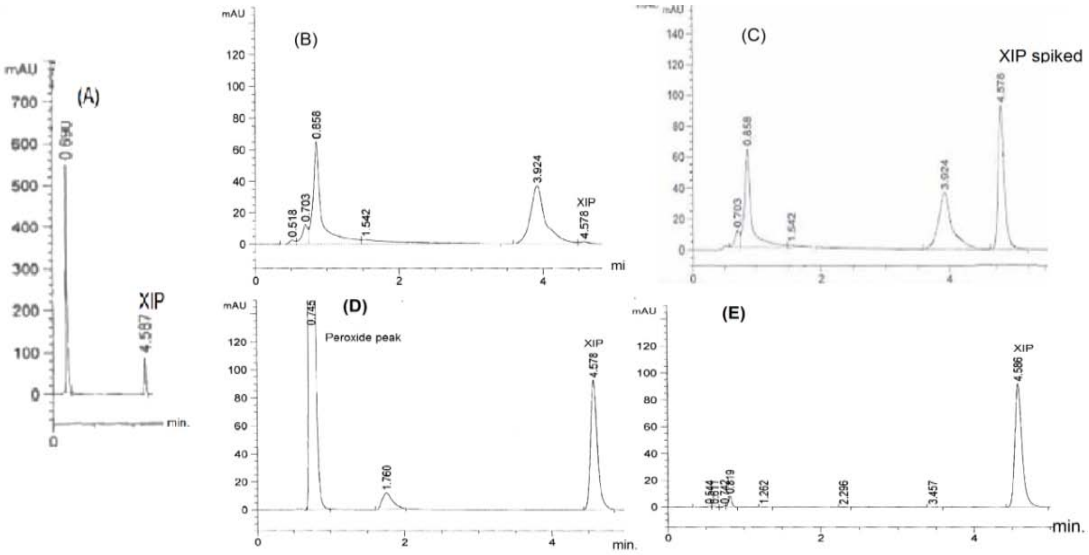

Fig. 4: **HPLC chromatogram of XIP degradation using (A) 2 N HCl (B) 0.1 N NaOH (C) 0.1 N NaOH spiked with 100 µg/ml XIP standard solution (D) 30% H_2O_2 for 2 h at 80 °C. (E) Sunlight exposure for 4 h**

Linearity

Under the optimal experimental chromatographic conditions a good linearity of the calibration curve was obtained between the peak areas of XIP and the concentration range which was verified by the high correlation coefficient as mentioned in table 1.

LOD and LOQ

LOD was determined by evaluating the lowest concentration of XIP that can be readily detected.

LOQ was determined by establishing the lowest concentrations that can be quantified.

The values of LOQ and LOD were calculated according to the following equations:

LOD = 3.3 SD/b

LOQ = 10 SD/b

Where SD is the standard deviation of the intercept of the regression line and b is the slope of the calibration graph.

Specificity and accuracy

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. The comparison between the chromatogram of the raw XIP and that of extracted XIP from Epitens® tablets indicates that the excipients in the formulation did not interfere with the determination of XIP which indicates the specificity of the method (fig. 5).

Table 1: Regression and statistical parameters for the determination of XIP using the proposed HPLC method

System suitability	
t_R±SD (min)	$4.543±2.39×10^{-2}$
N	10402
k´	3.549
Linearity and Regression Data	
Linearity range (µg/ml)	1-100
LOD (µg/ml)	0.088
LOQ (µg/ml)	0.267
Slope (b)*	5.6523
Intercept (a)*	-0.8476
Correlation coefficient (r^2)*	0.9999

* Regression equation for the peak area of XIP against the concentration of XIP.

The accuracy of the proposed method was expressed in terms of % recovery of 5 different concentrations (injected in triplicates). Results show high accuracy with good recoveries of 99.92±1.02 (table 2).

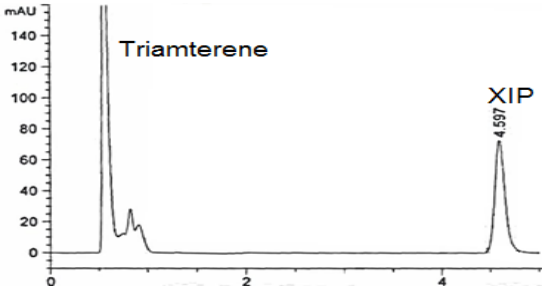

Fig. 5: Application of the proposed HPLC method for the determination of XIP in Epitens® tablets under the optimized chromatographic conditions

In addition, the results obtained using the proposed HPLC method were statistically compared with those obtained with the reported HPLC method [7]. The results in table 3 indicate that the calculated *t* and *F* values are less than the corresponding tabulated ones [16]. This proves that there is no significant difference between the suggested method and reference one, regarding accuracy and precision.

Precision

Results in table 4 show that there were high intraday and interday precisions (both within 1.4%). Intra-day precision was assessed by injection of the standard solution at three concentrations three times during a day. The same was done for interday precision test except that the injection of the samples was every day for three days.

Table 2: Application of the proposed HPLC method for determination of XIP in Epitens® tablets

Taken concentration (µg/ml)	Found concentration (µg/ml)	Recovery (%)*
40.00	40.07	100.17
50.00	50.15	100.29
60.00	59.89	99.82
90.00	90.94	101.04
100.00	98.29	98.29
Mean		99.92
SD		1.02
RSD		1.016

* Average of three experiments

Table 3: Determination of XIP by the proposed HPLC method compared with reported method

Parameter	HPLC method	Reported method
Mean ± SD	99.53 ± 0.90	98.31 ± 1.39
RSD (%)	0.908	1.42
Student-t-test	1.77 (1.78)*	--
F-test	2.38 (3.84)*	--
n	9	5

*$p<0.05$.

Table 4: Intraday and interday precision for the simultaneous determination of XIP using the proposed HPLC method

Added concentration (µg/ml)	Mean* % recovery±SD	RSD (%)	Mean* % recovery±SD	RSD (%)
30	101.19±0.54	0.537	99.82±0.75	0.753
60	101.39±1.40	1.382	99.78±0.24	0.237
90	101.58±0.94	0.922	100.65±0.32	0.322

* Average of three experiments

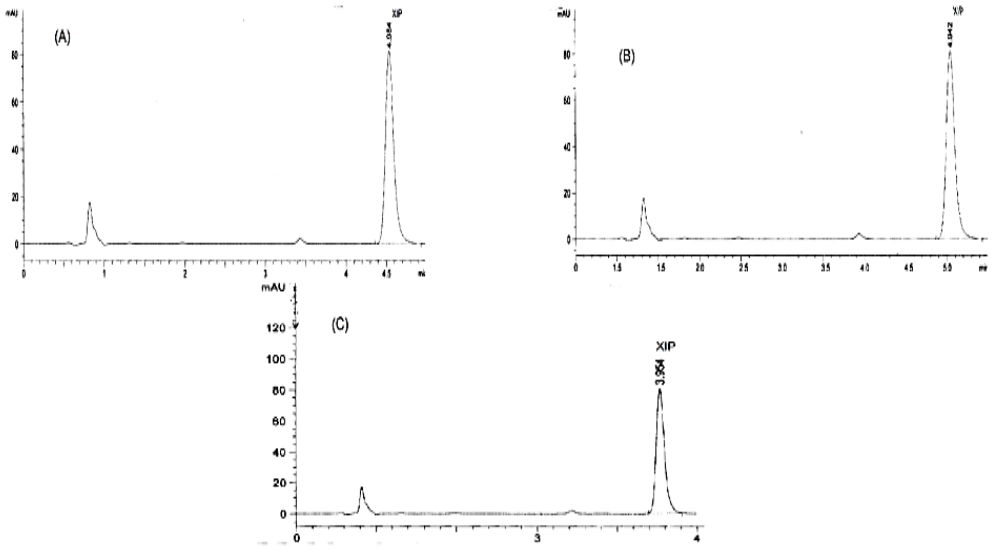

Fig. 6: Robustness of the proposed HPLC method indicated from a typical chromatogram of a 10 µl injection of a standard mixture of 90 µg/ml XIP using (A) pH 2.4 (B) acetonitrile 39% (C) pH 2.8 and the other conditions as optimized

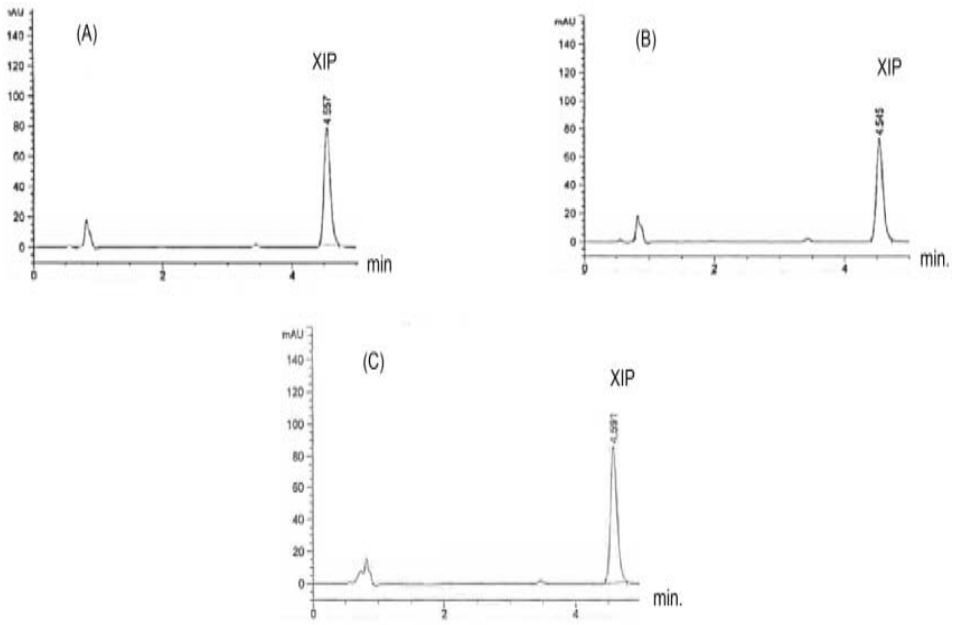

Fig. 7: Robustness of the proposed HPLC method indicated from a typical chromatogram of a 10 µl injection of a standard mixture of 90 µg/ml XIP using (A) Flow rate 1.4 ml/min (B) Detection wavelength 221 nm (C) Detection wavelength 219 nm and the other conditions as optimized

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by changes in pH, flow rate, % of acetonitrile and wavelength of detection. pH was changed from 2.6 to 2.4 and 2.8. The flow rate of the mobile phase was changed from 1.5 ml/min to 1.4 and 1.6 ml/min. The organic strength was varied by±1% and wavelength of detection was changed by ±1 nm. The slight variations in the examined factors had no significant effect on the shape of the peak.

The slight decrease in pH and % of acetonitrile led to increasing in retention time (fig. 6) (A) and (B). In addition, the slight increase in pH and % of acetonitrile led to decrease in retention time with no effect on peak area in both cases (fig. 6) (C) and (D). The small change in flow rate and wavelength of detection had no significant effect on retention time and peak area (fig. 7) (A) and (B) except the peak area that increased in case of increasing flow rate and decreasing wavelength of detection, (fig. 7) (C) and (D).

CONCLUSION

A simple, precise, selective and stability indicating RP-HPLC method was developed and validated for the determination of XIP and its degradation products. The degradation behavior of XIP was studied under acid, alkali, oxidation and photolysis conditions. Short chromatographic run time in less than 5 min allowing the analysis of a large number of samples in a short period of time so the proposed LC method can be used for the quality control of the cited drug.

ACKNOWLEDGEMENT

The authors are grateful to the EIPICO Pharmaceutical Industries Company, Cairo, Egypt for providing a gift sample of bulk drug.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Budavaried S**.** The Merck Index, An Encyclopedia of Chemicals, Drugs *and Biologicals*. 14th ed. Merck and Co, Inc: Whitehouse Station, NJ, USA; 2006.
2. Sweetman SC. Martindale, the complete drug reference. 36th ed. The Pharmaceutical Press; 2009.
3. Rang HP, Date MM, Ritter JM. Pharmacology. 4th ed. Churchill Livingstone: Edinburgh London, New York, Philadelphia; 1999.
4. El Guindi NM, Abd-Elhamid SM, El-Bakry WMM, El-Shahat MF. Quantitative determination of xipamide in pure form and in tablets through complexation with iron (III) chloride. J Drug Res 2010;31:93–9.
5. Wagieh NE, Abbas SS, Abdelkawy M, Abdelrahman MM. Spectrophotometric and spectro- densitometric determination of triamterene and xipamide in pure form and in a pharmaceutical formulation. Drug Test Anal 2010;2:113–21.
6. Omar MA. Spectrophotometric and spectrofluorimetric determination of certain diuretics through ternary complex formation with eosin and lead (II). J Fluoresc 2010;20:275–81.
7. Maher HM, Youssef RM, El-Kimary EI, Hassan EM, Barary MA. Bioavailability study of triamterene and xipamide using urinary pharmacokinetic data following single oral dose of each drug or their combination. J Pharm Biomed Anal 2012;61:78–85.
8. Ruiz-Angel MJ, Torres-Lapasio JR, Garcia-Alvarez-Coque MC. Effects of pH and the presence of micelles on the resolution of diuretics by reversed-phase liquid chromatography. J Chromatogr A 2004;1022:51–65.
9. Rosado-Maria A, Gasco-Lopez AI, Santos-Montes A, Izquierdo-Hornillos R. High-performance liquid chromatographic separation of a complex mixture of diuretics using a micellar mobile phase of sodium dodecyl sulfate. Application to human urine samples. J Chromatogr B: Biomed Sci Appl 2000;748: 415–24.
10. Legorburu MJ, Alonso RM, Jimenez RM. Determination of the non-thiazide diuretic xipamide in pharmaceuticals and urine by HPLC with amperometric detection. J Liq Chromatogr Relat Technol 1999;22:735–46.
11. Bonet-Domingo E, Torres-Lapasio JR, Medina-Hernandez MJ, Garcia-Alvarez-Coque MC. Chromatographic monitoring of diuretics in urine samples using a sodium dodecyl sulfate-propanol micellar eluent. Anal Chim Acta 1994;287:201–10.
12. Bodenan S, Paillet M, Christen MO. Rapid determination of xipamide in human plasma and urine by high-performance liquid chromatography. J Chromatogr B: Biomed Sci Appl 1990;533:275-81.
13. Hempelmann FW. Studies on Xipamide (4-chloro-5-sulfamoyl-2', 6'-salicyloxylidide). Part 1:Physico-chemical and chemical properties. Arzneimittelforschung 1977;27:2140–3.

14. Belal F, El-Brashy A, El-Enany N, Tolba M. Conventional and first derivative synchronous fluorometric determination of ethamsylate in pharmaceutical preparations and biological fluids. Application to stability studies. J Fluoresc 2011;21:1371–84.
15. ICH guidelines for validation of analytical procedures: text and methodology. Q2 (R1) ICH, Geneva; 2005.
16. Miller, JN, Miller, JC. Statistics and chemometrics for analytical chemistry. 5th ed. PrenticeHall, England; 2005.