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Original research paper

High performance liquid chromatography for simultaneous determination of xipamide, triamterene and hydrochlorothiazide in bulk drug samples and dosage forms

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A novel, simple and robust high-performance liquid chromatography (HPLC) method was developed and validated for simultaneous determination of xipamide (XIP), triamterene (TRI) and hydrochlorothiazide (HCT) in their bulk powders and dosage forms. Chromatographic separation was carried out in less than two minutes. The separation was performed on a RP C-18 stationary phase with an isocratic elution system consisting of 0.03 mol L^{-1} orthophosphoric acid (pH 2.3) and acetonitrile (ACN) as the mobile phase in the ratio of 50:50, at 2.0 mL min^{-1} flow rate at room temperature. Detection was performed at 220 nm. Validation was performed concerning system suitability, limits of detection and quantitation, accuracy, precision, linearity and robustness. Calibration curves were rectilinear over the range of 0.195–100 µg mL^{-1} for all the drugs studied. Recovery values were 99.9, 99.6 and 99.0 % for XIP, TRI and HCT, respectively. The method was applied to simultaneous determination of the studied analytes in their pharmaceutical dosage forms.

Keywords: xipamide, triamterene, hydrochlorothiazide, HPLC, simultaneous analysis

Xipamide (XIP, 4-chloro-2,6-dimethyl-5-sulfamoylsalicylanilide) (1) is used for high blood pressure and edema control with a moderately powerful diuretic action (2). Several procedures have been developed for its determination, which include spectrophotometry (3–5), spectrofluorimetry (5,6), TLC-densitometry (4) and HPLC (7–12).

Triamterene (TRI, 6-phenyl-2,4,7-triaminopteridine) (1) is a relatively inefficient antihypertensive when used alone; hence it is used in combination with some potent diuretic (*e.g.*, anthranilic acid or thiazide derivative) to give a synergistic action (13). Literature survey reveals that TRI has been determined by spectrophotometric (4, 14–18), fluorimetric (19, 20) and chromatographic (7, 21, 22) methods.

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Hydrochlorothiazide (HCT, 6-chloro-3,4-dihydro-2*H*-1,2,4- benzothiadiazine-7-sulphonamide-1,1-dioxide) (1) is used as an effective diuretic and antihypertensive agent. Previous methods described for HCT determination include spectrophotometry (15–17, 23), HPTLC with fluorimetric detection (23) and HPLC (17, 21, 24–26).

Literature review revealed that a few methods were developed for determination of XIP and TRI together (4, 7) or TRI with HCT (15–17, 21); however, these methods suffer either from low sensitivity or long separation time. In addition, the single-pill, triple-combination antihypertensive therapy has been widely accepted as an effective, well-tolerated, and convenient strategy that can help hypertensive patients (27). Nevertheless, nowadays there is no one dosage form that includes the three drugs analyzed in this study all together. Therefore, the development of a simple, reproducible and sensitive HPLC method for their simultaneous determination in a mixture of bulk drug samples or in a combined dosage form would be very important.

Chemical structures of all three analytes are given in Fig. 1.

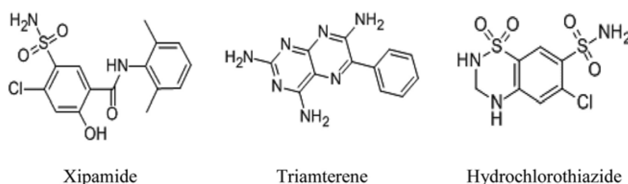

Fig. 1. Chemical structures of the analytes.

EXPERIMENTAL

Materials and reagents

Analytical HPLC grade solvents were used in all experiments, including methanol and ACN (POCH S.A., Poland). Orthophosphoric acid 85 % and sodium hydroxide (Burdick & Jackson, USA) were used. Double distilled water was used. Xipamide (99.09 %), triamterene (99.91 %) and hydrochlorothiazide (99.50 %) were kindly supplied by EIPICO Pharmaceutical Industries Company, Egypt. The pharmaceutical dosage forms used were Epitens® (Egyptian International Pharmaceutical Industry Co, Egypt, 30 mg TRI and 10 mg XIP per tablet) and Moduretic® (Kahira Company for Pharmaceuticals, Egypt, 50 mg HCT and 5 mg amiloride per tablet). There is no formulation in the Egyptian market that includes HCT only. This is why Moduretic®, containing HCT and amiloride, was analyzed for HCT contents.

Chromatographic conditions

HPLC apparatus (Agilent 1100, Agilent, USA) composed of a quaternary pump G1310A with quaternary solvent cabinet, vacuum degasser G1322A and a four-channel gradient pump, autosampler G1313A, variable wavelength detector G1314A with standard 10-mm pathlength-flow cell (14 μL volume, 4×10^6 Pa maximum pressure). Separations were performed on an Agilent 5-μm Hypersil BDS C18 column (150 x 4.6 mm i.d.). A pH meter Jenway 4330 (Jenway, UK) was used.

Validation

For the determination of XIP, TRI and HCT, an isocratic mobile phase consisting of ACN : 0.03 mol L^{-1} H_3PO_4 (pH 2.3) (50:50, *V*/*V*) at a flow rate of 2.0 mL min^{-1} was applied. pH readings always belong to the aqueous part of the mobile phase. For each run, 10 μL of sample was injected. Detection was achieved at 220 nm.

The proposed analytical method was validated through linearity, limits of detection and quantitation, accuracy, precision and robustness according to the guidelines (28–30). The limits of detection (*LOD*) and quantification (*LOQ*) (29, 30) were defined as the injected quantities giving a signal-to-noise of 3 and 10, respectively, in terms of peak height (Table I).

To obtain calibration graphs, drug concentrations in the range of 0.195–100 μg mL^{-1} were used. Peak areas were plotted against the corresponding injected concentrations.

Checking of chromatographic parameters was done using the standard mixture solution. They include retention time, resolution (R_s), selectivity factor (α), symmetry and N (theoretical plates) (Table I).

Table I. Chromatographic, linear regression and limiting data for the determination of XIP, TRI, and HCT using the proposed HPLC method

Parameter	Xipamide	Triamterene	Hydrochlorothiazide
Chromatographic parameters			
t_R ± SD (min)	1.853 ± 0.002	1.493 ± 0.002	0.961 ± 0.001
Resolution (R_s)	3.17	4.50	–
Selectivity (α)	1.415	2.575	–
Symmetry	0.860	0.630	0.680
k'	1.973	1.397	0.54
N (150 mm column)	5607	2197.5	1252.75
Linearity and regression data			
Linearity range (μg mL^{-1})	0.195–100	0.195–100	0.195–100
LOD (μg mL^{-1})	0.012	0.024	0.012
LOQ (μg mL^{-1})	0.04	0.08	0.04
Slope ± RSD[a]	47.518 ± 0.01	40.429 ± 0.02	32.806 ± 0.03
Intercept ± RSD[a]	0.4696 ± 1.6	3.8595 ± 1.3	2.3953 ± 2.5
Coefficient of determination (R^2)	0.9999	0.9999	0.9999

[a] RSD (%)

The precision study was performed by injection of the standard solution at four concentrations four times during a day or every day for four days in case of intra-day and inter-day precision, respectively (Table II).

Table II. Intra- and inter-day precision determination of XIP, TRI and HCT using the proposed HPLC method

Drug	Intra-day			Inter-day	
	Added conc. ($\mu g\ mL^{-1}$)	Recovery (%)[a]	RSD (%)	Recovery (%)[a]	RSD (%)
Xipamide	12.50	96.8	0.1	99.7	1.5
	25.00	97.6	0.1	98.8	1.1
	50.00	99.4	0.4	99.7	0.2
	100.00	101.5	0.1	101.3	1.0
Triamterene	12.50	98.8	0.7	97.3	1.9
	25.00	98.9	0.3	97.3	1.4
	50.00	99.3	0.4	98.4	0.8
	100.00	101.1	0.4	100.2	0.8
Hydrochloro-thiazide	12.50	99.7	0.7	99.9	1.8
	25.00	99.4	0.5	98.1	1.3
	50.00	99.1	0.2	99.3	0.3
	100.00	100.7	0.2	100.9	0.5

[a] Recovery of the label claim. Mean of four determinations.

Stability of the standard solution was checked through injection of the prepared solution at intervals into the chromatograph for up to about 7 days.

Robustness of the method was studied for small changes in the flow rate (± 0.1 mL min^{-1}), % of ACN (± 0.5 %), wavelength of detection (± 1 nm) and injection volume (± 0.5 μL).

Preparation of standard solutions

Ten mg of XIP, TRI and HCT were dissolved in 10 mL methanol and then completed to 100 mL with H_2O to give the stock solution (0.1 mg mL^{-1}). Working standard solutions were prepared by diluting aliquots of stock solutions with the same diluting solvent (10 % methanol in water) to obtain concentrations ranging from 0.195–100 $\mu g\ mL^{-1}$ for all the studied drugs.

Assay of tablets

Ten tablets of Epitens® and Moduretic® were finely powdered, separately. An accurately weighed quantity of the powders equivalent to 30 mg TRI and 10 mg XIP in case of Epitens® and equivalent to 50 mg HCT in case of Moduretic® tablets were transferred into 100-mL volumetric flasks and extracted using 10 mL methanol and then completed to 100 mL with water. The flasks were shaken for 15 min using an ultrasonic shaker and then filtered into dry conical flasks. Aliquots of the filtrates (25 and 10 mL) of Epitens® and Moduretic®, respectively, were separately transferred into 100-mL volumetric flasks and diluted to volumes with the diluting solvent to prepare solutions containing 25 $\mu g\ mL^{-1}$ XIP,

75 μg mL^{-1} TRI in case of Epitens® tablets and 50 μg mL^{-1} HCT in case of Moduretic® tablets.

Epitens® tablets extract (XIP and TRI) and Moduretic® tablets extract (HCT) were determined separately.

Also, a laboratory prepared mixture composed of tablet solutions of XIP and TRI (Epitens®) and HCT (Moduretic®) was prepared and then analyzed.

RESULTS AND DISCUSSION

Method optimization

Optimization of different experimental conditions was carried out at the detection wavelength of 220 nm, which gives the best sensitivity for all three analytes.

Elution of TRI, XIP and HCT was achieved at reasonable retention times (2 minutes) using 50 % ACN in the mobile phase (Fig. 2). Longer retention times were obtained at lower concentrations of ACN, while increasing the ACN concentration to 60 % led to overlap of XIP with TRI peaks (eluted together at 1.571 min). pH 2.3 of the aqueous part of the mobile phase was chosen as the optimum pH providing the best peak symmetry and sharpness adding to reasonable separation, retention times and peak shape.

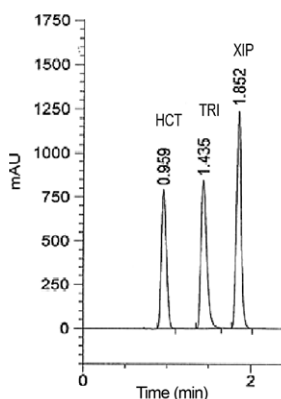

Fig. 2. Typical chromatogram of a 10-μL injection of a standard mixture of 100 μg mL^{-1} XIP, TRI and HCT using 0.03 mol L^{-1} orthophosphoric acid (pH 2.3) and ACN (50:50) as the mobile phase at a flow rate of 2.0 mL min^{-1} and detection at 220 nm.

Validation

Linearity and limiting values. – The results indicate that there is a wide linearity range (0.195–100 μg mL^{-1} with $R^2 > 0.999$) as well as low *LOD* (0.01–0.02 μg mL^{-1}) and *LOQ* (0.04–0.08 μg mL^{-1}) values. Resolution is greater than 1.5 and symmetry factors for all analytes are 0.6–0.9 (see Table I).

Selectivity and specificity of the method. – The presence of amiloride co-formulated with HCT did not affect separation and quantitation of HCT. We assume that the peak at 1.3 min may be ascribed to amiloride. We can also conclude that there was no interference of other additives in the formulations (Fig. 3). Detection and validated quantitation of amiloride is our next task.

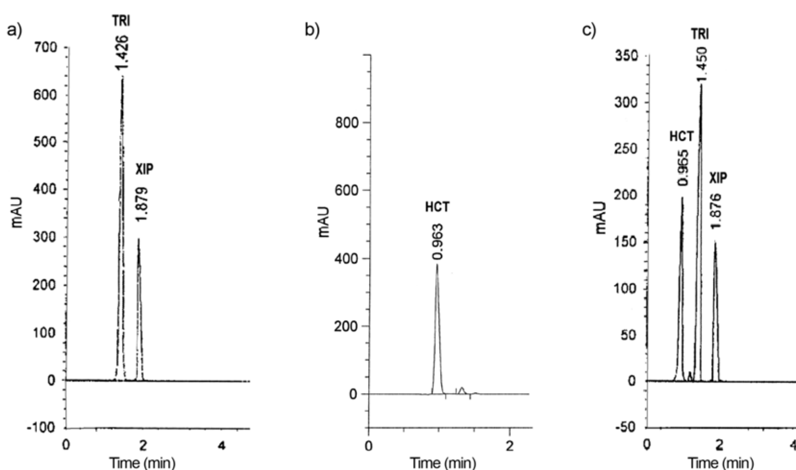

Fig. 3. Application of the proposed HPLC method for the determination of:a) XIP, TRI in Epitens® tablets, b) HCT in Moduretic® tablets, c) laboratory prepared mixture of the tablet solutions (XIP and TRI from Epitens®, HCT from Moduretic®, under optimized chromatographic conditions.

Precision. – Good intra- and inter-day precision was indicated by RSD values of 0.1–0.7 and 0.2–1.9 %, resp. (Table II).

Stability and robustness. – The results of stability tests reveal that the RSD of the peak area was within 1.0 %. The results of robustness studies indicated that the method is much more sensitive to slight variations in the flow rate (Figs. 4a and b) and % of ACN (Figs. 4c and d) with RSD values within 3.9 %. The decrease in ACN percentage led to an increase of retention times for all the studied drugs. Small changes in the wavelength of detection and injection volume had no significant effect on the peak area and retention time for all the studied drugs (RSD values lower than 1.0 %, Table III).

Accuracy of the new method and application for analysis of XIP, TRI and HCT in tablets

Laboratory prepared mixture (containing all analytes) was composed of Epitens® and Moduretic® tablet solutions. The analysis has shown good accuracy with good recoveries of the label claim (mean ± RSD, $n = 3$) 98.9 ± 1.1, 97.8 ± 1.4 and 99.1 ± 1.5 % for XIP, TRI and HCT, respectively (Table IV). For all three analytes, the model accuracy obtained from standard solutions within the linearity range covers the interval of 97 to 102 % (Table II).

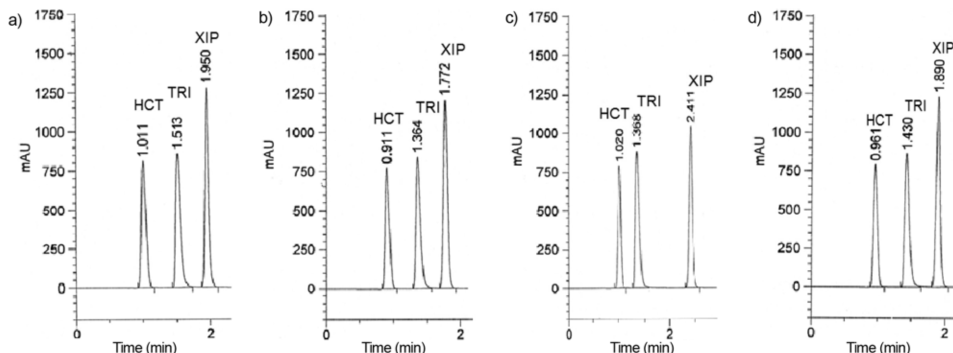

Fig. 4. Robustness of the proposed HPLC method from a typical chromatogram of a 10-μL injection of a standard mixture of 100 μg mL^{-1} XIP, TRI and HCT: a) flow rate 1.9 mL min^{-1}, b) flow rate 2.1 mL min^{-1}, c) 49.5 % ACN, d) 50.5 % ACN. Other conditions are as optimized.

Table III. Robustness of the proposed HPLC method

Analyte concentration (μg mL^{-1})		Injection volume (μL)		Detection wavelength (nm)		Flow rate (mL min^{-1})	
		9.5	10.5	219	221	1.9	2.1
XIP	50	0.2	0.0	0.1	0.0	3.4	3.4
	100	0.0	0.2	0.1	0.9	3.7	3.1
TRI	50	0.1	0.1	0.0	0.1	3.6	3.9
	100	0.1	0.0	0.1	0.1	3.6	3.7
HCT	50	0.3	0.1	0.3	0.2	3.6	3.9
	100	0.1	0.1	0.1	0.4	3.7	3.7

Table IV. Application of the proposed HPLC method for simultaneous determination of XIP, TRI and HCT in a laboratory prepared mixture of Epitens® and Moduretic® tablets[a]

XIP		TRI		HCT	
Taken conc. (μg mL^{-1})	Recovery (%)	Taken conc. (μg mL^{-1})	Recovery (%)	Taken conc. (μg mL^{-1})	Recovery (%)
8.33	99.8	25.00	96.8	16.66	98.1
12.50	98.2	37.50	98.7	25.00	100.2
Mean[a]	98.9		97.8		99.1
RSD (%)	1.2		1.4		1.5

[a] Average of four experiments.

When the results of the method for determination of the three analytes were statistically compared with those obtained from the reported HPLC methods (7, 24), it was found that there was no significant difference between them regarding accuracy and precision, since the calculated *t*- and *F*-values were lower than the corresponding tabulated ones (Table V).

Table V. Determination of XIP, TRI and HCT by the proposed HPLC method compared to reported methods

Drug		This paper	Reported methods
Xipamide	Mean (%)	99.9	98.3 (ref. 7)
	RSD (%)	1.9	1.4
	Student's *t* -test	1.53 (1.83)[a]	–
	F-test	1.77 (5.19)[a]	–
	n	6	5
Triamterene	Mean (%)	99.6	100.4 (ref. 7)
	RSD (%)	0.7	0.9
	Student's *t* -test	1.6(1.86)[a]	–
	F-test	1.61 (6.39)[a]	–
	n	5	5
Hydrochlorothiazide	Mean (%)	99.4	99.7 (ref. 24)
	RSD (%)	1.0	0.5
	Student's *t* -test	0.54 (1.83)[a]	–
	F-test	4.16(5.19)[a]	–
	n	5	6

[a] The figures in parentheses are the theoretical values for *t*- and *F*-tests ($p < 0.05$).

CONCLUSIONS

A simple, fast and specific HPLC method has been described for simultaneous determination of xipamide, triamterene and hydrochlorothiazide in their pure form and in pharmaceutical preparations. The proposed method is of great interest for routine sample analysis and quality control of the analyzed drugs because of the short chromatographic run time (2 minutes) allowing a large sample throughput. When compared to the previously reported methods, the new method is simple, cost effective, precise, sensitive and accurate.

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