Microchemical Journal 212 (2025) 113407

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Dual pH-optimized HPTLC method for simultaneous quantification and enhanced fluorescence detection of triamterene and losartan in human plasma: Toward achieving maximum separate fluorescence intensity

Ahmed A. Khorshed [a,b,*], Fatma M. Abdelnaeem [a], Mohamed Oraby [a,c], Dalia M. Nagy [d], Sayed M. Derayea [d]

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Sohag University, Sohag 82524, Egypt
[b] Department of Biomedical Engineering, University of Alberta, Edmonton, Alberta T6G 1H9, Canada
[c] Pharmaceutical Chemistry Department, College of Pharmacy, Al-Esraa University, Baghdad, Iraq
[d] Analytical Chemistry Department, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

ARTICLE INFO

Keywords:
Triamterene
Losartan
HPTLC
Fluorescence
Plasma

ABSTRACT

A novel and selective High Performance Thin Layer Chromatographic (HPTLC) method was developed for the simultaneous determination of triamterene (TRM) and losartan (LOS) in their pure forms and spiked human plasma using fluorescence detection. The challenge of differing optimal pH values for maximum fluorescence in TRM and LOS detection was addressed by devising a strategy for detection in various pH media. TRM was measured in a neutral medium and achieved maximum fluorescence at 540 nm upon excitation at 365 nm, with a 66.7-fold sensitivity increase compared to previous methods. LOS, which exhibited weak fluorescence in a neutral medium, showed maximum fluorescence in a strong acidic environment. After perchloric acid treatment, LOS achieved maximum response at 400 nm after excitation at 260 nm, with an 83.3-fold sensitivity increase. Pre-coated silica gel 60-F254 was used as the stationary phase, with a mobile phase of toluene: ethyl acetate: methanol: acetone: ammonia (6:1.5:1.5:0.9:0.1, v/v/v/v/v). Rf values for LOS and TRM were 0.16 and 0.32, respectively. The method exhibited good linearity for TRM (3–150 ng/band) and LOS (6–150 ng/band), with LODs of 0.70 and 1.41 ng/band and LOQs of 2.13 and 4.26 ng/band. The technique demonstrated excellent compliance with ICH guidelines for accuracy, precision, repeatability, and robustness. The method achieved high recovery percentages and low standard deviations in analyzing both bulk drug and plasma samples. The current work is a valuable tool for routine clinical laboratory use, enabling efficient monitoring of TRM and LOS from a single sample preparation.

1. Introduction

Hypertension is a long-term, chronic, pathological illness characterized by increased blood pressure which can raise morbidity and mortality and harm bodily organs [1]. For the treatment of hypertension and its related pathologic diseases, such as edema and congestive heart failure, first-choice medications include diuretics, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor antagonists [2].

Losartan potassium (LOS), chemically named as 2-butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl) benzyl] imidazole-5-methanol monopotassium salt, is a member of angiotensin II receptor antagonists (AIIRAs) [3]. Its hypotension action results from inhibition of the binding of angiotensin (AT) II to the AT1 receptor in vascular smooth muscle [4]. LOS is particularly beneficial for individuals who suffer from cough related to angiotensin-converting-enzyme (ACE) inhibitors and has also been trialed for the treatment of myocardial infarction and heart failure [5].

Triamterene (TRM) (6-phenyl-2,4,7-triaminopteridine) is a natriuretic drug that is used to treat a number of illnesses including nephritic syndrome, cirrhosis of the liver, idiopathic and drug-induced oedema, and oedema related to congestive heart failure [6]. It belongs to the potassium-sparing family and acts directly on distal tubular cells to decrease potassium ion release and to prevent the re-absorption of

* Corresponding author at: Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Sohag University, Sohag 82524, Egypt.
E-mail address: ahmed.khorshed.analytical@gmail.com (A.A. Khorshed).

https://doi.org/10.1016/j.microc.2025.113407
Received 22 January 2025; Received in revised form 15 March 2025; Accepted 17 March 2025
Available online 21 March 2025

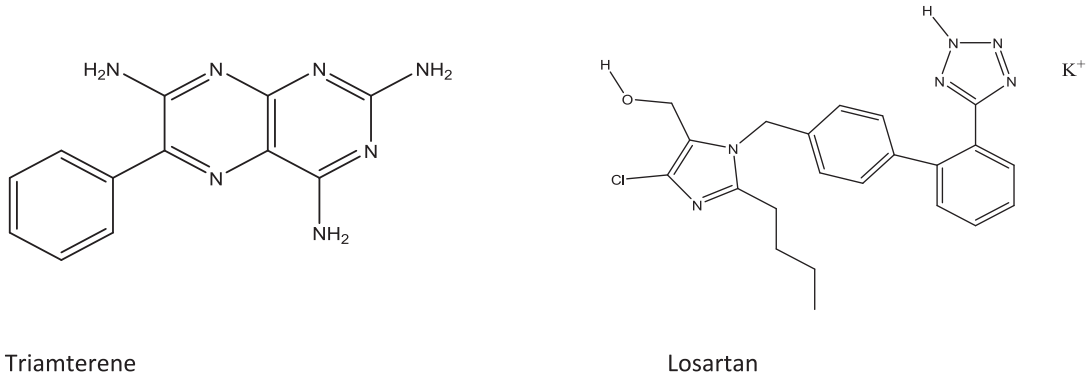

Fig. 1. Chemical structure of the studied drugs

chloride ions [7]. After oral administration, TRM is absorbed and metabolized by hydroxylation and subsequent immediate conjugation, resulting in its main metabolite hydroxy triamterene sulfate. Since TRM is not very effective as a treatment for hypertensive, it is typically used in conjunction with stronger diuretics (thiazide, anthranilic acid or AIIRAs like Losartan) to lessen their potassium-wasting effects. This combination has a synergistic impact that increases both diuretic and hypotensive effects [8,9].

Medical studies and reviews indicate that combined therapy, such as the administration of TRM and LOS together, has produced very successful results with fewer side effects and cheaper health treatment costs than monotherapy [10]. When used in concert, they effectively treat hypertension and lower the primary causes of cardiovascular morbidity and mortality [11].

According to the literature, various analytical techniques have been utilized to determine TRM including spectrophotometric methods [12,13], spectrofluorimetric method [8] and TLC with UV detection [12,14,15]. However, the sensitivity of these techniques is insufficient to detect TRM in biological matrices. Only one HPTLC method using fluorescence detection has been described for the single measurement of TRM in spiked plasma [2].

Moreover, TRM has been previously determined using electrochemical [16], UPLC [17] and HPLC techniques [10,18,19]. Notably, a voltametric technique has been used to measure TRM and LOS concurrently in urine and pharmaceutical formulations [20]. Even with their high sensitivity and selectivity, these techniques are not practical for quick or on-site TRM assays. They are expensive, time-consuming, and necessitate extensive extraction procedures prior to analysis, which make them less feasible for rapid testing particularly during Olympic competitions.

The determination of LOS in biological fluids or pharmaceutical formulations has been accomplished using a variety of analytical techniques, such as spectrophotometry [21,22], Spectrofluorimetry [23,24], HPLC [25,26], HPTLC [27,28], capillary electrophoresis and capillary electrochromatography [29].

On the other hand, due to its advantages of using less solvent, cleaning up analysis, and being cost-effective, high-performance thin-layer chromatography (HPTLC) is a valuable analytical technique [28,30–36]. According to the aforementioned review of the literature, until now, there is currently no published HPTLC method for the simultaneous determination of TRM and LOS (Fig. 1) that uses either UV or fluorescence detection. Therefore, the goal of this work is to establish an HPTLC method that is easy to use, sensitive, selective, and dependable for the simultaneous measurement of TRM and LOS in their synthetic combination and in human plasma that has been spiked using reflectance/fluorescence mode. Since obtaining blood samples from patients undergoing the investigated drug therapy was challenging, we opted to conduct the analysis using human plasma samples spiked in vitro with TRM and LOS. This approach allowed us to systematically assess the method's performance under controlled conditions, ensuring accurate evaluation of sensitivity, selectivity, and reproducibility. However, we acknowledge that the use of spiked samples may not fully replicate the complexity of real patient samples, particularly regarding the presence of metabolites. Future studies will focus on validating the method using real clinical samples to further assess its applicability and reliability in therapeutic drug monitoring and pharmacokinetic studies.

2. Experimental

2.1. Apparatus

WinCATS version 1.4.4.6337 software (CAMAG, Muttenz, Switzerland) and a Camag-HPTLC system with a Linomat V autosampler and TLC Scanner III were used to acquire the data. A high-pressure mercury vapor lamp served as the source of radiation for the reflectance/fluorescence scanning. A 100 μL Hamilton syringe (Hamilton, Bonaduz, Switzerland) and a gentle stream of nitrogen were utilized in conjunction with the Linomat for the sample application in order to facilitate the solvent's rapid evaporation. The scanning speed was set to 20 mm/s, while the scanner's slit dimensions were set to 3 × 0.45 mm. The twin-trough chamber (27.0 × 26.5 × 7.0 cm, length × height × breadth; Sigma-Aldrich, St. Louis, MO) was used to develop the plates. An ultrasonic bath (Cole-Parmer, Chicago, IL) and Andreas Hettich GmbH centrifuge (Tuttingen, Germany) were used during samples preparation.

2.2. Chemicals, standards, and samples

AL-Kahira Pharmaceutical Company (Cairo, Egypt) cheerfully provided triamterene, while Global Napi Pharmaceutical Co. (6th October City, Egypt) kindly provided losartan potassium. TRM and LOS both demonstrated a purity of at least 98.00 % according to routine TLC analysis. El-Nasr Pharmaceutical Chemicals in Abo-Zaabal, Cairo, Egypt, supplied solvents such as toluene, ethyl acetate, acetone, and ammonia and acids as perchloric acid. HPLC methanol was obtained by Fischer Scientific Company (Loughborough, UK). Fresh human plasma samples were obtained from Misr hospital (Sohag, Egypt) and immediately frozen at −20 °C until further processing.

2.3. Preparation of standard solution

A weight equal to 10.0 mg of each drug were dissolved in 7 mL of HPLC methanol, sonicated for 10 min, and then the volume was completed to 10 ml using the same solvent to get 1.0 mg/mL of TRM or LOS stock solutions. In order to get standard working solutions containing 1, 3, 10, 20, 30, and 50 μg/ml of TRM and 2, 4, 8, 15, 35, and 50 μg/ml of LOS, specific quantities of both medications were combined and diluted with HPLC methanol. A 3 μL aliquot of each drug's

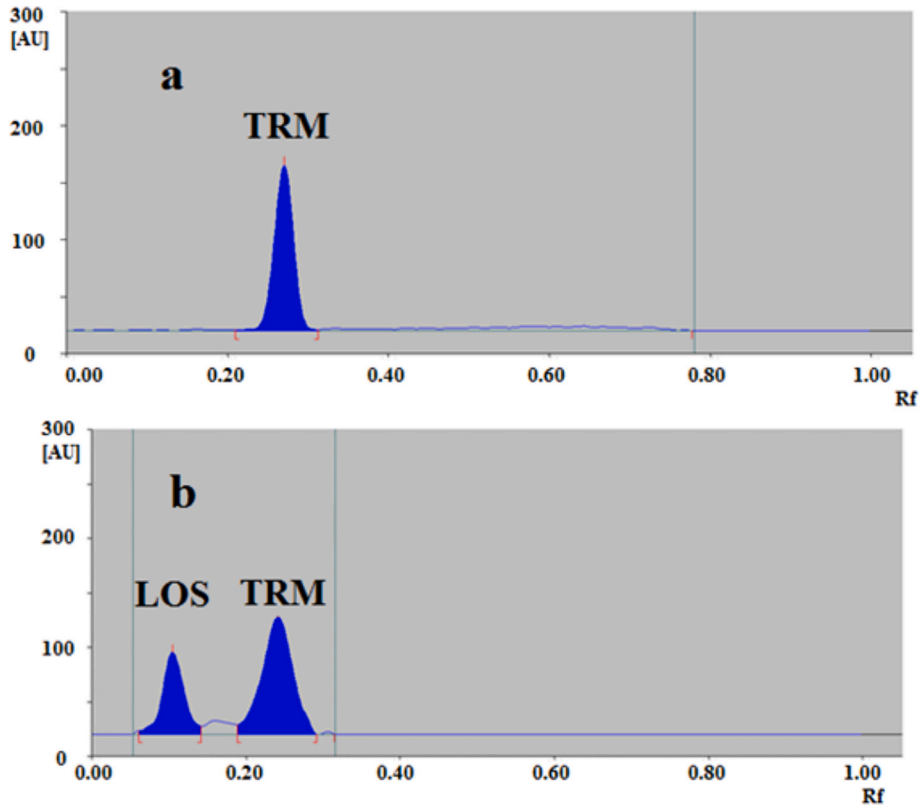

Fig. 2. HPTLC chromatogram of 24 ng/band of LOS and 30 ng/band of TRM (a) before and after (b) spraying with 0.2 M perchloric acid and measured by reflectance/fluorescence mode.

previously stated working solutions was applied to the HPTLC plates, resulting in final concentrations of 3, 9, 30, 60, 90, and 150 ng/band for TRM and 6, 12, 24, 45, 105, and 150 ng/band for LOS.

2.4. Preparation of plasma samples

Spiked human plasma samples were prepared by mixing 200 μL of fresh human plasma with 150 μL of TRM standard solution, and 150 μL of LOS standard solution. After that, 300 Âμl of acetonitrile and 700 Âμl of HPLC methanol were added and mixed for 4 min by vortexing. The mixture was centrifuged at 14000 rpm and −4° C for 15 min. Six replicates were conducted for each concentration using the supernatant according to the stated chromatographic conditions and finally the recovery and standard deviation were calculated.

2.5. Chromatographic conditions

Various aliquots of standard solutions of TRM and LOS were applied to the precoated HPTLC plates, previously washed with HPLC methanol, following the preceding instructions. The plates were developed, dried, and scanned using the reflectance/fluorescence detection. HPTLC plates (Merck, Darmstadt, Germany) had a stationary phase thickness of 200 μm and a particle size of 5 μm. They were pre-coated with silica gel 60 F_{254}. Plates were cut into 20 × 6 cm sections. The optimum mobile phase composition was toluene: ethyl acetate: HPLC methanol: acetone: ammonia (6: 1.5: 1.5: 0.9: 0.1, v/v/v/v/v). The development compartment was filled with 10 ml of mobile phase and allowed to saturate with the mobile phase vapor for 20 min at room temperature. Before fluorescence measurements, the plate was developed, allowed to dry in the air for 10 min, and then put into the TLC scanner using a 540 nm emission optical filter and excitation wavelength of 365 nm. TRM was first identified at neutral medium (HPLC methanol) which gave its maximum fluorescence. However, highly acidic conditions were required for LOS detection by spraying the developed plate with 0.2 M perchloric acid. The emitted fluorescence was measured using an emission filter set at 400 nm and excitation wavelength of 260 nm.

3. Results and discussion

The primary objective of this work was to develop a simultaneous HPTLC method for the determination of LOS and TRM, with enhanced sensitivity to enable its application in human plasma samples. A significant challenge in this process was the distinct fluorescence behaviors of LOS and TRM, as each drug exhibited maximum fluorescence under different pH conditions. TRM showed maximum fluorescence intensity in methanol at neutral pH and is therefore amenable to analysis in the medium. However, LOS demonstrates low fluorescence in a neutral medium with maximum fluorescence occurring in a very acidic medium. Plates were therefore should be sprayed with 0.2 N perchloric acid in order to provide for maximum fluorescence of LOS. The technique provides effective detection and quantitation of both compounds under optimum conditions. Therefore, a novel and innovative strategy was developed to detect each drug at its optimal pH, ensuring maximum fluorescence for each, thereby enabling their concurrent detection and quantification with high sensitivity. This proposed method introduces the first HPTLC strategy to simultaneously measure TRM and LOS in both pure mixtures and spiked human plasma. It offers high sensitivity and selectivity, while being easy to apply, cost-effective, and time-saving. TRM exhibits strong native fluorescence in HPLC methanol (neutral medium) and is directly detected after plate development at a 365 nm excitation wavelength (Fig. 2a). At such conditions, LOS displays weak fluorescence and does not initially appear in the chromatogram (Fig. 2a). However, protonation significantly enhances LOS fluorescence. To achieve this purpose, the plates were sprayed with 0.2 M perchloric acid, allowed to dry, and then re-scanned at a 260 nm excitation wavelength. Consequently, the LOS peak becomes clearly

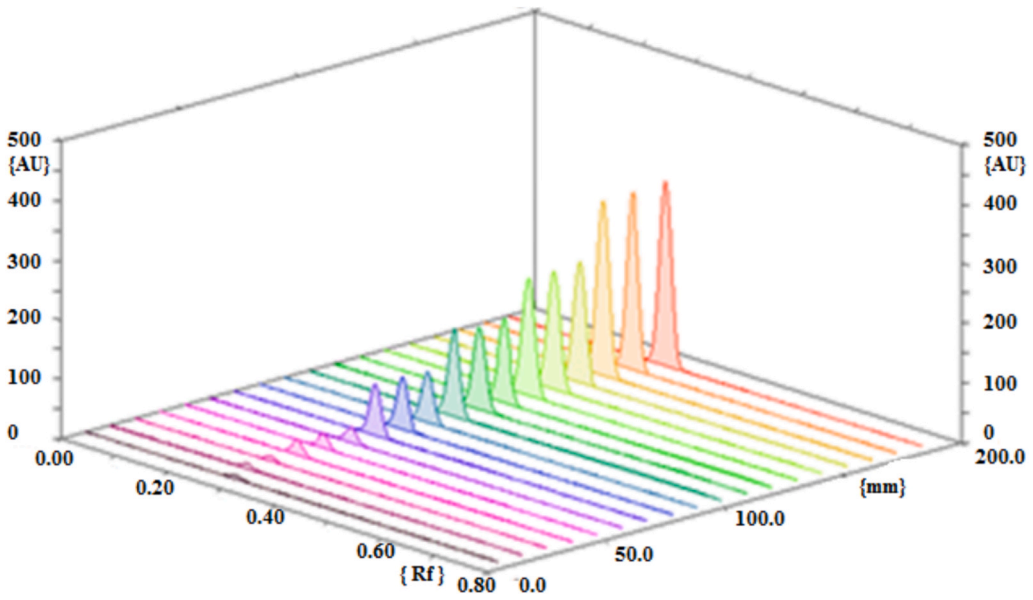

Fig. 3. Three-dimensional graph showing recorded intensities against Rf values for (3–150 ng/band) for TRM before spraying with 0.2 M perchloric acid, measured by reflectance/fluorescence mode.

detectable, as shown in (Fig. 2b).

The acidic nature of LOS is primarily due to the presence of the tetrazole ring, with a corresponding pKa value of 3.15. [37]. An acidic medium is necessary to convert the tetrazole ring of LOS to the fully non-ionized form. In this state, resonance allows the proton to delocalize over the four nitrogen atoms, thus enhancing the fluorescence intensity [38]. The intensity of LOS's fluorescence in acidic solutions is at least five times that of water [39].

It should be noted that these strong acidic conditions required for enhancement of LOS fluorescence cannot be applied in HPLC as they lead to destruction of the column (stationary phase). However, the stationary phase in TLC is disposable, which enables the determination of LOS under drastic conditions.

Using this method, it was possible to analyze two different medications at different pH values simultaneously, which increased the selectivity of the method. Compared with the previously published researches for HPTLC technique, the proposed method increased the sensitivity of TRM by ~ 66.67-fold [12] and by nearly 83.33-fold for LOS [40].

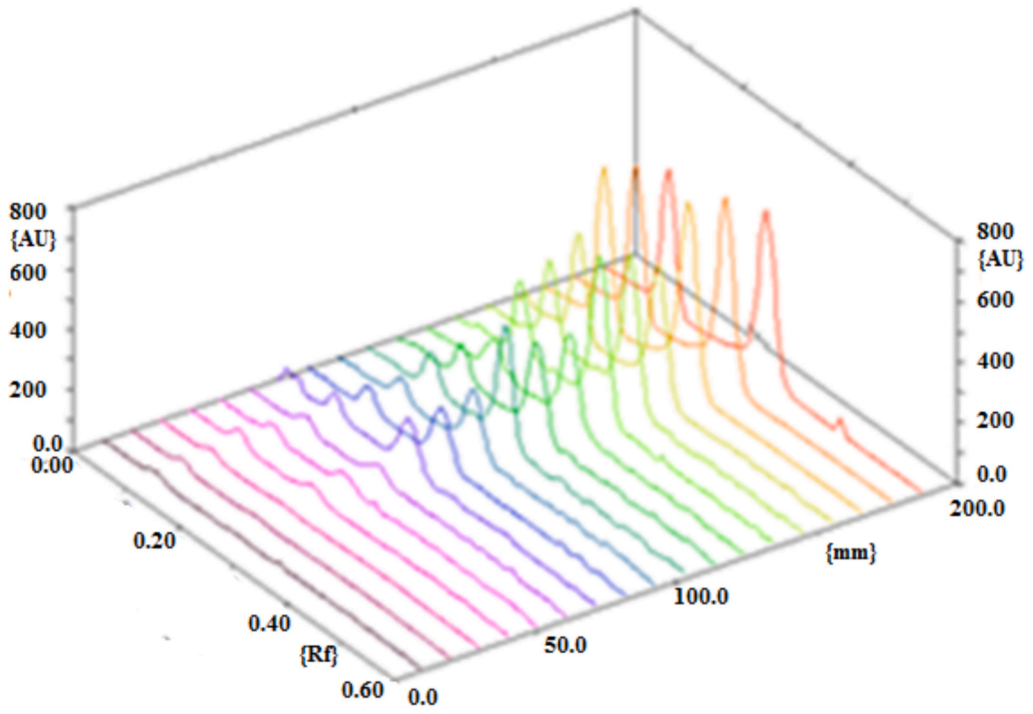

Fig. 4. Three-dimensional graph showing recorded intensities against Rf values for (6–150 ng/band) for LOS and (3–150 ng/band) for TRM after spraying with 0.2 M perchloric acid, measured by reflectance/fluorescence mode.

Table 1
Statistical data of some analytical parameters of the proposed TLC-fluorescence method for simultaneous determination of Triamterene and Losartan.

Parameter	Losartan	Triamterene
Linear range (ng/band)	6–150	3–150
Slope ± SD (a)	44.73 ± 0.48	20.84 ± 0.78
Intercept ± SD (a)	67.20 ± 19.05	192.07 ± 4.44
Correlation coefficient, r	0.9997	0.9972
Determination Coefficient, r^2	0.9995	0.9943
R_F	0.16	0.32
LOD (ng/band)	1.41	0.70
LOQ (ng/band)	4.26	2.13

(a) Average of three determinations.

3.1. Chromatographic conditions optimization

Numerous attempts have been made to find an appropriate mobile phase that could effectively and selectively separate TRM from LOS. Thus, a wide range of mobile phases with different polarities, compositions, and ratios were tested, including dichloromethane: methanol: acetic acid resulted in band tailing of TRM and migration of LOS with the solvent front. Toluene: methanol: acetic acid gave no separation between the investigated drugs. A huge tailing of TRM was obtained when using a mobile phase that consisted of toluene: ethyl acetate: methanol: acetic acid. There was no discernible migration of TRM from the baseline when a mixture of toluene: ethyl acetate: acetone: acetic acid was used as a mobile phase. Likewise, band tailing of TRM occurred with toluene: ethyl acetate: methanol: acetone: acetic acid, and finally the use of toluene: ethyl acetate: methanol: acetone resulted in acceptable separation between the two concurrently investigated drugs but band tailing of TRM still present. Tailing of TRM could occur because the highly polar primary amine groups of TRM are attracted to the negatively charged silanol groups of silica [41]. Making the mobile phase alkaline may lessen the ionization of this amine group and thus reduce its polarity, promoting band separation and reducing tailing. Concentrated ammonia solution was added to the mobile phase mixture with varying volumes, and the results showed clearly separated and distinct bands of both TRM and LOS.

The final mobile phase composition was toluene: ethyl acetate: HPLC methanol: acetone: ammonia (6: 1.5: 1.5: 0.9: 0.1, v/v/v/v/v). which gave Rf values of 0.16 for LOS and 0.32 for TRM, as illustrated in Fig. 2a and b.

3.2. Method validation

The suggested method for the simultaneous determination of TRM and LOS was validated in terms of linearity, detection and quantitation limits, accuracy, precision, and robustness by adhering to the International Conference on Harmonization's (ICH) 2005 recommendations for bioanalytical method validation [42]. The 95 % confidence level was used for the statistical analysis of the data using Excel 2019 (Microsoft, Richmond, WA).

Linearity

In order to get calibration plots, peak areas were plotted against the matching drug concentration (ng per band). Correlation coefficient values were 0.9972 for TRM and 0.9997 for LOS over the concentration ranges of (3–150 ng/band) for TRM (Fig. 3) and (6–150 ng/band) for LOS (Fig. 4). The results of peak purity ensure the specificity, which indicated that no impurities or degradation products were coeluted (Table 1).

Limit of detection and quantitation

The sensitivity of measurements of TRM and LOS was estimated in terms of the limit of quantitation (LOQ). The smallest amount that can be detected for each drug was also calculated under the chromatographic conditions in terms of the limit of detection (LOD). LOQ and LOD were calculated by the use of the following equations: LOD = 3.3 × SD/S and LOQ = 10 × SD/S, where SD is the standard deviation of the intercept, and S is the slope of the corresponding calibration plot. LOQ and LOD for TRM were found to be 2.13 and 0.70 ng/band, respectively, and for LOS the values were 4.26 and 1.41 ng/band, respectively (Table 1).

Table 2
Assessment of the accuracy of the proposed TLC-fluorescence method for simultaneous determination of Triamterene and Losartan.

Drug	Conc. of drug (ng/band)	Amount found (ng/band)	% Recovery (a) ± SD
Losartan	12	12.16	101.31 ± 2.09
	45	45.02	100.04 ± 1.79
	105	104.71	99.73 ± 0.93
Triamterene	9	8.84	98.26 ± 2.71
	60	60.57	100.95 ± 2.52
	90	87.95	97.72 ± 1.00

(a) Average of six determinations.

Table 3
Evaluation of the precision of the proposed TLC-fluorescence method for simultaneous determination of Triamterene and Losartan at the intra- and inter-day levels.

Drug	Conc. of drug (ng/band)	Intra-day	Inter-day
		%RSD (a)	%RSD (b)
Losartan	12	2.11	2.62
	45	2.38	1.80
	105	2.85	2.08
Triamterene	3	2.97	1.98
	60	2.98	2.14
	90	2.57	2.67

(a) Estimated from six determinations at each concentration level.
(b) Estimated from 18 determinations at each concentration level over 3 days.

Table 4
Investigation of the robustness of proposed TLC-fluorescence method for simultaneous determination of Triamterene and Losartan.

Parameter	Losartan	Triamterene
Optimal parameters	100.04 ± 1.79	100.95 ± 2.52
Composition of the mobile phase Toluene: ethyl acetate: methanol: acetone: ammonia		
(6: 1.5: 1.7: 0.7: 0.0.1, v/v/v/v/v)	98.56 ± 1.44	99.37 ± 2.09
(5.8: 1.7: 1.5: 0.9: 0.1, v/v/v/v/v)	101.32 ± 2.51	97.68 ± 0.94
Concentration of perchloric acid		
0.19 M	102.25 ± 1.81	101.64 ± 0.68
0.21 M	99.04 ± 1.67	98.91 ± 1.82

(a) Average of six determinations.

Accuracy

The accuracy of the recommended HPTLC approach was assessed using three different concentration levels for each drug under study (9, 60, and 90 ng/band for TRM and 12, 45, and 105 ng/band for LOS). For each concentration level, six measurements were taken. The method used was highly accurate, as evidenced by the proximity of recovery values to 100 % (Table 2).

Precision

Analysis of standard solutions of TRM and LOS with concentrations covering the whole linearity range allowed for the evaluation of precision. Each solution was analyzed six times on the same day in order to establish the method's precision at the level of intraday variation using concentrations of (3, 60 and 90 ng/band) for TRM and (12, 45 and 105 ng/band) for LOS. These solutions were also analyzed on three separate days to assess the interday precision. Table 3 displays the precision study findings showing that RSD is below 3, confirming a good precision of the proposed HPTLC strategy.

Robustness

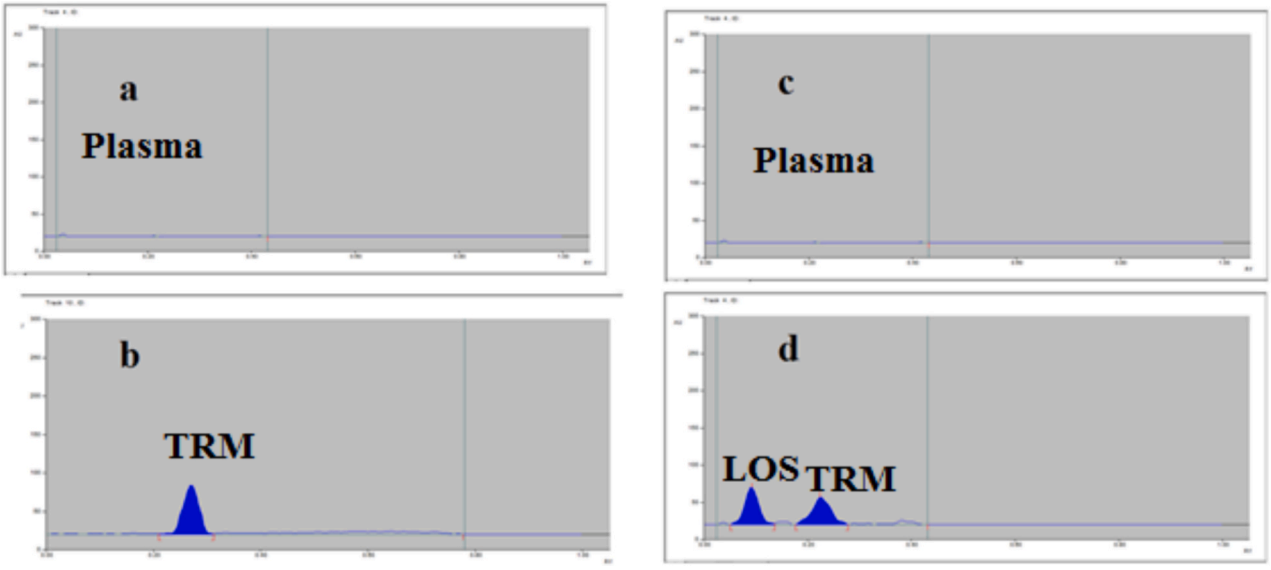

Fig. 5. HPTLC densitogram of a blank plasma sample without TRM and LOS spiking (a, c), and a plasma sample spiked with 30 ng/band of TRM and 12 ng/band of LOS (b, d).

Table 5
Application of the proposed TLC-fluorescence method for simultaneous determination of Triamterene and Losartan in spiked human plasma.

Drug	Conc. of drug (ng/band)	Amount found (ng/band)	% Recovery (a) ± SD
Losartan	12	12.64	105.37 ± 1.92
	24	22.04	91.83 ± 2.33
	45	43.09	95.75 ± 1.75
	105	108.55	103.38 ± 2.24
	150	148.03	98.68 ± 2.27
Triamterene	3	2.89	96.18 ± 2.81
	9	8.59	95.47 ± 2.55
	30	34.02	113.40 ± 2.73
	90	104.01	115.56 ± 1.91
	150	151.91	101.27 ± 1.59

(a) Average of six determinations.

By estimating the effect of minor changes to particular experimental parameters, such as the composition of the mobile phase and the concentration of perchloric acid, on the analytical performance, the degree of robustness or method dependability was evaluated. The results shown in Table 4 indicate that these minor changes had little effect on the method's performance because the recovery percentage remained high. Specifically, the percentage recovery consistently ranged from 97.68 ± 0.94 to 102.25 ± 1.81 percent. This outcome demonstrates the outstanding robustness and reliability of the recommended approach for determining TRM and LOS.

3.3. Application to spiked human plasma

The high sensitivity of the described approach enabled the analysis of the cited drugs in human plasma. As shown in Fig. 5 and Table 5, the results demonstrate the method's ability to estimate the examined medications in human plasma without interference from plasma components.

The blood levels of the drugs being studied after co-administration are extremely important to know. Since it was difficult to get blood samples from patients receiving the drug therapy under investigation, we chose to perform the analysis using human plasma samples that had been in vitro mixed with TRM and LOS.

An aliquot of plasma sample that had not been tampered with the targeted drugs was analyzed as a method blank, as illustrated in Fig. 5a and c. The drugs' mixture under investigation was added to the plasma samples, and analyzed by the proposed method and resulting chromatogram is illustrated in Fig. 5b and d. The percentages of recovery ranged from 95.47 ± 2.55 to 115.56 ± 1.91 for TRM and from 91.83 ± 2.33 to 105.37 ± 1.92 for LOS, demonstrated the strong selectivity and dependability of the suggested approach (Table 5).

4. Conclusion

This study presents a rapid, accurate, and reproducible HPTLC densitometric method for the simultaneous estimation of triamterene (TRM) and losartan (LOS) using reflectance/fluorescence mode for the first time. To overcome the challenge of differing optimal pH conditions for the maximum fluorescence of each drug, we developed a novel and innovative approach to detect each drug at its respective optimal pH, ensuring enhanced fluorescence and enabling their simultaneous detection and quantification with high sensitivity. Triamterene was measured at neutral pH, followed by the determination of LOS in a strongly acidic medium, achieved by spraying the plate with 0.2 M perchloric acid. Compared with previously reported HPTLC techniques, the proposed method significantly enhanced the sensitivity of TRM determination by approximately 66.67-fold and LOS by nearly 83.33-fold. The approach has proven its flexibility and efficiency in quantifying these medications in human plasma samples, with peak shape and location remaining unaffected by the biological matrix. The present approach is a valuable tool for routine clinical laboratory use, where a single sample preparation can monitor multiple drugs efficiently.

CRediT authorship contribution statement

Ahmed A. Khorshed: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **Fatma M. Abdelnaeem:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mohamed Oraby:** Investigation, Writing – review & editing. **Dalia M. Nagy:** Supervision. **Sayed M. Derayea:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] J. Petersen, M. Benzeval, Untreated hypertension in the UK household population—Who are missed by the general health checks? Prev. Med. Rep. 4 (2016) 81–86.
[2] A.A. Khorshed, et al., HPTLC method for the ultrasensitive detection of triamterene in plasma, J. Chromatogr. Sci. 60 (3) (2022) 267–273.
[3] D.A. Sica, T.W. Gehr, S. Ghosh, Clinical pharmacokinetics of losartan, Clin. Pharmacokinet. 44 (2005) 797–814.
[4] D. Tsvetkova, D. Obreshkova, Application of validated TLC-densitometric method for simultaneous identification and determination of losartan potassium, telmisartan, and valsartan in tablets, JPC-J. Planar Chromatogr.-Modern TLC 25 (4) (2012) 326–330.
[5] N. Attaa Bakr, et al., First-derivative synchronous spectrofluorimetric method for estimation of losartan potassium and atorvastatin in their pure forms and in tablets, Luminescence 35 (4) (2020) 561–571.
[6] M. Arvand, et al., Direct determination of triamterene by potentiometry using a coated wire selective electrode, J. Pharm. Biomed. Anal. 33 (5) (2003) 975–982.
[7] F.A. Ibrahim, et al., Validated spectroscopic methods for the estimation of triamterene in bulk powder and capsule form via derivatization reactions, Luminescence 33 (8) (2018) 1423–1431.
[8] M. Luis, et al., Application of PLS regression to fluorimetric data for the determination of furosemide and triamterene in pharmaceutical preparations and triamterene in urine, Talanta 62 (2) (2004) 307–316.
[9] G. Ibañez, et al., Determination of triamterene in pharmaceutical formulations and of triamterene and its main metabolite hydroxytriamterene sulfate in urine using solid-phase and aqueous solution luminescence, Anal. Chim. Acta 538 (1–2) (2005) 77–84.
[10] A.M. Ramezani, G. Absalan, R. Ahmadi, Green-modified micellar liquid chromatography for isocratic isolation of some cardiovascular drugs with different polarities through experimental design approach, Anal. Chim. Acta 1010 (2018) 76–85.
[11] S.E. Kjeldsen, et al., Effects of losartan on cardiovascular morbidity and mortality in patients with isolated systolic hypertension and left ventricular hypertrophy: a Losartan Intervention for Endpoint Reduction (LIFE) substudy, J. Am. Med. Assoc. 288 (12) (2002) 1491–1498.
[12] N.E. Wagieh, et al., Spectrophotometric and spectrodensitometric determination of triamterene and xipamide in pure form and in pharmaceutical formulation, Drug Test. Anal. 2 (3) (2010) 113–121.
[13] K. Mohammadpour, M.R. Sohrabi, A. Jourabchi, Continuous wavelet and derivative transform applied to the overlapping spectra for the quantitative spectrophotometric multi-resolution of triamterene and hydrochlorothiazide in triamterene-H tablets, Talanta 81 (4–5) (2010) 1821–1825.
[14] A.F. Khorshid, M.M. Amin, Instantaneous determination of antihypertensive drugs utilizing TLC: Densitometric methods, Int. J. Front. Life Sci. Res. 2 (2022) 026–036.
[15] A. Maślanka, J. Krzek, M. Stolarczyk, Simultaneous analysis of hydrochlorothiazide, triamterene, furosemide, and spironolactone by densitometric TLC, Jpc–J. Planar Chromatogr.–Modern TLC 22 (2009) 405–410.
[16] F.F. Hudari, J.C. Souza, M.V.B. Zanoni, Adsorptive stripping voltammetry for simultaneous determination of hydrochlorothiazide and triamterene in hemodialysis samples using a multi-walled carbon nanotube-modified glassy carbon electrode, Talanta 179 (2018) 652–657.
[17] N. Fares, et al., Ultra-performance liquid chromatographic and densitometric methods for sensitive determination of xipamide and triamterene in pure and pharmaceutical dosage forms, J. AOAC Int. 105 (1) (2022) 19–25.
[18] H. Ahmad Panahi, M. Ejlali, M. Chabouk, Two-phase and three-phase liquid-phase microextraction of hydrochlorothiazide and triamterene in urine samples, Biomed. Chromatogr. 30 (7) (2016) 1022–1028.
[19] S.S. Abd El-Hay, H. Hashem, A.A. Gouda, High performance liquid chromatography for simultaneous determination of xipamide, triamterene and hydrochlorothiazide in bulk drug samples and dosage forms, Acta Pharm. 66 (1) (2016) 109–118.
[20] A.A. Ensafi, R. Hajian, Determination of losartan and triamterene in pharmaceutical compounds and urine using cathodic adsorptive stripping voltammetry, Anal. Sci. 24 (11) (2008) 1449–1454.
[21] R.M. Maggio, P.M. Castellano, T.S. Kaufman, A multivariate approach for the simultaneous determination of losartan potassium and hydrochlorothiazide in a combined pharmaceutical tablet formulation, Anal. Bioanal. Chem. 391 (2008) 2949–2955.
[22] M.C. Abeysekera, et al., Development, validation, and concentration determination of losartan potassium using 1D UV visible spectrophotometry, Syst. Rev. Pharm. 13 (2) (2022) 116–121.
[23] S.R. El-Shaboury, et al., Spectrofluorimetric method for determination of some angiotensin II receptor antagonists, J. Pharm. Anal. 2 (1) (2012) 12–18.
[24] S. Shalan, J.J. Nasr, Simultaneous evaluation of losartan and amlodipine besylate using second-derivative synchronous spectrofluorimetric technique and liquid chromatography with time-programmed fluorimetric detection, R. Soc. Open Sci. 6 (4) (2019) 190310.
[25] L. Kristoffersen, et al., Simultaneous determination of 6 beta-blockers, 3 calcium-channel antagonists, 4 angiotensin-II antagonists and 1 antiarrhytmic drug in post-mortem whole blood by automated solid phase extraction and liquid chromatography mass spectrometry: method development and robustness testing by experimental design, J. Chromatogr. B 850 (1–2) (2007) 147–160.
[26] M.A. Obando, J.M. Estela, V. Cerdà, Simultaneous determination of hydrochlorothiazide and losartan potassium in tablets by high-performance low-pressure chromatography using a multi-syringe burette coupled to a monolithic column, Anal. Bioanal. Chem. 391 (2008) 2349–2356.
[27] S. Sathe, S. Bari, Simultaneous analysis of losartan potassium, atenolol, and hydrochlorothiazide in bulk and in tablets by high-performance thin-layer chromatography with UV absorption densitometry, Acta Chromatogr. 19 (2007) 270–278.
[28] A.A. Khorshed, et al., Enhancing simultaneous determination of some angiotensin II receptor antagonists and amlodipine in plasma using HPTLC with fluorescence densitometry: Independent fluorescence detection of the co-administrative drugs in the mixture across various pH conditions, J. Chromatogr. B (2024) 124162.
[29] M. Quaglia, et al., Determination of losartan and hydrochlorothiazide in tablets by CE and CEC, J. Pharm. Biomed. Anal. 29 (6) (2002) 981–987.
[30] A.A. Khorshed, et al., Ultrasensitive TLC determination of montlukast and loratadine mixture in human plasma utilizing fluorescence detection at dual pH values: Toward attaining separate maximum fluorescence intensity, J. Chromatogr. B 1193 (2022) 123161.
[31] A.A. Khorshed, et al., Simultaneous determination of amlodipine besylate and azilsartan mixture in human plasma utilizing high-performance thin-layer chromatography with ultraviolet detection, Jpc–J. Planar Chromatogr.–Modern TLC (2024) 1–9.
[32] S. Hussein, et al., Application of high-performance thin-layer chromatography for screening and simultaneous determination of some angiotensin II receptor antagonists in dosage forms and plasma, JPC-J. Planar Chromatogr.-Modern TLC 27 (3) (2014) 192–198.
[33] S.-A.-E.-R. Hussein, et al., Development of two high-performance thin-layer chromatographic methods for the determination of irbesartan in tablets and plasma, Jpc–J. Planar Chromatogr.–Modern TLC 28 (2015) 83–89.
[34] S.-A.-E.-R. Hussein, et al., HPTLC with fluorescence densitometry for simultaneous determination of some angiotensin II receptor blockers in tablets and plasma, J. AOAC Int. 98 (2) (2015) 354–360.
[35] A.H. Ibrahim, M. Oraby, A.A. Khorshed, HPTLC determination of ergosterol in wheat and structure elucidation by NMR: Toward confirming method selectivity, J. Food Compos. Anal. 114 (2022) 104763.
[36] G.A. Salah, et al., Two selective HPTLC methods for determination of some angiotensin II receptor antagonists in tablets and biological fluids, Biomed. Chromatogr. 31 (7) (2017) e3916.
[37] E. Cagigal, et al., pKa determination of angiotensin II receptor antagonists (ARA II) by spectrofluorimetry, J. Pharm. Biomed. Anal. 26 (3) (2001) 477–486.
[38] Y. Kohara, et al., A new class of angiotensin II receptor antagonists with a novel acidic bioisostere, Bioorg. Med. Chem. Lett. 5 (17) (1995) 1903–1908.
[39] R.A. Shaalan, T.S. Belal, Simultaneous spectrofluorimetric determination of amlodipine besylate and valsartan in their combined tablets, Drug Test. Anal. 2 (10) (2010) 489–493.
[40] K. Santhana Lakshmi, S. Lakshmi, Simultaneous analysis of losartan potassium, amlodipine besylate, and hydrochlorothiazide in bulk and in tablets by high-performance thin layer chromatography with UV-absorption densitometry, J. Anal. Methods Chem. 2012 (1) (2012) 108281.
[41] S.A. Shah, et al., Simultaneous determination of losartan and hydrochlorothiazide in combined dosage forms by first-derivative spectroscopy and high-performance thin-layer chromatography, J. AOAC Int. 84 (6) (2001) 1715–1723.
[42] I.C.H.H.T. Guideline Validation of analytical procedures: text and methodology. Q2 (R1) **1**(20) (2005) 05.