Journal of Chromatography B 1241 (2024) 124162

Contents lists available at ScienceDirect

Journal of Chromatography B

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

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

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

[a] Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Sohag University, Sohag 82524, Egypt
[b] Department of Biomedical Engineering, University of Alberta, Edmonton, AB T6G 1H9, Canada
[c] Analytical Chemistry Department, Faculty of Pharmacy, Minia University, 61519 Minia, Egypt

ARTICLE INFO

Keywords:
Densitometry
Fluorescence
HPTLC
Amlodipine
AIIRAs
Plasma

ABSTRACT

A novel and highly sensitive high-performance thin-layer chromatographic (HPTLC) method was developed and validated to quantify a combination of five pharmaceutical mixtures spiked to human plasma. The compounds comprised Amlodipine (AML) along with five angiotensin II receptor antagonist drugs (AIIRAs), namely Olmesartan (OLM), Telmisartan (TLM), Candesartan (CAN), Losartan (LOS), and Irbesartan (IRB). HPTLC was performed on silica gel 60 F254 plates using a mobile phase of Toluene: ethyl acetate: methanol: acetone: acetic acid (6:1.5:1:0.5:1, v/v/v/v/v). In a pioneering move, a reflectance/fluorescence detection mode was employed to identify two concurrently administered drugs at different pH levels *for the first time*. This method utilized the same chromatographic system, incorporating a specific measurement for AML at a neutral medium to achieve its maximum fluorescence at a 360 nm excitation wavelength, and measuring emission using a 540 nm optical filter. The process involved obtaining a very low fluorescence response from AIIRA. Subsequently, to enhance AIIRA's fluorescence, the plate was sprayed with perchloric acid to transition to a strong acidic medium, ultimately attaining the maximum fluorescence of AIIRA using various excitation wavelengths and a 400 nm emission filter. Through this strategic process, we could optimize the fluorescence signals of both drugs, thereby elevating the sensitivity of detection for this drug combination. AML demonstrated a linear range of 18–300 ng/band, while AIIRAs drugs exhibited a linear range of 6–150 ng/band. The method satisfied the International Conference on Harmonization (ICH) criteria for recovery, precision, repeatability, and robustness, showcasing exceptional sensitivity. The approach was successfully applied to quantify AML and AIIRAs drugs in both bulk drug and plasma samples, achieving high recovery percentages and minimal standard deviations.

1. Introduction

Hypertension is a leading contributor to cardiovascular disease and premature death on a global scale [1]. Various risk factors, including obesity, alcohol consumption, physical inactivity, high sodium intake, low potassium intake, and unhealthy diets, contribute to hypertension [1]. The treatment of hypertension primarily involves five main classes of medications: calcium channel blockers, angiotensin II receptor antagonists (AIIRAs), angiotensin-converting enzyme inhibitors, beta-blockers, and diuretics [2]. AIIRAs, such as Losartan (LOS), were introduced in the late 1990s and quickly followed by Irbesartan (IRB), Valsartan, Candesartan (CAN), Telmisartan (TLM), and Olmesartan (OLM) [2]. AIIRAs represent a significant advancement in hypertension management, and they are recommended as the first-line treatment according to World Health Organization (WHO) guidelines due to their low side effect profile, patient compliance, and therapeutic effectiveness, especially when compared to angiotensin-converting enzyme inhibitors [3]. Calcium channel blockers (CCBs) are widely used in hypertension treatment and are considered the second-line treatment after AIIRAs [4]. CCBs are known for their lower side effects compared

* 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.jchromb.2024.124162
Received 21 March 2024; Received in revised form 10 May 2024; Accepted 17 May 2024
Available online 22 May 2024
1570-0232/© 2024 Published by Elsevier B.V.

to other antihypertensive medications and their effectiveness in reducing blood pressure [5].

Combining antihypertensive drugs can enhance overall treatment tolerance, especially when the pharmacological characteristics of one medication counteract the side effects of another [6]. This study focuses on the management of severe and resistant hypertension, including pseudo-resistance, secondary forms of hypertension, and appropriate pharmacological drug combination therapy [7]. Combining AIIRAs such as OLM, TLM, CAN, LOS, and IRB with CCBs like Amlodipine (AML) has been shown to effectively reduce and control blood pressure [8,9]. AIIRAs mitigate the risk of increased heart rate associated with AML and reduce peripheral edema, a common side effect [10]. The AIIRAs studied in this work contain tetrazole and imidazole rings, as well as an extended conjugated biphenyl moiety, responsible for their native fluorescence [11].While several analytical techniques, including spectrophotometry [12–17], spectrofluorometry [18–21] and square wave voltammetry [22–25], have been employed to determine these drugs, they were primarily applied to dosage forms rather than biological fluids. Chromatographic techniques, specifically HPLC [26–30] and GC–MS [31–33], were explored for the simultaneous detection of AIIRA and AML mixtures. Despite their potential, HPLC methods involved cumbersome sample preparations, including solid-phase extraction [34,35]and liquid–liquid extraction [36,37]. In parallel, GC methods introduced complexity with intricate derivatization steps [38], complicating the analytical process.

High-Performance Thin-Layer Chromatography (HPTLC) emerged as a promising alternative for drug analysis. Its exceptional resolution capability facilitated precise separation of complex mixtures, ensuring accuracy. HPTLC's efficiency, cost-effectiveness, and simultaneous analysis of multiple compounds on a single plate heightened its appeal in pharmaceutical applications. Certain reports utilized HPTLC to detect mixtures of AIIRA with AML, employing Reflectance Absorbance mode for detection [39–43] and the application was limited to dosage forms,

Notably, HPTLC in fluorescence mode exhibited remarkable sensitivity, allowing for the detection of drugs in biological fluids such as plasma with high sensitivity. This advantageous characteristic eliminates the necessity for complex sample treatments typically employed in HPLC when working with plasma samples, underscoring the practical utility of HPTLC in in-vivo analysis and pharmacokinetic studies[44,45]. The fluorescence intensity of the investigated AIIRA drugs reached its peak in a strong acidic medium. However, these stringent conditions, necessary for employing a fluorescence detector in their determination, cannot be applied in HPLC due to the potential damage they inflict on the stationary phase. Furthermore, the disposable stationary phase in HPTLC allowed for the use of drastic conditions, such as a strong acidic medium, enhancing method response and sensitivity—a capability absent in HPLC. The Reflectance/Fluorescence mode was employed for individual AIIRA detection in plasma [46,47]. However, there was a great need for simple HPTLC method for simple simultaneous determination of AIIRA with AML.

The challenge stemmed from the unique fluorescence characteristics of the two drugs in the mixture, each reaching optimal intensity at different pH levels. AML showed maximum fluorescence in a neutral medium but underwent quenching in an acidic environment. On the other hand, AIIRA, the companion drug typically co-administered with AML, displayed peak fluorescence in a strong acidic medium but experienced quenching in a neutral environment. This inherent divergence made achieving simultaneous detection under consistent conditions particularly challenging.

However, we capitalized on a distinctive feature of HPTLC: its non-destructive nature. Unlike HPLC, where compounds are discarded in waste after separation and entering the detector therefore both compounds should be measured under the same pH conditions. Preserving the separated samples on the TLC plate allowed us to address the challenge of measuring both compounds in the mixture at different pH conditions required to attain maximum fluorescence of each compound.

This study aimed to develop and test a new fluorescence densitometry High-Performance Thin Layer Chromatography (HPTLC) system for their simultaneous determination for the first time. Unlike previous reports, which limited use to pharmaceutical formulations, the high sensitivity obtained from this current method allowed detection of the studied drugs in plasma samples. Time, solvents, effort, and expense are all advantages of the existing process. The conditions impacting the fluorescence of the studied drugs were investigated and optimized. According to ICH criteria, the proposed approach was validated.

2. Experimental

2.1. Apparatus

Data was collected using Camag-HPTLC system with a Linomat V auto-sampler and TLC Scanner III, as well as winCATS version 1.4.4.6337 software (CAMAG, Muttenz, Switzerland). The source radiation for the reflectance/fluorescence scanning was high-pressure mercury vapor lamp. For the sample application, the Linomat was used in conjunction with a 100 mL Hamilton very precise syringe (Hamilton, Bonaduz, Switzerland) and a mild stream of nitrogen to allow the solvent to evaporate fast. The scanner's slit dimensions were set to 3 0.45 mm, and the scanning speed was set to 20 mm/s. The plates were developed in twin-trough chamber (27.0 x 26.5 x 7.0 cm, length x height x width; respectively Sigma-Aldrich, St. Louis, MO). The samples were prepared using an ultrasound bath device (Cole-Parmer, Chicago, IL) and Andreas Hettich GmbH centrifuge (Tuttingen, Germany).

2.2. Chemicals and materials

Olmesartan medoxomil was graciously provided by Chemipharm Pharmaceutical Co. (6th of October, Egypt). Telmisartan was sourced from Sigma Pharmaceutical Company in Qewaisna, Egypt. Candesartan cilexetil, losartan potassium, and amlodipine besylate were generously supplied by Global Napi Pharmaceutical Co. (6th of October, Egypt). Irbesartan was kindly supplied by Memphis Pharmaceutical Co. (Cairo, Egypt).

The purity of the investigated drugs underwent testing through TLC measurements, yielding results exceeding 98.00 %. Toluene, ethyl acetate, methanol, acetone, and acetic acid were procured from El-Nasr Pharmaceutical Chemicals in Abo-Zaabal, Cairo, Egypt.

2.3. Standard solution preparation

A 1.0 mg/ml concentration of the analyzed drugs (OLM, TLM, CAN, LOS, IRB, and AML) was prepared by accurately weighing 10 mg of each drug. The drugs were then placed into a 10 ml volumetric flask containing 8 ml of methanol, followed by a 10-minute sonication. The remaining volume was completed with methanol to reach a final volume of 10 ml. Subsequently, stock solutions of each drug were diluted with methanol to create working standard solutions: (2, 4, 8, 15, 35, and 50 μg/ml) for the studied AIIRAs drugs and (7, 14, 25, 40, 70, and 100 μg/ml) for AML.

A syringe was employed to apply a 3 μL aliquot of the prepared working solutions to the silica gel plate, resulting in final concentrations of (6, 12, 24, 45, 105, 150 ng/band) for the studied AIIRAs drugs and (21, 42, 75, 120, 210, 300 ng/band) for AML.

2.4. Plasma samples preparation

In an Eppendorf centrifuge tube from Hamburg, Germany, 150 μL of plasma sample was combined with 150 μL of a specific concentration of the investigated AIIRAs drugs and 150 μL of a particular concentration of AML standard solutions. Subsequently, the mixture underwent vortex mixing for 2 min. Following this, 300 μL of acetonitrile was added, and the mixture was vortex-mixed for 5 min. The final step involved

centrifugation at 14,000 rpm for 15 min at – 4 °C. The clear supernatant from each centrifuge tube was withdrawn and analyzed in accordance with the proposed method outlined under "Chromatographic conditions." This procedure was repeated in six replicates for each concentration, employing three different concentrations to cover the linear range of each drug.

2.5. Chromatographic conditions for the separation of the studied mixtures

The mobile phase employed to separate the analyzed mixtures consisted of 10 ml of Toluene: ethyl acetate: methanol: acetone: acetic acid (6: 1.5: 1: 0.5: 1, v/v/v/v/v), saturated for 20 min at room temperature. AML was initially detected based on its maximum fluorescence, observed upon excitation at 360 nm and using a 540 nm emission optical filter.

In contrast, the studied AIIRAs drugs exhibited their maximum fluorescence under strong acidic conditions, necessitating the plate to be sprayed with 0.2 M perchloric acid. Excitation wavelengths of 250, 300, 260, 260, and 262 nm were applied for OLM, TLM, CAN, LOS, and IRB, respectively. A 400 nm emission filter was utilized to measure the emitted fluorescence.

3. Results and discussion

The studied AIIRAs and AML were effectively used in the management of hypertension, Fig. 1. As all the documented HPTLC methods for the simultaneous determination of the studied AIIRA with AML were with UV detection, there is a pressing need to create a new HPTLC method with fluorescence densitometry for the determination of the studied mixtures. This current work provides a simple, quick, and sensitive method for simultaneous determination of AML and the studied

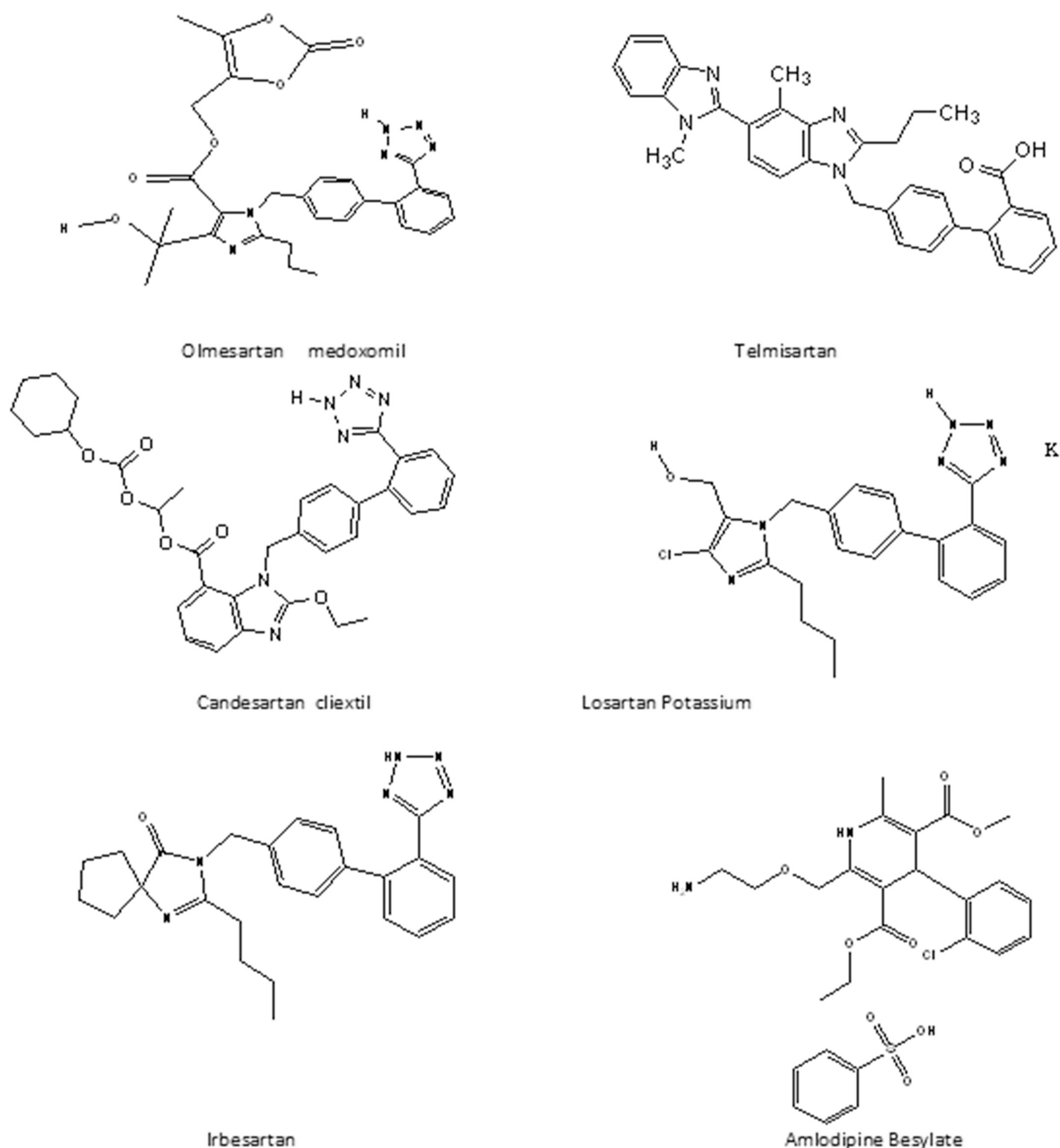

Fig. 1. Chemical structures of the studied drugs.

AIIRAs “using fluorescence detection” in spiked human plasma. The present method is highly sensitive since all of the reported HPTLC methods relied on the reflectance/ absorbance mode and thus were only applied in pharmaceutical dosage forms, but not biological samples.

The use of mobile phase of Toluene: ethyl acetate: methanol: acetone: acetic acid (6: 1.5: 1: 0.5: 1, v/v/v/v/v), gave well-separated, compacted and highly resolved bands for all the studied drugs.

AML had R_f value of (0.22 ± 0.002), and (0.50, 0.40, 0.62, 0.69 and 0.73 ± 0.001) for OLM, TLM, CAN, LOS and IRB, respectively.

AML exhibits fluorescence due to the presence of its dihydropyridine nucleus [48], and the analyzed AIIRAs demonstrate fluorescence due to various fluorescent functional groups, known as fluorophores, within their chemical structures. These groups include tetrazole, biphenyl, and/or imidazole [19].

Given that AIIRA and AML are administered together, there is a significant need to develop a sensitive method enabling their simultaneous detection in plasma after co-administration. The sensitivity attributed to the fluorescence detection mode facilitates their determination in plasma after optimizing conditions to attain maximum fluorescence intensity for both AIIRA and AML. While AML exhibits maximum fluorescence in a neutral medium, AIIRA has very low fluorescence intensity in a neutral medium, resulting in AIIRA not emerging in the chromatogram immediately after development, as shown in Fig. 2 a, illustrating the chromatogram for AML after development of the plate.

The literature distinctly affirms that the Relative Fluorescence Intensity (RFI) of AIIRA should be evaluated in acids or acidic media to optimize the detection of the inherent fluorescence of these drugs with maximum sensitivity. The acidic nature of these drugs, influenced by the presence of the tetrazole ring (with a pKa around 5), governs their acid-base characteristics. The respective pKa values for OLM, TLM, CAN, LOS, IRB are 4.14, 4.45, 6.0, 3.15, 4.70 [49]. Hence, an acidic medium is essential to ensure protonation and complete non-ionization of the tetrazole ring. This condition enables the localization of the proton over the four nitrogen atoms through resonance, consequently enhancing fluorescence intensity [50]. AIIRAs’ fluorescence intensity in an acidic media is at least five times more than in water [51].

Apart from possessing a 1,4-dihydropyridine ring structure, AML features two ester groups with four oxygen atoms in its configuration, capable of forming hydrogen bonds in a neutral medium. This safeguards the lowest excited singlet state of the fluorophore from non-radiative or potential quenching processes, resulting in a substantial increase in fluorescence intensity. However, in acidic solutions, there is a clear reduction in its fluorescence intensity due to the drug’s instability in acidic environments. As a consequence, the two drugs in each of the five studied mixtures exhibit maximum fluorescence at distinct pH conditions, with neutrality favoring AML and strong acidity favoring AIIRA. The optimal conditions for enhancing the fluorescence of one AIIRA lead to the quenching of AML, and vice versa.

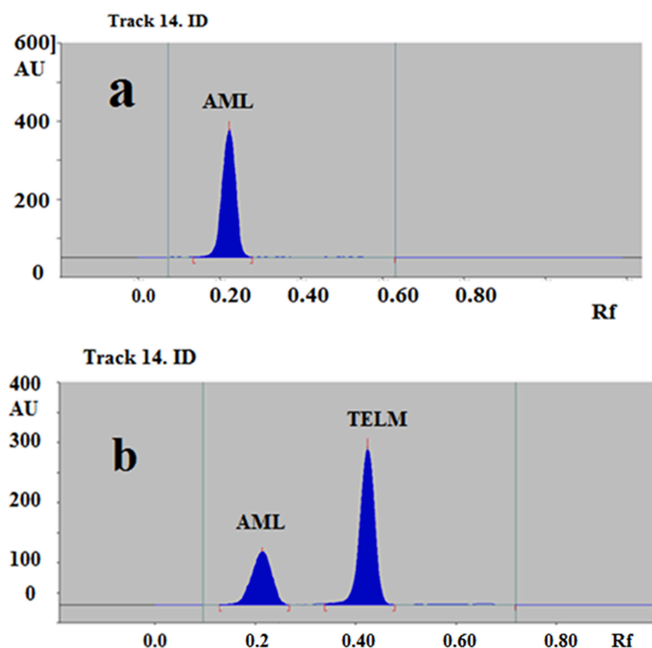

Fig. 2. HPTLC densitogram of mixture of AML (120 ng/band) and TELM (50 ng/band) (a) before (b) after spraying with 0.2 M perchloric acid.

HPTLC offers the advantage of scanning the plate multiple times under different reaction conditions. This unique capability allows for the high-sensitivity measurement of AML under its optimal conditions, which differ from those required for the quantification of the studied AIIRAs. After determining AML under the mentioned conditions, the plates were sprayed with acid to achieve the pH required to obtain maximum fluorescence of AIIRA. We tried several acids to enhance the fluorescence intensity such as acetic, phosphoric, hydrochloric, and sulfuric acids. Perchloric acid yielded the best results with minimal destructive effects on the stationary phase as shown in Fig. S1.

We utilize specific excitation wavelengths to determine each drug in the mixture, employing incident light from a mercury lamp. We utilize emission filters of 540 nm for AML and 400 nm for AIIRAs to eliminate interference from the excitation light and capture only the emitted light. Fluorescence detection enhances sensitivity compared to the previously reported UV detection methods [40,43,49,50]. We detect AML by excitation at 360 nm and using 540 nm as an emission optical filter. After that the plate was sprayed with 0.2 N perchloric acid and thoroughly dried. Subsequently, they were scanned at the specified excitation wavelength for each AIIRAs drug mentioned above and at 400 nm as the optical emission filter. By providing the necessary conditions for maximum AIIRA fluorescence, the peaks of the studied AIIRAs became clearly visible, as illustrated in (Fig. 2b). The HPTLC densitogram illustrating the determination of AML in mixture with CAND, IRB, LOS, and OLM are displayed in Fig. S2a, S3a, S4a, and S5a. Additionally, Fig. S2b, S3b, S4b, and S5b shows the densitogram of CAND, IRB, LOS, and OLM respectively after spraying with perchloric acid.

The high acidity conditions required for determining the studied AIIRAs to enhance their fluorescence are not feasible with HPLC, as it may damage the stationary phase. In contrast, the disposable nature of the stationary phase in HPTLC allows for the detection of these drugs under such severe and challenging circumstances.

3.1. Method development

Several solvent compositions had been tried, for the best separation of the studied mixtures. (Table S1). All of the samples were evenly distributed throughout the plate. All the targeted AIIRAs have a weakly acidic nature. The mobile phase used in the proposed method requires the addition of an acid to convert the tetrazole ring to its non-ionized form. This step ensures the formation of compact spots at suitable Rf values for effective separation of the co-administered drugs. It’s crucial because the ionized form of the tetrazole ring may interact with the polar silanol groups present in the silica gel of the HPTLC plates, leading to band tailing and very low Rf values. The solvent system of Toluene: ethyl acetate: methanol: acetone: acetic acid (6: 1.5: 1: 0.5: 1, v/v/v/v/v) was utilized as mobile phase for the quantification of the studied mixtures, which had an Rf value of (0.22 ± 0.002) for AML, and (0.50, 0.40, 0.62, 0.69 and 0.73 ± 0.001) for OLM, TLM, CAN, LOS and IRB, respectively, as shown in Fig. 2 using mixture AML – TLM as a representative example. This solvent system provided compacted and highly characterized bands with well separation between the studied drugs and AML. The volume of all solvents in the solvent system had a significant effect on the Rf value and separation as illustrated in **(Table S1).** The HPTLC plates were thoroughly dried using a hot-air drier after development. AML was detected first by scanning at 360 nm excitation wavelength and 540 nm emission optical filter. While the studied AIIRAs

drugs show maximum fluorescence by excitation at 250, 300, 260, 260 and 262 nm for OLM, TLM, CAN, LOS and IRB, respectively and 400 nm emission filter after spraying the plates with 0.2 M perchloric acid.

3.2. Validation of the method

The method was validated using ICH criteria for validating analytical processes, and all findings were reported as percentages in terms of linearity, accuracy, precision, limits of detection, limits of quantification and robustness. The statistical analysis was carried out with the 95 % confidence level.

4. Linearity

The peak area response and the corresponding concentration were used to create a calibration curve. For each concentration of the studied mixtures, three measurements were constructed. The calibration curve's linear regression data revealed an acceptable linearity for the relationship between peak area and the corresponding drugs concentrations in a range of (18–300 ng/band) for AML and (6–150 ng/band) for the studied AIIRAs drugs (Fig. 3 a, b), as shown in (Table 1). The correlation

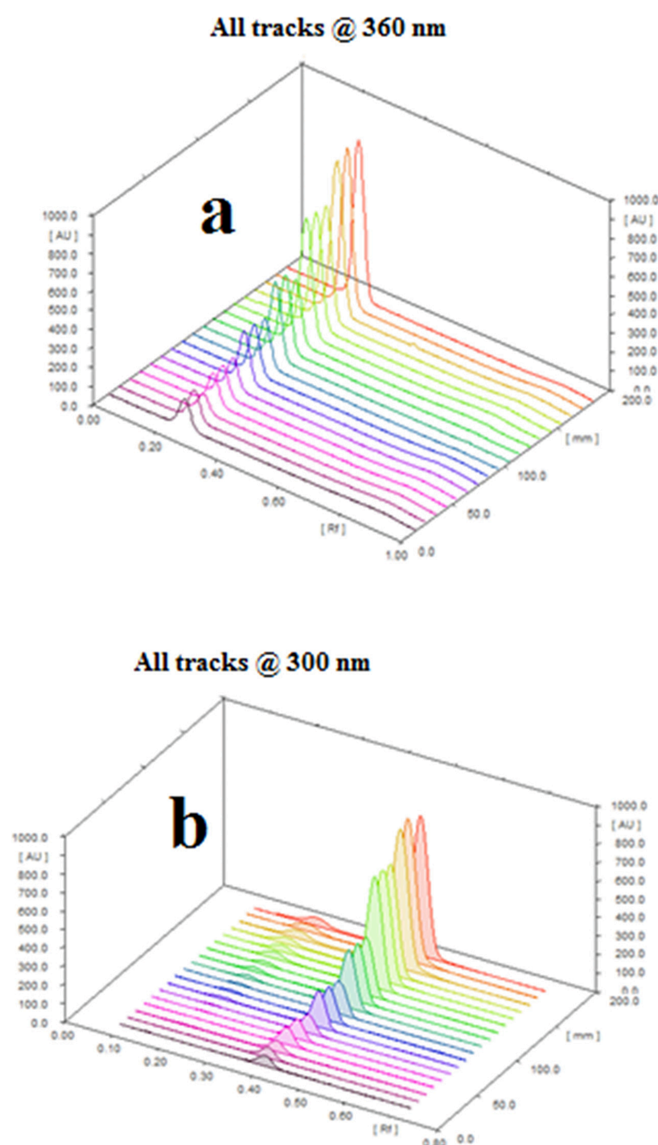

Fig. 3. 3D graph of the calibration of AML (21–300 ng/band) and TELM (6–150 ng/band) (a) before and (b) after spraying with.

coefficients were in the range 0.9974–0.9996, indicating good linearity of the proposed method. The calibration curve was carried out in spiked plasma samples and the drugs show good linearity (Table S2) over the ranges of (42–120) for AML and (12–105) for AIIRA.

5. Detection and quantification limits

The proposed method utilizes fluorescence detection to concurrently analyze five AIIRAs alongside AML. Previous approaches for their simultaneous determination relied on reflectance absorbance mode for detection [42,43], which exhibited significantly lower sensitivity. To the author's knowledge, there is no existing HPTLC method for the simultaneous determination of the co-administered AIIRAs and AML utilizing fluorescence detection. Reflectance/fluorescence mode was solely employed for determining angiotensin II receptor antagonists individually in plasma samples[47]. The method's sensitivity was assessed by determining the limit of detection (LOD) and the limit of quantitation (LOQ) using the standard deviation of the intercept's response and the calibration curve slope (LOQ). The LOD and LOQ were calculated based on the formulas LOD = 3 Ϭ/ b and LOQ = 10 Ϭ/ b, where Ϭ represents the intercept's standard deviation, and b is the calibration curve slope. The calculated LOD and LOQ values, as presented in Table 1 for synthetic mixture of the standard drugs. The Calculated LOD and LOQ values in spiked plasma are given in Table S2.

6. Accuracy

Six replicate measurements of three different concentrations of each drug were assessed to determine the accuracy of the proposed HPTLC method. The results were expressed as a percentage of recovery ± standard deviation. (Refer to Table 2**)** The proposed method's great accuracy was demonstrated by the close agreement of percent recoveries to 100 % with minimum values of standard deviation. The percentage recoveries ranged from 97.23 % to 102.55 % which indicated good accuracy of the proposed HPTLC method.

7. Precision

Repeatability and intermediate precision were used to evaluate the precision of the suggested approach in terms of Intra-day and inter-day precision. The repeatability of the proposed HPTLC method was assessed by analyzing three different concentrations of each drug six times intra-daily. The intermediate precision of the proposed methodology was then examined by repeating the previously mentioned steps six times in three different days. The precision of the recommended technique at the two examined levels was expressed as relative standard deviation (RSD) and the results were given in **(**Table 3**)**. For all concentration levels of each drug, the RSD didn't exceed 2.88 % which indicating good precision of the proposed HPTLC method.

8. Robustness

Robustness means an analytical procedure's capacity to remain unchanged by minor changes in optimum technique parameters without altering quantitation. The mobile phase composition, excitation wavelength, saturation time, and perchloric acid concentration were chosen as the four essential factors to be checked in the robustness testing to determine the method's robustness. The outcomes are displayed in **(**Table 4**)**. It was discovered that none of these variables had a substantial effect on the drug determination. The suggested HPTLC approach is considered robust as a result of this indication of the proposed method's reliability.

8.1. Application of the proposed HPTLC method in spiked human plasma

This novel HPTLC approach has a high sensitivity, allowing

Table 1
Statistical data for the simultaneous HPTLC determination of some AIIRAs – amlodipine mixtures.

Parameters	OLM – AML		TLM – AML		CAN – AML		LOS – AML		IRB – AML	
	OLM	AML	TLM	AML	CAN	AML	LOS	AML	IRB	AML
Linear range (ng/band)	6–150	21–300	6–150	21–300	6–150	21–300	6–150	18–300	6–150	18–300
Correlation coefficient, r	0.9980	0.9987	0.9993	0.9989	0.9996	0.9983	0.9992	0.9991	0.9982	0.9974
Determination Coefficient, r2	0.9960	0.9975	0.9986	0.9979	0.9992	0.9967	0.9985	0.9982	0.9963	0.9948
Slope ± SD (a)	57.509 ± 1.819	11.684 ± 0.287	95.114 ± 1.716	11.313 ± 0.256	49.812 ± 0.676	10.840 ± 0.309	49.135 ± 0.754	10.30 ± 0.176	73.33± 2.229	9.78 ± 0.286
Intercept ± SD (a)	−407.883 ± 7.58	10.223 ± 7.097	556.687 ± 16.726	72.679 ± 8.62	883.356 ± 11.56	211.047 ± 9.493	108.325 ± 6.37	92.00 ± 3.933	142.564 ± 22.45	98.83 ± 6.80
Rf	0.50 ± 0.001	0.22 ± 0.002	0.40 ± 0.001	0.22 ± 0.002	0.62 ± 0.001	0.22 ± 0.002	0.69 ± 0.001	0.22 ± 0.002	0.73 ± 0.001	0.22 ± 0.002
LOD (ng/band)	0.44	2.00	0.58	2.51	0.76	2.88	0.43	1.26	1.01	2.29
LOQ (ng/band)	1.32	6.07	1.76	7.62	2.32	8.75	1.30	3.82	3.06	6.95

(a) Average of three determinations.

Table 2
Evaluation of the accuracy of the proposed HPTLC method for simultaneous determination of the studied AIIRAs– amlodipine mixtures.

Studied mixtures	AIIRAs OLM, TLM, CAN, LOS, IRB			AML		
	Conc. (ng\band)	Amount found (ng/band)	% Recovery (a) ± SD	Conc. (ng\band)	Amount found (ng/band)	% Recovery (a) ± SD
OLM – AML	18	17.66	98.14 ± 0.39	42	41.24	98.21 ± 1.16
	60	58.34	97.23 ± 0.87	120	122.07	101.72 ± 2.26
	150	152.55	101.70 ± 0.46	300	299.47	99.82 ± 1.19
TLM – AML	12	11.73	97.72 ± 0.55	42	41.04	97.72 ± 1.34
	45	46.14	102.52 ± 1.93	120	120.71	100.59 ± 1.61
	105	104.76	99.77 ± 1.30	210	215.35	102.55 ± 0.55
CAN – AML	12	12.26	102.16 ± 2.34	75	74.63	99.50 ± 2.29
	45	45.28	100.62 ± 0.97	210	210.78	100.37 ± 1.70
	150	149.63	99.76 ± 1.77	300	296.58	98.86 ± 0.99
LOS – AML	18	18.05	100.25 ± 1.84	30	29.99	99.97 ± 2.07
	60	60.76	101.27 ± 1.91	150	152.50	101.66 ± 2.64
	150	149.30	99.53 ± 1.78	300	296.17	98.72 ± 1.44
IRB – AML	9	9.07	100.82 ± 2.15	60	60.24	100.40 ± 2.28
	60	59.85	99.76 ± 0.84	90	90.71	100.79 ± 1.68
	105	105.71	100.68 ± 1.66	210	209.52	99.77 ± 0.80

(a) Average of six determinations at each concentration level.

Table 3
Precision of the proposed HPTLC method for simultaneous determination of the studied AIIRAs– amlodipine mixtures at the intra- and inter-day levels.

Studied mixtures	AIIRAs OLM, TLM, CAN, LOS, IRB			AML		
	Conc. (ng\band)	Intra-day RSD % (a)	Inter-day RSD % (b)	Conc. (ng\band)	Intra-day RSD % (a)	Inter-day RSD % (b)
OLM – AML	18	2.79	2.14	42	1.66	2.73
	60	1.05	0.58	120	2.04	2.12
	150	0.48	1.60	300	1.54	1.86
TLM – AML	12	1.78	2.20	42	1.12	2.11
	45	1.11	1.21	120	0.84	1.17
	105	1.06	1.88	210	0.62	1.36
CAN – AML	12	2.32	2.34	75	1.89	1.66
	45	1.80	2.21	210	1.50	2.16
	150	2.17	1.18	300	1.02	0.95
LOS – AML	18	2.25	1.74	30	2.35	2.15
	60	2.88	2.28	150	1.10	1.64
	150	2.56	1.46	300	1.57	0.48
IRB – AML	9	1.62	2.52	60	2.21	2.30
	60	0.82	2.50	90	1.49	1.99
	105	0.50	2.88	210	1.19	0.76

(a) Average of 6 determinations at each concentration level.
(b) Average of 18 determinations at each concentration level over three distinct days.

verification of the examined drugs in spiked human plasma in a very simple and low-cost method. Due to the difficulty in obtaining blood from hypertensive patients who had been treated with the studied drugs, we opted to test our proposed approach on spiked human plasma samples to establish its potential to identify residual levels of the studied drugs after delivery in human plasma. The usefulness of this current methodology for studying these anti-hypertensive drugs in spiked plasma, was confirmed by the high estimated mean percent recovery (Table 5). Three different concentration spiking levels were analyzed of each mixture according to the proposed method described above. The obtained recovery percentages ranging from 93.35 to 107.08. The retrieved data's standard deviation was within the range of 0.56–2.73 for all the studied mixtures within the analytical methods allowed limits. These figures demonstrated the minimal effect of matrix, instrumental and personal errors contributed to the low variability Fig. 4a. The results obtained in Table 5 demonstrate that the suggested approach can reliably calculate AML and the studied AIIRAs drugs in human plasma while avoiding any substantial interference from the matrix of plasma (Fig. 4 **b, c**).

9. Conclusion

The suggested HPTLC approach with fluorescence detection is speedy, simple, affordable, and sensitive for simultaneous determination of AML and the studied AIIRAs drugs with high accuracy and precision. There is no reported HPTLC method which is general for the

Table 4
Results for the investigation of the robustness of the proposed HPTLC method for simultaneous determination of the studied AIIRAs– amlodipine mixtures.

Parameters	OLM – AML		TLM – AML		CAN – AML		LOS – AML		IRB – AML	
	OLM	AML	TLM	AML	CAN	AML	LOS	AML	IRB	AML
Optimum parameters	101.70 ± 0.46	99.82 ± 1.19	99.77 ± 1.30	100.59 ± 1.61	100.62 ± 0.97	100.37 ± 1.70	99.53 ± 1.78	99.97 ± 2.07	99.76 ± 0.84	100.40 ± 2.28
Composition of the mobile phase Toluene: ethylacetate: methanol: acetone: acetic acid (5.8: 1.7: 1: 0.5: 1, v/v/v/v/v)	99.15 ± 1.45	100.75 ± 2.24	102.13 ± 1.68	98.44 ± 0.75	97.92 ± 2.17	99.46 ± 1.59	100.36 ± 1.48	101.44 ± 2.29	99.28 ± 1.55	100.57 ± 1.38
Toluene: ethylacetate: methanol: acetone: acetic acid (5.8: 1.5: 1.1: 0.5: 1.1, v/v/v/v/v)	100.39 ± 1.54	99.46 ± 1.82	99.62 ± 1.77	102.32 ± 2.36	98.85 ± 1.79	101.34 ± 1.49	98.70 ± 1.38	100.30 ± 1.57	101.44 ± 0.67	99.60 ± 2.34
Toluene: ethylacetate: methanol: acetone: acetic acid (6: 1.5: 0.8: 0.7: 1, v/v/v/v/v)	98.26 ± 1.39	100.65 ± 2.07	101.72 ± 1.88	99.36 ± 1.69	97.87 ± 0.98	99.16 ± 1.57	99.54 ± 2.34	102.25 ± 1.56	98.42 ± 1.83	99.79 ± 2.67
Scanning wavelength, 360 nm		98.39 ± 1.46		100.66 ± 1.23		99.76 ± 1.22		98.59 ± 0.94		101.12 ± 1.56
358	–	100.79 ± 1.28	–	99.49 ± 1.73	–	98.48 ± 1.34	–	98.68 ± 2.13		100.45 ± 0.78
362	–		–		–		–			
248	100.78 ± 1.59									
252	101.14 ± 0.78	–	–	–	–	–	–	–		
298			99.28 ± 1.54							
302	–	–	98.96 ± 2.26	–	–	–	–	–	–	–
258					101.36 ± 2.07		99.24 ± 1.53			
262	–	–	–	–	100.61 ± 1.89	–	98.58 ± 1.25	–	–	–
260									101.39 ± 1.77	
264	–	–	–	–	–	–	–	–	99.68 ± 1.65	–
Concentration of perchloric acid										
0.19 M	99.37 ± 1.56	98.68 ± 2.18	99.55 ± 1.33	100.57 ± 2.11	101.72 ± 1.80	98.78 ± 2.46	97.68 ± 0.69	102.21 ± 1.43	99.30 ± 1.41	98.52 ± 1.70
0.21 M	99.10 ± 1.25	100.40 ± 1.17	98.86 ± 1.51	100.39 ± 0.75	102.09 ± 1.26	99.66 ± 1.40	99.67 ± 2.35	100.25 ± 1.90	99.71 ± 0.86	100.47 ± 1.63
Saturation time										
18 mins	100.39 ± 0.57	101.02 ± 1.60	99.58 ± 1.49	97.83 ± 1.20	99.34 ± 1.55	102.30 ± 1.42	98.46 ± 2.59	99.52 ± 0.73	97.86 ± 1.40	100.56 ± 2.03
22 mins	99.40 ± 1.92	100.37 ± 1.60	98.76 ± 1.23	98.96 ± 2.48	101.32 ± 1.11	100.62 ± 0.88	98.78 ± 2.44	102.07 ± 1.38	98.69 ± 0.97	99.08 ± 1.50

(a) Average of six determinations.

Table 5
Application of the proposed HPTLC method for simultaneous determination of the studied AIIRAs– amlodipine mixtures in spiked human plasma.

Studied mixtures	AIIRAs OLM, TLM, CAN, LOS, IRB			AML		
	Conc. (ng\band)	Amount found (ng/band)	% Recovery (a) ± SD	Conc. (ng\band)	Amount found (ng/band)	% Recovery (a) ± SD
OLM – AML	12	11.20	93.35 ± 1.82	21	21.51	102.43 ± 1.96
	45	43.38	96.39 ± 2.33	42	44.36	105.61 ± 2.57
	105	106.54	101.47 ± 1.45	120	121.66	101.38 ± 1.69
TLM – AML	24	24.31	101.28 ± 2.68	42	44.23	105.30 ± 1.24
	45	44.41	98.68 ± 2.72	75	72.67	96.88 ± 0.83
	105	112.03	106.70 ± 1.35	120	117.05	97.54 ± 2.73
CAN – AML	12	11.53	96.09 ± 0.96	42	42.71	101.69 ± 1.54
	24	23.31	97.11 ± 1.07	75	78.09	104.12 ± 1.38
	105	100.38	95.60 ± 1.58	120	128.50	107.08 ± 0.56
LOS – AML	18	18.84	104.65 ± 1.36	30	31.12	103.74 ± 0.79
	60	58.21	97.01 ± 0.78	60	61.22	102.03 ± 1.22
	105	110.49	105.22 ± 2.16	90	93.26	103.62 ± 1.79
IRB – AML	9	8.58	95.33 ± 2.47	60	58.76	97.94 ± 1.26
	45	43.70	97.11 ± 1.29	90	93.62	104.02 ± 1.48
	60	61.60	102.67 ± 0.68	120	122.92	102.43 ± 0.89

(a) Average of six determinations at each concentration level.

simultaneous determination of AML with different members of AIIRAs. As with all prior HPTLC procedures, the reflectance/absorbance mode and the dosage forms were used instead of biological fluids. To obtain good separation, the mobile phase Toluene: ethyl acetate: methanol: acetone: acetic acid (6: 1.5: 1: 0.5: 1, v/v/v/v/v) was used. After development and AML detection, spray the chromatogram using 0.2 M perchloric acid to increase the fluorescence of the studied AIIRAs drugs. In the present study, the reflectance/fluorescence mode used for

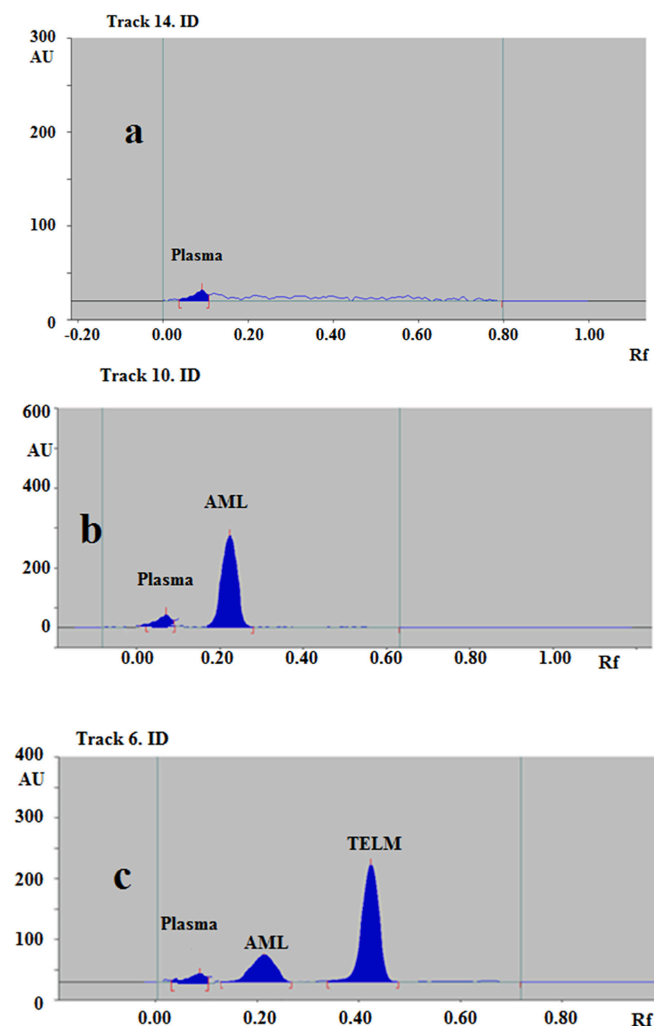

Fig. 4. HPTLC densitogram of (a) blank plasma sample and plasma sample spiked with mixture of AML (70 ng / band) and TELM (25).

analytes detection for the first time. The constructed approach was able to analyze AML and the studied AIIRAs drugs in the human plasma due to the proposed method's high sensitivity and the selectivity and simplicity of HPTLC. Because of the excellent recovery of the studied drugs from plasma, this method is suitable for their determination without interference from various plasma constituents and can be employed in pharmacokinetic studies.

CRediT authorship contribution statement

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

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

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jchromb.2024.124162.

References

[1] K.T. Mills, A. Stefanescu, J. He, The global epidemiology of hypertension, Nat. Rev. Nephrol. 16 (4) (2020) 223–237.
[2] S. Laurent, **Antihypertensive drugs**, *Pharmacol. Res.* 124 (2017) 116–125.
[3] M. Burnier, H. Brunner, Angiotensin II receptor antagonists, Lancet 355 (9204) (2000) 637–645.
[4] S. Honma, I. Tani, M. Sakai, I. Soma, K. Toriyabe, M. Yoshida, Effect of N-acetyl cysteine on renal interstitial fibrosis in mice, Biol. Pharm. Bull. 43 (12) (2020) 1940–1944.
[5] A. Graudins, H.M. Lee, D. Druda, Calcium channel antagonist and beta-blocker overdose: antidotes and adjunct therapies, Br. J. Clin. Pharmacol. 81 (3) (2016) 453–461.
[6] D.A. Sica, Rationale for fixed-dose combinations in the treatment of hypertension: the cycle repeats, Drugs 62 (2002) 443–462.
[7] W.B. White, J.R. Turner, D.A. Sica, J.D. Bisognano, D.A. Calhoun, R.R. Townsend, H.D. Aronow, D.L. Bhatt, G.L. Bakris, Detection, evaluation, and treatment of severe and resistant hypertension: proceedings from an American Society of Hypertension Interactive forum held in Bethesda, MD, USA, October 10th 2013, J. Am. Soc. Hypertens. 8 (10) (2014) 743–757.
[8] L. Ma, W. Wang, Y. Zhao, Y. Zhang, Q. Deng, M. Liu, H. Sun, J. Wang, L. Liu, Combination of amlodipine plus angiotensin receptor blocker or diuretics in high-risk hypertensive patients: a 96-week efficacy and safety study, Am. J. Cardiovasc. Drugs 12 (2012) 137–142.
[9] T. Ishimitsu, A. Numabe, T. Masuda, T. Akabane, A. Okamura, J. Minami, H. Matsuoka, Angiotensin-II receptor antagonist combined with calcium channel blocker or diuretic for essential hypertension, Hypertens. Res. 32 (11) (2009) 962–968.
[10] G.L. Bakris, Combined therapy with a calcium channel blocker and an angiotensin II type 1 receptor blocker, J. Clin. Hypertension 10 (2008) 27–32.
[11] Z.K. Gryczynski, I. Gryczynski, Practical fluorescence spectroscopy, CRC Press, 2019.
[12] P.S. Patil, P.D. Kulkarni, M.S. Burkul, H.N. More, S.A. Pishawikar, Simultaneous estimation of amlodipine besylate and olmesartan medoxomil by first order derivative spectroscopy from tablet, Int. J. PharmTech Res. 3 (2) (2011) 668–675.
[13] N. Jain, R. Jain, A. Jain, S.P. Pandey, D.K. Jain, Spectrophotometric method development and validation for quantitative estimation of amlodipine besylate in bulk drug and their dosage forms by using hydrotropic agent, Eurasian J. Anal. Chem. 5 (3) (2010) 212–217.
[14] M. Celebier, S. Altinoz, Determination of olmesartan medoxomil in tablets by UV-Vis spectrophotometry, Die Pharmazie – Int. J. Pharm. Sci. 62 (6) (2007) 419–422.
[15] A. Pandey, H. Sawarkar, M. Singh, P. Kashyap, P. Ghosh, UV-spectrophotometric method for estimation of telmisartan in bulk and tablet dosage form, Int. J. ChemTech Res. 3 (2) (2011) 657–660.
[16] N. Ahmad Charoo, M. Bashir, E. Abdalla, K.I.H. Ali, Determination of candesartan cilexetil in tablet dosage forms and dissolution testing samples by first derivative UV spectrophotometric method, Anal. Lett. 42 (14) (2009) 2232–2243.
[17] P.R. Patil, S.U. Rakesh, P. Dhabale, K. Burade, Simultaneous UV spectrophotometric method for estimation of losartan potssium and amlodipine besylate in tablet dosage form, Asian J. Res. Chem. 2 (2) (2009) 183–187.
[18] A.A. Abdelmonem, G.H. Ragab, H.A. Hashem, E.A. Bahgat, Spectrofluorimetric and spectrophotometric determination of irbesartan and bisoprolol hemifumarate independently in their tablets, Pharm. Biosci. J. (2016) 43–52.
[19] S.R. El-Shaboury, S.A. Hussein, N.A. Mohamed, M.M. El-Sutohy, Spectrofluorimetric method for determination of some angiotensin II receptor antagonists, J. Pharm. Anal. 2 (1) (2012) 12–18.
[20] 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.
[21] T.S. Belal, M.S. Mahrous, M.M. Abdel-Khalek, H.G. Daabees, M.M. Khamis, Validated spectrofluorimetric determination of two pharmaceutical antihypertensive mixtures containing amlodipine besylate together with either candesartan cilexetil or telmisartan, Luminescence 29 (7) (2014) 893–900.
[22] İ.M. Palabıyık, A. Dogan, İ. Süslü, Simultaneous determination of amlodipine and Irbesartan in their pharmaceutical formulations by square-wave voltammetry, Comb. Chem. High Throughput Screen. 25 (2) (2022) 241–251.
[23] N.A. Alarfaj, Square-wave adsorptive stripping voltammetric determination of antihypertensive agent telmisartan in tablets and its application to human plasma, J. Anal. Chem. 68 (2013) 335–340.
[24] İ. Süslü, N. Özaltin, S. Altinöz, Voltammetric determination of candesartan cilexetil in its Cu (II) complex and application to pharmaceutical formulations, J. AOAC Int. 93 (2) (2010) 562–568.

[25] M.C.G. Santos, C.R.T. Tarley, L.H. Dall'Antonia, E.R. Sartori, **Evaluation of boron-doped diamond electrode for simultaneous voltammetric determination of hydrochlorothiazide and losartan in pharmaceutical formulations**, Sens. Actuators B 188 (2013) 263–270.

[26] L.P. Peikova, B.G. Tsvetkova, B.D. Kostova, D.R. Rachev, Investigations and HPLC assay of model formulations containing amlodipine besylate and lisinopril, Int. J. Pharm. Sci. Rev. Res. 20 (2013) 11–15.

[27] A.M. Alanazi, A.S. Abdelhameed, N.Y. Khalil, A.A. Khan, I.A. Darwish, HPLC method with monolithic column for simultaneous determination of irbesartan and hydrochlorothiazide in tablets, Acta Pharm. 64 (2) (2014) 187–198.

[28] H.A. Alhazmi, A.M. Alnami, M.A. Arishi, R.K. Alameer, M. Al Bratty, Z.U. Rehman, S.A. Javed, I.A. Arbab, A fast and validated reversed-phase HPLC method for simultaneous determination of simvastatin, atorvastatin, telmisartan and irbesartan in bulk drugs and tablet formulations, Sci. Pharm. 86 (1) (2018) 1.

[29] M.A. Kottai, R. Sankhla, S. Gupta, A. Smith, R. Manavalan, **Development and validation of a reversed phase HPLC method for simultaneous determination of amlodipine and telmisartan in pharmaceutical dosage form**, 2010.

[30] P.D. Hamrapurkar, K.K. Gadapayale, Optimization and validation of RP-HPLC stability indicating method for determination of olmesartan medoxomil and its degraded product, Int. J. Appl. Sci. Eng. 11 (2) (2013) 137–147.

[31] W. Wichitnithad, O. Sudtanon, P. Srisunak, K. Cheewatanakornkool, S. Nantaphol, P. Rojsitthisak, Development of a sensitive headspace gas chromatography–mass spectrometry method for the simultaneous determination of nitrosamines in losartan active pharmaceutical ingredients, ACS Omega 6 (16) (2021) 11048–11058.

[32] W. Qi, Q. Zhao, J. Jiang, P. Hu, Simultaneous determination of olmesartan and amlodipine in human plasma and urine by ultra performance liquid chromatography tandem mass spectrometry, J. Chromatogr. B 938 (2013) 27–34.

[33] T.N. Rao, T. Patrudu, A.S. Kumar, N.K. Rao, K. Apparao, A new analytical method validation and quantification of residual solvents in telmisartan bulk drug product by headspace gas chromatographic method, Res. J. Sci. Technol. 10 (2) (2018) 98–104.

[34] P.K. Yeung, P.T. Pollak, A. Jamieson, G.J. Smith, D. Fice, Determination of plasma concentrations of losartan in patients by HPLC using solid phase extraction and UV detection, Int. J. Pharm. 204 (1–2) (2000) 17–22.

[35] G. Castro, I. Rodríguez, M. Ramil, R. Cela, Selective determination of sartan drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography tandem mass spectrometry, Chemosphere 224 (2019) 562–571.

[36] S. Soltani, A.M. Ramezani, N. Soltani, A. Jouyban, Analysis of losartan and carvedilol in urine and plasma samples using a dispersive liquid–liquid microextraction isocratic HPLC–UV method, Bioanalysis 4 (23) (2012) 2805–2821.

[37] E.F. Elkady, A.A. Mandour, F.K. Algethami, A.A. Aboelwafa, F. Farouk, Sequential liquid-liquid extraction coupled to LC-MS/MS for simultaneous determination of amlodipine, olmesartan and hydrochlorothiazide in plasma samples: Application to pharmacokinetic studies, Microchem. J. 155 (2020) 104757.

[38] C.-H. Feng, Y.-M. Chang, J.-R. Weng, Rapid methylation of valsartan in human plasma using evaporative derivatization reagent to improve its sensitivity in MALDI-TOF mass spectrometry, Anal. Methods 14 (37) (2022) 3694–3701.

[39] 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 (2012).

[40] S. Saeed, A.H. Nadim, A.M. Yehia, A.A. Moustafa, A versatile high-performance thin-layer chromatographic method for the simultaneous determination of five antihypertensive drugs: method validation and application to different pharmaceutical formulations, JPC – J. Planar Chromatogr.-Modern TLC 34 (2021) 467–477.

[41] S.K. Shah, D.P. Kawade, A.J. Asnani, D.R. Chaple, Simultaneous quantitative analysis of olmesartan medoxomil and amlodipine besylate in plasma by Hptlc technique, European J. Pharm. Med. Res. 3 (9) (2016) 410–515.

[42] T.B. Deshmukh, S.S. Deo, F.S. Inam, T.L. Lambat, Development and validation of novel HPTLC method for the simultaneous estimation of amlodipine besylate and telmisartan in tablet dosage form using ICH Q2 (R1) directions, J. Chinese Adv. Mater. Soc. 6 (4) (2018) 329–340.

[43] M.A. Hinge, D. Patel, Simultaneous estimation of amlodipine besylate and candesartan cilexitil by HPTLC method in their combined dosage form, Int. J. Pharm. Res. (09752366) 13 (2) (2021).

[44] A.A. Khorshed, F.M. Abdelnaeem, S.M. Derayea, D.M. Nagy, M. Oraby, 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.

[45] A.A. Khorshed, F.M. Abdelnaeem, S.M. Derayea, M. Oraby, D.M. Nagy, Concurrent estimation of montelukast and loratadine mixture in spiked human plasma utilizing high-performance thin-layer chromatography with ultraviolet detection, JPC–J. Planar Chromatogr.-Modern TLC 35 (6) (2022) 617–625.

[46] G.A. Salah, H.M. Abd El-Wadood, M. Khairy, A.A. Khorshed, Two selective HPTLC methods for determination of some angiotensin II receptor antagonists in tablets and biological fluids, Biomed. Chromatogr. 31 (7) (2017) e3916.

[47] S.-A.-E.-R. Hussein, H.M.A. El-Wadood, M.-A.-E.-W. Abdallah, A.-A.-E.-H. Khorshed, 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.

[48] D.J. Triggle, The 1, 4-dihydropyridine nucleus: a pharmacophoric template part 1. Actions at ion channels, Mini Rev. Med. Chem. 3 (3) (2003) 215–223.

[49] E. Cagigal, L. Gonzalez, R. Alonso, R. Jimenez, pKa determination of angiotensin II receptor antagonists (ARA II) by spectrofluorimetry, J. Pharm. Biomed. Anal. 26 (3) (2001) 477–486.

[50] Y. Kohara, E. Imamiya, K. Kubo, T. Wada, Y. Inada, T. Naka, A new class of angiotensin II receptor antagonists with a novel acidic bioisostere, Bioorg. Med. Chem. Lett. 5 (17) (1995) 1903–1908.

[51] 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.