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Dual TLC approaches for ICU drug monitoring: A comparative study of densitometry and portable smartphone-based detection of piperacillin, tazobactam, and paracetamol

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ABSTRACT

Broad-spectrum β-lactam antibiotics piperacillin (PIP) and tazobactam (TAZ) are widely co-administered in intensive care units (ICUs), frequently with paracetamol (PAR) for analgesic and antipyretic therapy. PAR may also appear as an adulterant in counterfeit medicines, posing serious risks such as reduced antibiotic efficacy, hepatotoxicity, and masking of clinical symptoms. These concerns are amplified in ICUs, where patients often experience severe or life-threatening infections. Therefore, reliable and practical analytical tools enabling simultaneous determination of these drugs are essential.

This study introduces two thin-layer chromatography (TLC)-based methods for simultaneous determination of PIP, TAZ, and PAR in pharmaceutical formulations and human plasma. The first method is based on conventional TLC–densitometry, while the second relies on a rapid smartphone-assisted detection approach after iodine visualization. Effective chromatographic separation was achieved using a mobile phase of n-butanol, glacial acetic acid, water, and ammonia (4:2:2:1, *v*/v).

The TLC-densitometric method showed linearity ranges of 1.00–20.00 μg/band for PIP, 0.50–20.00 μg/band for TAZ, and 3.00–20.00 μg/band for PAR, indicating good sensitivity at low concentrations. Conversely, the smartphone-based method demonstrated linearity ranges of 40.00–80.00, 100.00–500.00, and 60.00–100.00 μg/band for PIP, TAZ, and PAR, respectively. Although less sensitive, it provides a portable, low-cost, and user-friendly option for on-site analysis.

Both methods were fully validated and successfully applied to pharmaceutical products and spiked human plasma. High Blue Applicability Grade Index and Click Analytical Chemistry Index scores confirmed their sustainability, practicality, and suitability for quality control, clinical laboratories, and rapid ICU drug monitoring. The approaches support improved therapeutic safety and rapid decision making.

1. Introduction

Piperacillin (PIP) is a ureidopenicillin-class β-lactam antibiotic with a broad spectrum of activity, enhanced by a polar side chain that facilitates penetration into Gram-negative bacteria and reduces susceptibility to β-lactamase enzymes [1,2]. These characteristics confer activity against *Pseudomonas aeruginosa*, a major nosocomial pathogen, earning PIP the designation of an antipseudomonal penicillin [2,3]. Tazobactam (TAZ), a β-lactamase inhibitor, is co-formulated with piperacillin to enhance its antibacterial efficacy against β-lactamase–producing and resistant bacterial strains [4]. The PIP/TAZ combination is recommended for the treatment of hospital-acquired and ventilator-associated pneumonia in intensive care units (ICUs) [5–7].

This antibacterial combination is commonly co-administered with paracetamol (acetaminophen) (PAR), one of the most widely used analgesic and antipyretic agents in ICU and postoperative care settings [8–10]. Due to its low cost and symptomatic relief properties, paracetamol may also be illicitly added to counterfeit or substandard antibiotic

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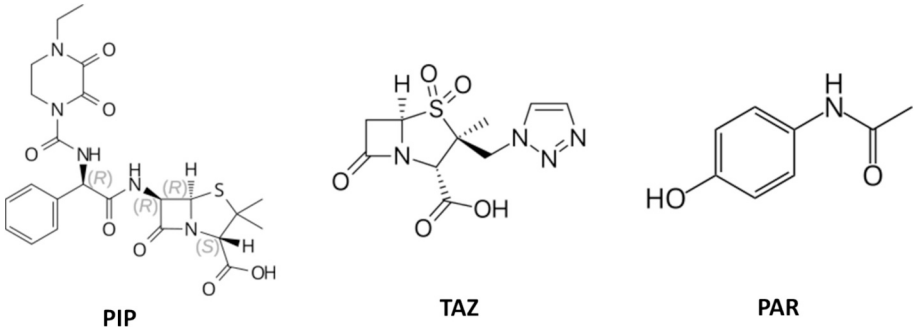

Fig. 1. Chemical structures of piperacillin (PIP), tazobactam (TAZ), and paracetamol (PAR).

formulations to mask symptoms and create a false perception of therapeutic efficacy [11–13]. Such adulteration can compromise appropriate antibiotic therapy, promote antimicrobial resistance, and increase the risk of paracetamol overdose and hepatotoxicity, particularly in critically ill patients [11–14]. These risks underscore the need for reliable analytical tools capable of detecting paracetamol both as a co-administered drug and as a potential adulterant. Addressing this analytical need requires methods that are not only selective and sensitive, but also economical, rapid, and environmentally sustainable. The chemical structures of the studied drugs -Tazobactam (TAZ), Piperacillin (PIP) and paracetamol (PAR) - are presented in Fig. 1.

Thin-layer chromatography (TLC) is a versatile, cost-effective, and environmentally favorable analytical technique widely used for the separation, identification, and quantification of compounds in diverse matrices [15]. TLC is highly versatile, permitting the analysis of a wide range of compounds, including both polar and nonpolar substances, through the use of various stationary phases and solvent systems. It provides rapid results, is inexpensive, requires minimal training, and does not rely on complex instrumentation, making it an accessible and efficient analytical tool for a broad range of applications [16]. From a green analytical chemistry perspective, TLC enables simultaneous multi-sample analysis using minimal solvent volumes, reduced waste generation, and simplified sample preparation, making it particularly suitable for routine pharmaceutical quality control and resource-limited settings [17–19]. Furthermore, TLC visualization approaches provide rapid, portable, and low-cost screening tools, especially when coupled with smartphone-based image acquisition and processing [18–20]. This technique eliminates the need for energy-intensive or sophisticated instrumentation [20,21]. Images acquired under visible or UV light are subsequently analyzed using dedicated readily available applications or software that apply simple image processing algorithms to quantify spot intensity [22]. This approach provides rapid, portable, low-cost, and on-site screening tools [23].

Several analytical methods have been reported for simultaneous determination of piperacillin and tazobactam in pharmaceutical formulations, including colorimetric techniques [24] and UV-spectrophotometric methods [25,26]. Additionally, methods for their determination in human plasma have been developed using HPLC with UV detection [27–30] or mass spectrometry [28,29], as well as capillary electrophoresis [31]. Paracetamol has been determined in combination with other drugs using chromatographic methods.

Although chromatographic and spectrometric methods have been reported for the determination of piperacillin (PIP) and tazobactam (TAZ) and paracetamol (PAR), either alone or with other drugs [24–31], no method has been described for their simultaneous determination in pharmaceutical formulations and human plasma. This analytical gap is particularly relevant for ICU drug monitoring and counterfeit medicine detection, where rapid, reliable multi-analyte screening is needed due to frequent co-administration and the risk of adulteration. Accordingly, this study develops and compares two TLC-based approaches - densitometric and smartphone-based - for the simultaneous determination of PIP, TAZ, and PAR in human plasma and for PIP and TAZ in dosage forms, with the ability to detect undeclared PAR. The proposed methods are simple, rapid, low-cost, and low-solvent alternatives to conventional chromatographic techniques, requiring minimal sample preparation and instrumentation while enabling high-throughput, on-site analysis suitable for quality-control and clinical laboratories.

2. Experimental

2.1. Instruments

The TLC system used comprised a Linomat autosampler (CAMAG, Muttenz, Switzerland), a CAMAG micro-syringe (100 μL), and a CAMAG TLC Scanner 3 operated using winCATS software. Visualization of the developed plates (Desaga, Wiesloch, Germany) was performed using a short-wavelength UV lamp emitting at 254 nm. TLC plates precoated with silica gel GF254 (20 × 10 cm, 0.25 mm thickness) were obtained from Merck (Darmstadt, Germany). Image acquisition for the smartphone-based detection method was carried out using an iPhone XR camera.

Plasma samples were vortexed using a vortex mixer (DLAB Scientific, China) and subsequently centrifuged using a benchtop centrifuge (Centurion Scientific Ltd., UK; 1200 series).

2.2. Materials and reagents

Piperacillin (PIP), tazobactam (TAZ) and paracetamol (PAR) were supplied by Rameda Co. (Giza, Egypt). The purities of PIP and PAR were evaluated in accordance with the British Pharmacopeia (BP) and found to be 100.04% and 99.58% respectively [32]. The purity of TAZ was assessed according to the United States Pharmacopeia (USP) and found to be 100.02% [33]. All solvents utilized in the study were of HPLC grade, and all reagents were of analytical pure grade. n-Butanol, methanol, and glacial acetic acid were obtained from Alfa Chemical Company (Cairo, Egypt). Ammonium hydroxide (NH4OH) and distilled water were supplied by PioChem Company (Giza, Egypt). The commercial pharmaceutical formulation Pipratаz® powder for I·V. infusion (4 g PIP/0.5 g TAZ) manufactured by Laboratorio Reig Jofre, S.A. (Barcelona, Spain) was imported by Hikma importation (Cairo, Egypt).

2.3. Solutions

2.3.1. Stock standard solutions

Stock standard solutions of 1 mg/mL of PIP, TAZ, and PAR were prepared for densitometric and smartphone-based methods. Accurately weighed amounts of 10.0 mg of each drug were transferred to separate 10 mL volumetric flasks, dissolved, and completed to the mark with methanol.

2.3.2. Laboratory - prepared mixtures

Different aliquots of PIP, TAZ and PAR stock solutions were

accurately transferred to a set of 10 mL volumetric flasks and diluted to the mark with methanol to prepare mixtures containing different ratios of the three drugs.

2.4. Procedures

2.4.1. Chromatographic conditions

Aliquots of sample solutions were applied to TLC plates using the autosampler in the form of bands of 5 mm width. The mobile phase consisted of n-butanol, glacial acetic acid, water and ammonia (4:2:2:1, by volumes). Linear ascending development was carried out in a twin-trough chamber pre-saturated with the mobile phase for 30 min at room temperature (25 ± 2 °C) to a distance of 8.0 cm. The developed plates were subsequently dried in air.

2.4.2. Construction of calibration curves and plate detection (densitometric method)

Aliquots from the stock standard solutions were applied to TLC plates to construct calibration curves. Volumes of 1.0 µL to 20.0 µL of PIP, 0.5 to 20.0 µL of TAZ, and 3.0 µL to 20.0 µL of PAR were applied to TLC plates, yielding final concentrations of 1–20 µg/band for PIP, 0.5–20 µg/band for TAZ, and 3–20 µg/band for PAR. Chromatographic development was performed as described previously. The developed plates were scanned at 254 nm using the TLC scanner, and calibration curves were constructed by plotting the integrated peak area against the corresponding concentration using polynomial regression to account for nonlinear detector response.

2.4.3. Construction of calibration curves and plate detection (smartphone-based method)

Aliquots from the stock standard solutions (1 mg/mL each) were applied to TLC plates as follows: 40.0–80.0 µL for PIP, 100.0–500.0 µL for TAZ, and 60.0–100.0 µL for PAR were applied to the TLC plates, corresponding to final concentrations of 40–80 µg/band, 100–500 µg/band, and 60–100 µg/band, respectively. Chromatographic development was performed as described previously. For spot visualization, a screw-capped TLC chamber containing a small amount of solid iodine crystals and powdered silica ("iodine chamber") was used. The developed plate was then placed in the iodine chamber for 10 min or until the spots turned yellow-brown. A photograph of the plate was captured using a smartphone camera at a distance of 10 cm against a white background under a desk lamp as the light source [14]. The intensity of each spot was quantified using the "Color Picker" software application (version 5.0.6, available at https://play.google.com/store/apps/details?id=gmikhail.colorpicker), the intensity of each spot color was determined. The calculated color intensity values were plotted against the corresponding concentrations to construct the calibration curves.

2.4.4. Application to pharmaceutical formulations

For TLC-densitometric method, an accurately weighed 1 g portion of Pipratаz® I.V. powder was transferred to a 25 mL volumetric flask and diluted to the mark with methanol. A 1 mL aliquot of this solution was further diluted to 25 mL with methanol. From this final dilution, the concentrations of PIP and TAZ were 1408 µg/mL and 176 µg/mL, respectively, corresponding to 14.08 µg/band of PIP and 1.76 µg/band of TAZ when 10 µL was applied per band.

For TLC-smartphone method, an accurately weighed 4.50 g portion of Pipratаz® I.V. powder was transferred to a 50 mL volumetric flask and dissolved in methanol to the mark. Different aliquots of this solution were further diluted to prepare concentrations of 8 mg/mL of PIP (equivalent to 80 µg/band) and 10 mg/mL of TAZ (equivalent to 100 µg/band), when 10 µL was applied per band.

2.4.5. Application to spiked plasma samples

Aliquots of 940.0 µL from plasma were spiked with 20.0 µL of each drug standard solutions and vortexed for 1 min to obtain plasma samples containing PIP, TAZ, and PAR at concentrations of 250: 25: 25, 300: 30: 30, and 350: 35: 35 µg/mL, respectively. These concentrations were selected to cover clinically relevant plasma levels. Reported peak plasma concentrations (Cmax) of piperacillin range from 264 to 368 µg/mL, while those of tazobactam range from 29 to 39 µg/mL following intravenous administration [34,35] whereas paracetamol exhibits Cmax values, typically 28–31 µg/mL after therapeutic dosing [36,37]. Protein precipitation was achieved by adding methanol a final volume of 5 mL, followed by vortexing for 5 min and centrifugation at 4500 rpm for 15 min.

2.4.5.1. TLC–densitometric analysis of spiked plasma samples. For the TLC–densitometric analysis, 150 µL aliquots of the resulting supernatant were applied to the TLC plates for determination of PIP, TAZ and PAR. To ensure that PAR concentrations fell within the linearity range, an addition technique was applied by adding 3.0 µL of a 1 mg/mL PAR standard solution to each spot. Accordingly, the tested concentrations applied to the TLC plate corresponded to 7.50: 0.75: 3.75, 9.00: 0.90: 3.90, and 10.5: 1.05: 4.05 µg/band for PIP, TAZ, and PAR, respectively.

2.4.5.2. TLC–Smartphone analysis of spiked plasma samples. Plasma samples for the TLC–smartphone method were prepared as described above. However, due to the higher linearity ranges of the smartphone-based method relative to the reported plasma Cmax values of the three drugs, a spiking technique was applied. Each plasma sample was spiked with 2 mL of a methanolic standard solution containing 1 mg/mL of PIP and PAR and 2 mg/mL of TAZ, followed by dilution to 5 mL with methanol. This ensured that the concentrations of the three drugs in the supernatant fell within the corresponding linearity ranges of the TLC–smartphone method. Aliquots of 150 µL were then applied to the TLC plates for analysis. Accordingly, the tested concentrations applied to the TLC plate corresponded to 67.50: 120.75: 60.75, 69.00: 120.90: 60.90, and 70.5: 121.05: 61.05 µg/band for PIP, TAZ, and PAR, respectively.

2.4.6. Practicality assessment of the proposed methods

The practical applicability of the proposed methods was evaluated using the Click Analytical Chemistry Index (CACI) [38] and the Blue Applicability Grade Index (BAGI) [39,40].

3. Results and discussion

Chromatographic techniques remain the most widely used analytical techniques, despite continuous advancements in analytical methods [23,41–43]. The present study aims to develop TLC-based methods that are sensitive, selective, accurate, and precise for the simultaneous determination of PIP, TAZ and PAR, fulfilling rigorous analytical quality standards. These methods also embody the principles of green analytical chemistry: they minimize solvent and reagent consumption, reduce waste generation, require only small sample volumes, and avoid energy-intensive instrumentation. Furthermore, smartphone-based detection provides a rapid, cost-effective, and portable approach, making it highly suitable for resource-limited settings [21,44]. Importantly, the method's simplicity and minimal training requirements enhance its practicality for routine pharmaceutical quality control, clinical laboratories, and on-site drug monitoring, offering a novel, sustainable alternative to conventional chromatographic techniques.

3.1. Development and optimization of TLC separation

Various compositions and ratios of mobile phases were tested to achieve optimal TLC separation, including ethyl acetate–-chloroform–glacial acetic acid–water, ethyl acetate–ethanol–ammonia, n-butanol–methanol–ammonia, ethanol–chloroform–diethyl amine, acetone–ethyl acetate–glacial acetic acid–water,

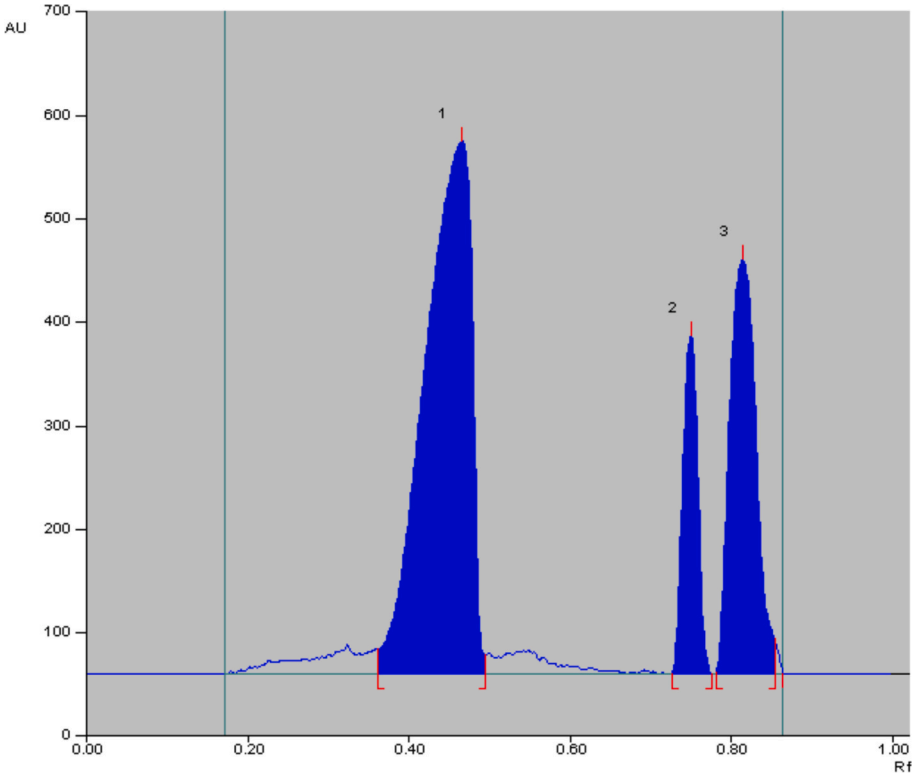

Fig. 2. Thin-layer chromatogram of a laboratory-prepared mixture of piperacillin (PIP, 1), tazobactam (TAZ, 2), and paracetamol (PAR, 3) developed using n-butanol–glacial acetic acid–water–ammonia (4:2:2:1, v/v) and scanned at 254 nm.

Table 1
System suitability tests parameters of TLC-densitometric method.

Parameters	PIP	TAZ	PAR	Reference value [33]
Retardation factor (R_f) ± 0.02	0.47	0.75	0.85	–
Resolution (R_s)[a]	NA	4.84	2.38	$R_s > 2$
Tailing factor (T)[b]	0.85	0.83	1.00	0.8–1.5
Capacity factor (k')[c]	1.13	0.33	0.18	0–10
Selectivity factor (α)[d]	NA	3.42	1.83	$\alpha > 1$

[a] $R_s = [2(z2 - z1)]/(w1 + w2)$, where z is migration distance and W is peak width at 50% from the baseline of the peak height.

[b] $T = W_{0.05}/2f$, where $W_{0.05}$ is the peak width at 5% height, and f is the distance from the peak maximum to the leading edge at the same height.

[c] $k' = 1\text{-}R_F /R_F$, where R_f is retardation factor.

[d] $\alpha = k'_2/k'_1$, where k' is the capacity factor; $k' = (1 - R_f) / R_f$.

chloroform–acetone–methanol–ammonia, and acetone–ethanol–ethyl acetate–2% sodium dodecyl sulfate–glacial acetic acid. Among these, the mobile phase comprising n-butanol, glacial acetic acid, water, and ammonia (4:2:2:1, by volumes) provided the most effective separation of PIP, TAZ, and PAR while using minimal amounts of organic solvents, aligning with green analytical chemistry principles. Initially this mobile phase was tested without ammonia. However, PIP contains basic functional groups, particularly the amine group (Fig. 1), which can interact strongly with the free acidic silanol groups on the silica gel surface causing the drug to adhere firmly and remain at the baseline [15,45,46].

Addition of ammonia as a basic reagent to the mobile phase effectively resolved this issue, enabling proper migration of the three drugs. Using the optimized system, the three compounds were successfully resolved with Rf of 0.47 ± 0.02, 0.75 ± 0.02, and 0.85 ± 0.02 for PIP, TAZ, and PAR, respectively (Fig. 2). System suitability parameters were calculated to confirm the reliability and robustness of the chromatographic system. The results, presented in Table 1, show that the values obtained were all within acceptable ranges [32,33]. The developed TLC method is not only environmentally sustainable due to low solvent and reagent consumption, but it also offers a rapid, cost-effective, and practical approach suitable for routine pharmaceutical quality control and clinical laboratory applications.

3.2. Optimization of TLC-densitometric scanning

Following chromatographic development, drug spectrodensitograms were recorded following the chromatographic process to determine the optimal scanning wavelength for the TLC-densitometric method, as illustrated in (Fig. 3). Wavelength selection was based on a compromise to achieve adequate sensitivity for all three drugs simultaneously, while maximizing the signal-to-noise ratio and minimizing baseline noise [47]. Selection of the optimal wavelength ensured maximum sensitivity and accuracy for the quantification of PIP, TAZ, and PAR while minimizing background noise and interference. This approach also supports green analytical chemistry principles by avoiding additional reagents or derivatization steps, thereby reducing chemical consumption and waste.

3.3. Optimization of the smartphone-based method

The rapid advancement of smartphone technology has created new opportunities for its use in analytical methods [48]. Smartphones offer a portable, accessible, and user-friendly detection tool, supporting sustainable and low-cost analytical practices [23,49,50]. In this study, a novel smartphone-based method was developed for on-site simultaneous detection and quantification of PIP, TAZ, and PAR (Fig. 4). The optimal distance between the TLC plate and the smartphone camera was found to be 10 cm, allowing for sharp and wide images while capturing the entire plate in a single frame. Increasing the distance slightly reduced image sharpness and resolution, while decreasing it limited the camera's field of view, necessitating division of the plate into multiple smaller sections for imaging. Iodine was used as a universal visualizing agent producing brown spots and enabling direct detection without additional reagents or energy-intensive instruments.

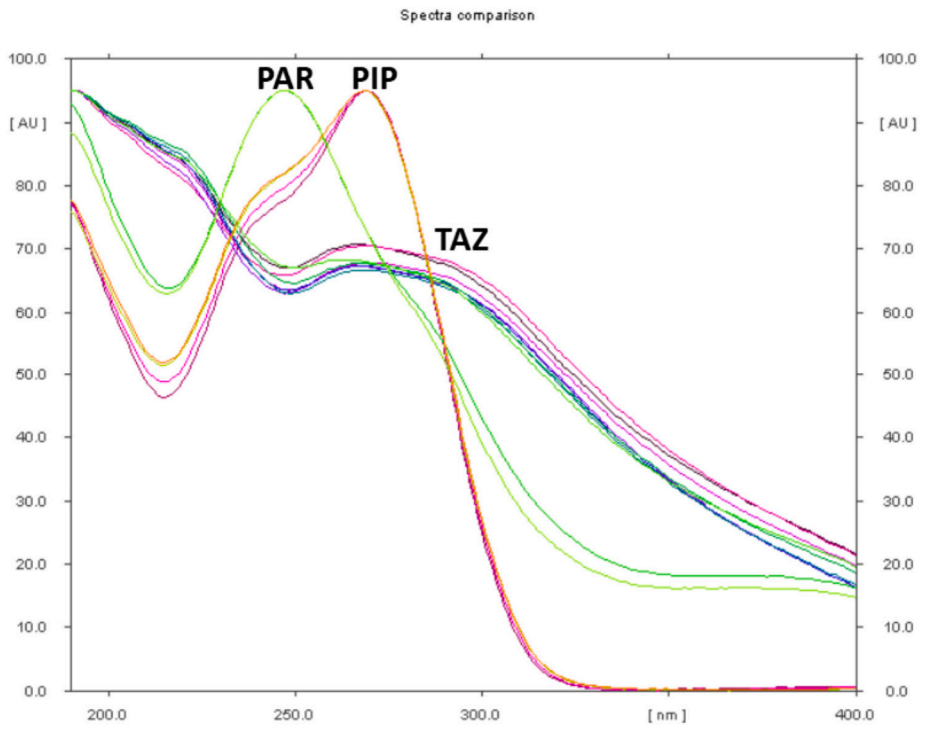

Fig. 3. Superimposed TLC spectrodensitograms of piperacillin (PIP), tazobactam (TAZ), and paracetamol (PAR) obtained after chromatographic separation, used for wavelength selection and peak purity assessment.

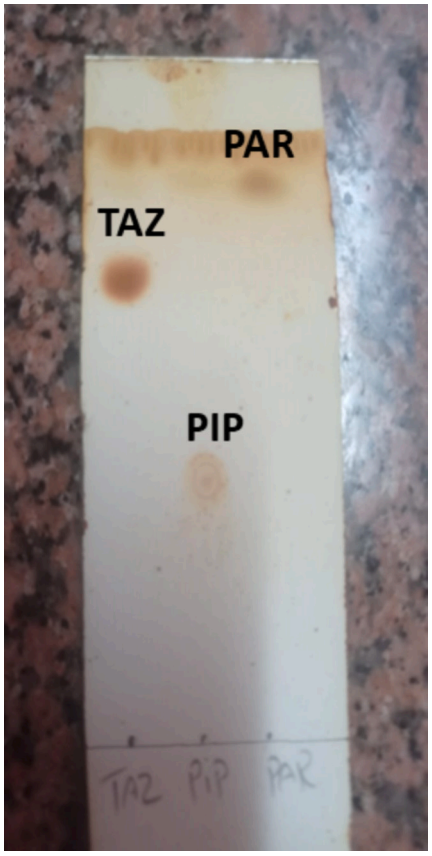

Fig. 4. TLC plate showing separation of tazobactam (TAZ, 400 μg/band), piperacillin (PIP, 60 μg/band), and paracetamol (PAR, 80 μg/band) after iodine vapor visualization.

Spot intensities were quantified using the "Color Picker" software application (version 5.0.6; available at: https://play.google.com/store/apps/details?id=gmikhail.colorpicker), which applies image-processing algorithms to ensure consistent and reproducible measurements. Multiple images of the same plate were analyzed to confirm reproducibility, demonstrating low variation in intensity values. This approach not only ensures accurate and sensitive quantification but also aligns with green analytical chemistry principles by minimizing chemical usage, waste, and energy consumption, making it highly practical for routine pharmaceutical quality control and resource-limited laboratory settings.

3.4. Methods validation

The proposed TLC-densitometric and TLC–smartphone methods were validated following ICH guidelines [51]. Key validation parameters—including linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), and robustness—were evaluated, and the results are summarized in **Table 2**.

3.4.1. Linearity and range

For TLC–densitometric method, calibration curves were constructed by plotting the integrated area against the corresponding concentration using polynomial regression to account for the nonlinear detector response observed at higher analyte concentrations where polynomial curves can provide a better fit to the data points, reducing errors in quantification [52]. The concentration ranges were 1–20 μg/band for PIP, 0.5–20 μg/band for TAZ, and 3–20 μg/band for PAR (**Fig. S1**).

For the TLC-smartphone method, calibration curves were constructed by plotting the luminance of each spot against its corresponding concentration over the range of 40–80 μg/band for PIP, 100–500 μg/band for TAZ, and 60–100 μg/band for PAR (**Fig. S2**).

Both methods demonstrate green analytical chemistry principles by requiring minimal sample volumes and small amounts of solvents to construct the calibration curves, thereby reducing chemical consumption and waste. The regression parameters along with their relative standard errors, limit of detections (LOD) and limit of quantifications

Table 2
Validation parameters for determination of PIP, TAZ, and PAR by the proposed method.

Parameter	TLC-densitometry			TLC-smartphone		
	PIP	TAZ	PAR	PIP	TAZ	PAR
Range (μg/band)	1.00–20.00	0.50–20.00	3.00–20.00	40.00–80.00	100.00–500.00	60.00–100.00
Slope	a = −45.58 b = 2279.80	a = −3.64 b = 441.11	a = −51.88 b = 2171	−0.312	−0.0249	−0.109
Intercept	2330.50	1586.70	−2079.60	47.44	42.71	54.84
Standard error of the slope	a = 2.53 b = 54.85	a = 0.48 b = 9.67	a = 1.60 b = 37.23	0.019	0.00078	0.008
Standard error of the intercept	234.18	34.09	170.01	1.16	0.25	0.64
Correlation coefficient (*r*)	0.9998	0.9999	0.9999	0.9892	0.9972	0.9845
LOD (μg/band)	0.22	0.16	0.87	12.76	33.00	19.38
LOQ (μg/band)	0.65	0.49	2.65	38.67	98.90	58.72
Accuracy (%Recovery ± %RSD)	100.31 ± 1.50	99.51 ± 1.38	99.83 ± 1.25	99.33 ± 1.51	98.25 ± 1.87	100.63 ± 1.03
Repeatability (%RSD)	1.42	1.60	0.92	1.30	1.71	0.86
Intermediate precision (%RSD)	1.55	1.71	1.06	1.83	1.86	1.02
Selectivity (mean ± %RSD)	100.57 ± 1.52	100.83 ± 1.60	99.79 ± 0.84	98.77 ± 1.66	100.01 ± 2.03	100.33 ± 1.47

(LOQ) are presented in Table 2.

3.4.2. Accuracy

The accuracy of the proposed methods was evaluated by analyzing PIP, TAZ, and PAR in triplicate at multiple concentration levels. For the TLC–densitometric method, PIP and TAZ were tested at 1, 5, 10, 15, and 20 μg/band, while PAR was assessed at 3, 5, 10, 15, and 20 μg/band. For the smartphone-based method, PIP was examined at 40, 50, 60, 70, and 80 μg/band; TAZ at 100, 200, 300, 400, and 500 μg/band; and PAR at 60, 70, 80, 90, and 100 μg/band. Both methods demonstrated excellent accuracy, with mean recovery values ranging from 98.69% to 100.31% (Table 2).

These results reflect the reliability of both approaches while supporting green analytical chemistry principles, as the analyses require minimal sample and solvent volumes, generate negligible waste, and avoid additional reagents, making the methods suitable for routine pharmaceutical quality control and clinical laboratory applications.

3.4.3. Precision

Repeatability (intra-day precision) and intermediate precision (inter-day precision) were evaluated by analyzing three concentrations of each drug in triplicate within the same day and across three consecutive days, respectively. For the TLC-densitometric method, concentrations of 5, 10 and 15 μg/band were measured for PIP, TAZ, and PAR. For the TLC–smartphone method, PIP was analyzed at 40, 60, and 80 μg/band; TAZ at 100, 200, and 400 μg/band; and PAR at 60, 80, and 100 μg/band. The low %RSD values obtained (Table 2) confirm the high precision and reproducibility of both methods.

3.4.4. Selectivity

The selectivity of the proposed methods was evaluated using five laboratory-prepared mixtures of PIP, TAZ, and PAR at various ratios as presented in **Table S1**, analyzed in triplicate. The good results of the mean % recoveries (Tables 2 & **S1)** demonstrate confirm that both methods can accurately determine each drug without interference from the others. Representative chromatograms of a well resolved mixture are shown in Fig. 3. The purity of the resolved peaks was further assessed using the spectral correlation tool in winCATS. UV-absorption spectra were recorded at three points along each peak for each drug: peak start slope (s), peak maximum (m), and peak end slope (e). Two correlation coefficients (rs,m and rm.,e) were calculated for each peak. Values greater than 0.9990 were obtained, indicating the homogeneity of the resolved peaks and the absence of masked or co-eluting components [53,54]. These results demonstrate the high selectivity and reliability of both TLC–densitometric and TLC–smartphone methods, supporting their application in routine pharmaceutical quality control and clinical laboratory analysis.

3.4.5. Robustness

Robustness of the proposed methods was evaluated by introducing small deliberate variations in chromatographic and detection conditions. For the TLC–densitometric method, these included changes in the mobile phase ratio, duration of mobile phase saturation (± 5 min), development distance (−5 mm) and scanning wavelength (± 2 nm). For

Table 3
Robustness of the proposed methods.

	Parameters	Original conditions	Variation	PIP		TAZ		PAR	
				R_f ± SD	%RSD*	R_f ± SD	%RSD*	R_f ± SD	%RSD*
TLC-densitometry	**Mobile phase ratio**	4:2:2:1	4.1:2:2:1	0.48 ± 0.01	2.09	0.76 ± 0.01	1.32	0.86 ± 0.01	1.16
			3.9:2:2:1	0.46 ± 0.01	2.17	0.74 ± 0.01	1.35	0.84 ± 0.01	1.19
	Saturation time (min)	30	25	0.45 ± 0.02	3.33	0.73 ± 0.02	2.05	0.83 ± 0.02	1.81
			35	0.48 ± 0.02	3.13	0.76 ± 0.02	1.97	0.86 ± 0.02	1.74
	Scanning wavelength (nm)	254	252	0.46 ± 0.01	2.17	0.74 ± 0.01	1.35	0.86 ± 0.01	1.16
			256	0.45 ± 0.01	2.22	0.76 ± 0.01	1.32	0.84 ± 0.01	1.19
	Distance development (mm)	80	75	0.44 ± 0.02	3.41	0.76 ± 0.02	1.97	0.83 ± 0.02	1.81
			85	0.46 ± 0.02	3.26	0.74 ± 0.02	2.03	0.86 ± 0.02	1.74
TLC-smartphone	**Mobile phase ratio**	4:2:2:1	4.1:2:2:1	0.46 ± 0.01	1.30	0.74 ± 0.01	1.35	0.84 ± 0.01	1.19
			3.9:2:2:1	0.46 ± 0.01	1.30	0.76 ± 0.01	1.32	0.83 ± 0.01	1.20
	Saturation time (min)	30	25	0.45 ± 0.02	3.33	0.73 ± 0.02	2.05	0.84 ± 0.01	1.19
			35	0.48 ± 0.02	3.13	0.76 ± 0.02	1.97	0.86 ± 0.01	1.16
	Camera plate distance (cm)	10	9	0.46 ± 0.01	2.17	0.74 ± 0.01	0.81	0.85 ± 0.01	0.71
			11	0.45 ± 0.01	2.22	0.75 ± 0.01	0.80	0.84 ± 0.01	0.71
	Image capture angle	90°	95°	0.46 ± 0.01	1.30	0.76 ± 0.01	1.32	0.83 ± 0.02	1.81
			85°	0.46 ± 0.01	1.30	0.77 ± 0.01	1.30	0.86 ± 0.02	1.74

* %RSD of peak area in TLC-densitometry, and %RSD of spot intensity in TLC-smartphone.

Table 4
Application of the proposed methods for determining the drugs in the spiked human plasma samples.

Plasma concentrations (μg/mL)	TLC-densitometry %Recoveries*			TLC-smartphone %Recoveries*		
(PIP: TAZ: PAR)	PIP	TAZ	PAR	PIP	TAZ	PAR
250: 25: 25	98.27%	97.33%	96.80%	96.36%	104.68%	100.16%
300: 30: 30	102.56%	100.01%	99.48%	95.18%	107.31%	101.66%
350: 35: 35	100.10%	96.19%	96.54%	97.81%	112.43%	103.16%

* Average of three determinations.

Table 5
Statistical comparison of the results obtained by the proposed methods and the official methods.

Parameter	TLC-densitometry			TLC-smartphone			Official methods [32,33]		
	PIP	TAZ	PAR	PIP	TAZ	PAR	PIP	TAZ	PAR
Mean	100.31	99.51	99.83	98.69	99.44	99.99	100.04	99.34	99.58
SD	1.51	2.37	1.25	1.49	1.86	1.03	1.22	1.70	1.10
Variance	2.28	5.62	1.56	2.22	3.46	1.06	1.49	2.89	1.21
n	5	5	5	5	5	5	5	5	5
Student's *t*-test (2.306)*	0.31	0.13	0.34	1.56	0.09	0.62	–	–	–
F-test (6.388)*	1.53	1.94	1.29	1.49	1.20	1.14	–	–	–

* The values between parentheses are the corresponding theoretical values of *t* and F at 95% confidence level.

the TLC–smartphone method, variations were applied to mobile phase ratio, saturation time, camera-to-plate distance and image capture angle. Percent RSD of peak areas or spot intensities and system suitability test (SST) parameters were calculated. The results of robustness, summarized in Table 3, demonstrate that the SST parameters within acceptable limits [55] despite these variations. The Rf values were almost unaffected, and the % RSD of peak areas or intensities did not exceed 2%, confirming that minor changes in experimental conditions do not compromise method performance. These findings indicate excellent robustness of both methods.

3.5. Application of the proposed methods to pharmaceutical preparation

The proposed methods were successfully applied to the determination of PIP, and TAZ in PIPRATAZ® powder for I.V infusion. The methods yielded mean recoveries of 96.97% ± 2.54 and 97.16% ± 0.57 using TLC-densitometry, and 99.42 ± 1.01 and 99.23 ± 1.87 using the TLC-smartphone method for PIP and TAZ, respectively. No PAR spots were observed in the analyzed dosage form.

3.6. Application of the proposed methods in human plasma

The proposed methods were successfully applied for the determination of PIP, TAZ, and PAR in human plasma, achieving good recoveries (Table 4) without any interference from endogenous plasma constituents.

3.7. Statistical analysis

A statistical comparison was carried out between the results obtained by the proposed techniques and the official titrimetric methods [32,33] for the determination of PIP, TAZ, and PAR. As shown in Table 5, no significant differences were observed, based on the calculated values of Student's *t*-test and F-test.

3.8. Limitations

3.8.1. Limitations of the TLC-densitometric method

Polynomial regression was applied in densitometric calibration to account for the nonlinear detector response observed at higher analyte concentrations, a well-documented characteristic of TLC densitometry [52]. Although this approach improved calibration accuracy across a wider concentration range and complied with ICH validation requirements, it introduces additional computational complexity compared with linear regression. This may slightly limit method transferability to laboratories lacking experience with nonlinear regression analysis.

3.8.2. Limitations of the smartphone-based TLC method

Despite the advantages of the proposed TLC–smartphone approach in terms of simplicity, portability, and rapid analysis, several limitations should be acknowledged.

i) Spot visualization

Iodine vapor employed as a visualization reagent in the TLC–smartphone method – due to its availability, rapid response, ease of use and effectiveness for spot detection - is volatile leading to gradual fading of the developed spots. Therefore, imaging acquisition must be performed immediately after visualization. This characteristic limits the applicability of the smartphone-based approach to rapid analysis and on-site screening.

ii) Reproducibility of Iimage acquisition

Image acquisition was standardized using a fixed camera-to-plate distance, uniform background, and controlled illumination. Nevertheless, smartphone-based quantification remains inherently sensitive to environmental conditions and device-specific parameters, which may introduce minor variability in pixel intensity despite careful experimental control.

iii) Linearity range and plasma applicability

The relatively higher linearity range compared with clinically relevant plasma concentrations encountered in ICU settings, required additional spiking to fall within the validated range limiting direct clinical monitoring at low concentrations. In contrast, the TLC- densitometric method demonstrated superior sensitivity and is therefore more appropriate for accurate plasma analysis.

iv) Matrix effects in smartphone detection

Recovery values exceeding 110% were observed at higher concentration levels during plasma analysis using the smartphone approach. These deviations are likely attributable to matrix effects and variability associated with iodine-based visualization, including potential enhancement of spot intensity by residual plasma components. While such deviations are acceptable for screening purposes, they highlight the inherent limitations of visualization-based detection when applied to complex biological matrices.

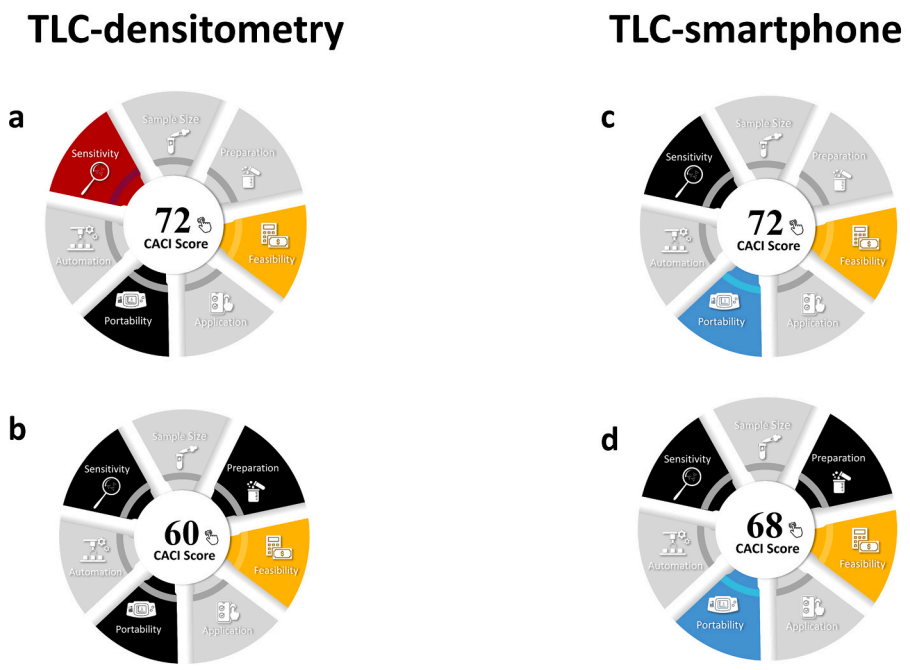

Fig. 5. Click Analytical Chemistry Index (CACI) pictograms evaluating the proposed TLC-densitometric and TLC–smartphone methods for (a, c) pharmaceutical dosage form and (b, d) spiked human plasma samples.

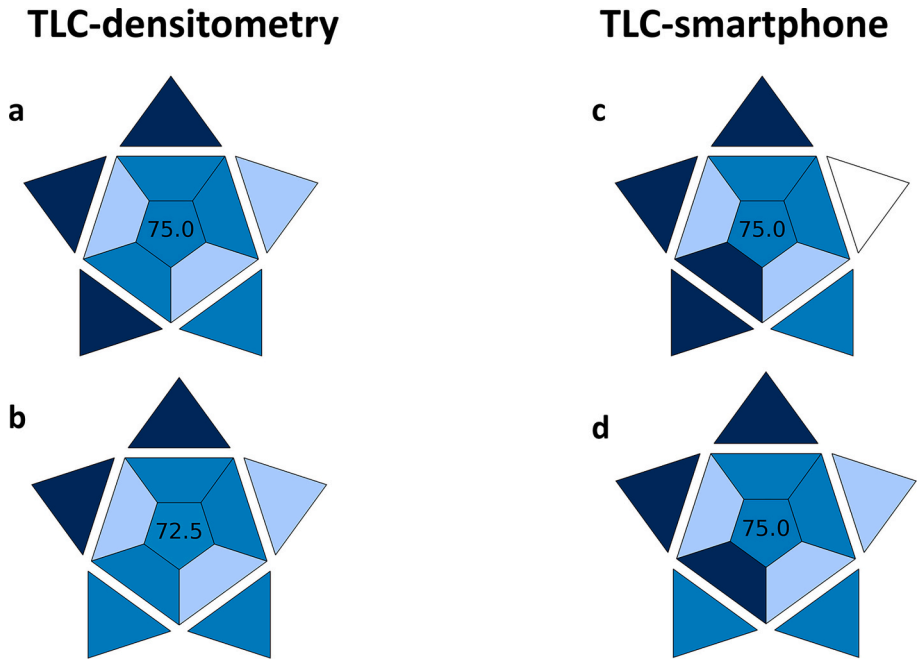

Fig. 6. Blue Applicability Grade Index (BAGI) pictograms illustrating the practical applicability of the proposed TLC-densitometric and TLC–smartphone methods for (a, c) pharmaceutical dosage form and (b, d) spiked human plasma samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.9. Practicality assessment using the click CACI and BAGI metrics

While conventional analytical performance metrics primarily emphasize accuracy and sensitivity, they often overlook the practical applicability of analytical methods, which is critical for routine laboratory and field use. In addition to analytical performance and statistical equivalence (Section 3.7), the practical applicability of the proposed methods was evaluated using the Click Analytical Chemistry Index (CACI) [38] and the Blue Applicability Grade Index (BAGI) [39], which offer complementary perspectives on method usability.

CACI focuses on the operational feasibility and efficiency of analytical methods through a color-coded pictogram that visually summarizes key criteria related to instrumentation, simplicity, and workflow suitability. The CACI pictograms (Fig. 5) indicate good compliance of both TLC-densitometric and TLC–smartphone methods with key operational criteria, including instrumentation accessibility, procedural simplicity, and workflow efficiency.

BAGI further complements this assessment by quantitatively

Table 6
Comparative evaluation of proposed TLC methods versus existing methods for PIP, TAZ, and PAR determination.

Parameter	UV / Colorimetry [24–26]	HPLC–UV [27,30]	LC–MS/MS [28,29]	Capillary Electrophoresis [31]	Proposed TLC–densitometry	Proposed TLC–smartphone
Analytes determined	PIP or PIP/TAZ only	PIP & TAZ (sometimes multiple antibiotics)	PIP & TAZ (± meropenem)	PIP &TAZ (± cefepime)	PIP, TAZ & PAR (simultaneous) (This work)	PIP, TAZ &PAR (simultaneous) (This work)
Matrix applicability	Pharmaceutical formulations	Plasma & serum	Plasma & pleural fluid	Plasma & formulations	Plasma & formulations	Plasma & formulations
Simultaneous PAR detection	Not reported	Not reported	Not reported	Not reported	First report	First report
Analytical principle	Absorbance / color reaction	Liquid chromatography (UV)	Liquid chromatography–mass spectrometry	Electrophoretic separation	Planar chromatography + densitometry	Planar chromatography + digital imaging
Linearity range	Narrow–moderate	ng–μg/mL	ng/mL	μg/mL	Low μg/band (high sensitivity)	High μg/band (screening level)
LOD / Sensitivity	Moderate	Good	Excellent (very low LOD)	Moderate–good	High (LOD ≤ 0.87 μg/band)	Lower sensitivity (LOD ≥ 12.8 μg/band)
Sample preparation	Simple dilution	Protein precipitation / extraction	Protein precipitation & cleanup	Protein precipitation	Protein precipitation	Protein precipitation + spiking
Instrumentation cost	Low	High	Very high	Moderate	Moderate (TLC scanner)	Very low (smartphone only)
Portability / on-site use	No	No	No	No	No	Portable (Field & ICU compatible)
Analysis time	Short	Moderate	Moderate–long	Moderate	Short; multiple samples per plate	Very short; multiple samples per plate rapid visual + imaging
Throughput	Low	Moderate	Moderate	Moderate	High (many samples per plate)	High (many samples per plate)
Need for derivatization	Sometimes required	No	No	No	No	Only iodine visualization
Green analytical aspects	Moderate	Low–moderate (solvent-intensive)	Low (energy- & solvent-intensive)	Moderate	High (low solvent, waste, energy)	Very high (low solvent, waste, & minimal energy)
Suitability for ICU drug monitoring	Limited	Reference labs	Specialized labs	Limited	Routine clinical labs	Point-of-care / resource-limited ICUs
Overall practicality (BAGI score)	Not assessed	Not assessed	Not assessed	Not assessed	72.5–75 (High applicability)	75 (High applicability)
Novelty	Established	Established	Established	Established	First TLC-densitometric method for simultaneous determination of ICU antibiotics (PIP, TAZ) with PAR	First TLC-smartphone method for simultaneous determination of ICU antibiotics (PIP, TAZ) with PAR

evaluating practical implementation aspects associated with White Analytical Chemistry, including sample preparation, instrument availability, throughput, reagent consumption, automation level, and sample volume. BAGI evaluation (Fig. 6) further supports CACI findings, yielding scores of 72.5-75 for both methods, which reflect strong practicality across critical factors such as sample preparation, throughput, reagent consumption, and multi-analyte capability.

Taken together, the CACI and BAGI results confirm that the proposed TLC approaches combine acceptable analytical performance with high practical applicability. This integrated evaluation supports their use as reliable and accessible alternatives to more complex chromatographic techniques, particularly where rapid analysis, minimal infrastructure, and operational flexibility are required.

3.10. Comparison of the proposed TLC methods with existing methods for PIP, TAZ, and PAR determination

Several analytical methods have been reported for the determination of piperacillin and tazobactam in pharmaceutical formulations and biological matrices, including UV-spectrophotometric and colorimetric techniques [24–26], HPLC with UV detection [27,30], LC–MS/MS methods [28,29], and capillary electrophoresis [31]. While these methods provide adequate sensitivity and selectivity for PIP/TAZ analysis, none have addressed the simultaneous determination of piperacillin, tazobactam, and paracetamol, despite the frequent co-administration of paracetamol in ICUs and its documented use as an adulterant in counterfeit pharmaceuticals. In contrast, the present work introduces, for the first time, two TLC-based approaches capable of resolving and quantifying the three drugs concurrently in plasma and pharmaceutical formulations. Compared to chromatographic and electrophoretic techniques, the proposed TLC-densitometric method offers comparable accuracy with lower solvent consumption and simpler sample preparation, while the TLC-smartphone method provides a uniquely portable, low-cost, and energy-efficient alternative suitable for on-site screening and rapid ICU drug monitoring (Table 6). Although LC–MS/MS remains superior in terms of sensitivity, its high cost, technical complexity, and limited accessibility restrict routine use. The high BAGI and favorable CACI scores obtained for the proposed methods further highlight their strong practicality, sustainability, and applicability, particularly in resource-limited settings and for counterfeit drug detection, where rapid and reliable multi-analyte screening is essential.

While the proposed TLC-densitometric and TLC–smartphone methods offer advantages in terms of simplicity, portability, cost-effectiveness, and compliance with green analytical chemistry principles, they do not match the sensitivity or selectivity of advanced chromatographic techniques such as LC–MS/MS. Accordingly, they should be considered as complementary tools particularly suitable for routine quality control, rapid ICU screening, and on-site analysis in resource-limited settings, rather than substitutes for high-end instrumental methods requiring ultra-trace detection.

4. Conclusion

This study presents a comparative evaluation of two TLC-based methods for the simultaneous determination of PIP, TAZ, and PAR in human plasma and pharmaceutical formulations. The proposed methods were successfully applied for the detection and quantification of the broad-spectrum beta-lactam antibiotics PIP and TAZ, along with PAR, which may be present as an adulterant in counterfeit products or as a co-administered drug in ICUs. Two quantification approaches were explored: a conventional densitometric method and a smartphone-based technique for rapid measurement under daylight illumination following a simple visualization step. While the densitometric method demonstrated superior sensitivity for low analytes concentrations, the TLC-smartphone approach offers a portable, low-cost, rapid, and simple alternative suitable for sustainable, on-site drug detection with minimal image processing. These methods offer promising tools for qualitative and quantitative analysis of PIP, TAZ, and PAR in both quality control and clinical laboratories. Furthermore, their practicality and applicability were systematically evaluated using both BAGI and CACI metrics.

CRediT authorship contribution statement

Reham A. Fekry: Writing – original draft, Validation, Methodology, Data curation, Conceptualization. **Khadiga M. Kelani:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Maha F. Abdel-Ghany:** Writing – review & editing, Visualization, Supervision, Formal analysis, Conceptualization. **Amr M. Mahmoud:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Hend Z. Yamani:** Writing – review & editing, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization.

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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.

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Appendix A. Supplementary data

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Data availability

Data will be made available on request.

References

[1] L.M. Lima, B.N.M. da Silva, G. Barbosa, E.J. Barreiro, β-Lactam antibiotics: an overview from a medicinal chemistry perspective, Eur. J. Med. Chem. 208 (2020) 112829, https://doi.org/10.1016/j.ejmech.2020.112829.
[2] S.E. Milne, P.M. Waterworth, Piperacillin, a new penicillin with high anti-pseudomonal activity, J. Antimicrob. Chemother. 4 (3) (1978) 247–254, https://doi.org/10.1093/jac/4.3.247.
[3] P.D. Tamma, S.L. Aitken, R.A. Bonomo, A.J. Mathers, et al., Infectious Diseases Society of America 2024 guidance on the treatment of antimicrobial-resistant gram-negative infections, Clin. Infect. Dis. (2023) ciae403, https://doi.org/10.1093/cid/ciad428.
[4] K.P. Daniel, L.C. Krop, Piperacillin–tazobactam: A β-lactam–β-lactamase inhibitor combination, Pharmacotherapy 16 (2) (1996) 149–162, https://doi.org/10.1002/j.1875-9114.1996.tb02933.x.
[5] G. Khilnani, K. Zirpe, V. Hadda, Y. Mehta, et al., Guidelines for antibiotic prescription in intensive care units, Indian J. Crit. Care Med. 23 (Suppl. 1) (2019) S1-S63, https://doi.org/10.5005/jp-journals-10071-23101.
[6] C.-H. Chen, C.-Y. Tu, W.-C. Chen, L.-K. Kuo, et al., Clinical efficacy of cefoperazone–sulbactam versus piperacillin–tazobactam, Infect. Drug Resist. 14 (2021) 2251–2258., https://doi.org/10.2147/IDR.S313828.
[7] I. El-Haffaf, J.A. Caissy, A. Marsot, Piperacillin–tazobactam in ICUs: a review of population pharmacokinetic analysis, Clin. Pharmacokinet. 60 (2021) 855–875, https://doi.org/10.1007/s40262-021-01013-1.
[8] C. Anderson, S. Boehme, J. Ouellette, C. Stidham, et al., Physical and chemical compatibility of injectable acetaminophen during simulated Y-site administration, Hosp. Pharm. 49 (1) (2014) 42–47, https://doi.org/10.1310/hpj4901-42.
[9] P. Young, M. Saxena, R. Bellomo, R. Freebairn, et al., Acetaminophen for fever in critically ill patients with suspected infection, N. Engl. J. Med. 373 (23) (2015) 2215–2224, https://doi.org/10.1056/NEJMoa1508375.
[10] M.M. de Maat, T.A. Tijssen, R.J. Brüggemann, H.H. Ponssen, et al., Paracetamol for intravenous use in medium- and intensive-care patients: pharmacokinetics and tolerance, Eur. J. Clin. Pharmacol. 66 (7) (2010) 713–719, https://doi.org/10.1007/s00228-010-0806-5.
[11] A.K. Deisingh, Pharmaceutical counterfeiting, Analyst 130 (2005) 271–279, https://doi.org/10.1039/B407759H.
[12] OECD/EUIPO, Trade in Counterfeit Pharmaceutical Products, Illicit Trade, OECD Publishing, Paris, 2020.
[13] P.N. Newton, M.D. Green, F.M. Fernández, N.P. Day, et al., Counterfeit anti-infective drugs, Lancet Infect. Dis. 6 (2006) 602–613.
[14] H. Yu, H.M. Le, E. Kaale, K.D. Long, T. Layloff, et al., Characterization of drug authenticity using thin-layer chromatography imaging with a mobile phone, J. Pharm. Biomed. Anal. 125 (2016) 85–93., https://doi.org/10.1016/j.jpba.2016.03.018.
[15] J. Sherma, B. Fried. Handbook of Thin-Layer Chromatography, 3rd ed., Boca Raton, CRC Press, 2003.
[16] C.F. Poole, The Essence of Chromatography, Elsevier, Amsterdam, 2003.
[17] E. Kaale, P. Risha, T. Layloff, et al., TLC for pharmaceutical analysis in resource-limited countries, J. Chromatogr. A 1218 (19) (2011) 2732–2736., https://doi.org/10.1016/j.chroma.2010.12.022.
[18] L.A. Hussein, M.F. Abdel-Ghany, H.Z. Yamani, Development of microwave-assisted extraction of trigonelline biomarker from trigonella foenum-graecum seeds followed by high-performance thin-layer chromatographic and high-performance liquid chromatographic analyses, JPC-J. Planar Chromatogr– Mod. TLC. 28 (2015) 373–379, https://doi.org/10.1556/1006.2015.28.5.5.
[19] A. Fiza, F. Shakeel, A. Khan, M.H. Alqarni, et al., Green high-performance thin-layer chromatography (HPTLC) approaches for pharmaceutical and drug analysis, TrAC Trends Anal. Chem. 195 (2026) 118606, https://doi.org/10.1016/j.trac.2025.118606.
[20] L. Shen, J.A. Hagen, I. Papautsky, Point of care colorimetric detection with a smartphone, Lab Chip 12 (2012) 420–424, https://doi.org/10.1039/C2LC40741H.
[21] M. Rezazadeh, S. Seidi, M. Lid, S. Pedersen-Bjergaard, et al., The modern role of smartphones in analytical chemistry, TrAC Trends Anal. Chem. 118 (2019) 548–555, https://doi.org/10.1016/j.trac.2019.06.019.
[22] M.-T. Liu, J. Zhao, S.-P. Li, Application of smartphone in detection of thin-layer chromatography: case of *salvia miltiorrhiza*, J. Chromatogr. A 1637 (2021) 461826, https://doi.org/10.1016/j.chroma.2020.461826.
[23] M.M. Ibrahim, K.M. Kelani, N.K. Ramadan, E.S. Elzanfaly, Smartphone as a portable detector for thin-layer chromatographic determination of some gastrointestinal tract drugs, ACS Omega 7 (27) (2022) 23815–23820, https://doi.org/10.1021/acsomega.2c02482.
[24] Z. Erdogan, H. Balcı, Design of a novel colorimetric method based on AuNPs for tazobactam and piperacillin detection, J. Res. Pharm. 27 (3) (2023) 1066–1075.
[25] S.P. Karpova, M.Y. Blazheyevskiy, O.O. Mozgova, Development and validation of UV spectrophotometric area-under-curve method for quantitative estimation of piperacillin, Int. J. Pharm. Sci Res. 9 (8) (2018) 3556–3560, https://doi.org/10.13040/IJPSR.0975-8232.9(8).3556-60.
[26] S. Sangeetha, M. Kumar, M.V. Kumudhavalli, S. Alexandar, et al., Development and validation of UV spectrophotometric area-under-curve method for quantitative estimation of piperacillin and tazobactam, Int. J. ChemTech Res. 10 (2) (2017) 988–994.
[27] T. Roth, L. Weber, M. Niestroj, F. Cipa, et al., Simultaneous determination of six antibiotics in human serum by HPLC with UV detection, Biomed. Chromatogr. 35 (3) (2021) e5010, https://doi.org/10.1002/bmc.5010.
[28] D. Krnáč, K. Reiffová, B.J. Rolinski, A new HPLC-MS/MS analytical method for quantification of tazobactam, piperacillin, and meropenem in human plasma, J. Sep. Sci. 44 (14) (2021) 2744–2753, https://doi.org/10.1002/jssc.202100067.
[29] N.D. Popowicz, S.J. O'Halloran, D. Fitzgerald, Y.C.G. Lee, et al., Rapid LC-MS/MS assay for quantification of piperacillin and tazobactam in human plasma and pleural fluid; Application to a clinical pharmacokinetic study, J. Chromatogr. B

Analyt. Technol. Biomed. Life Sci. 1081-1082 (2018) 58–66, https://doi.org/10.1016/j.jchromb.2018.02.027.
[30] K. Abdelkawy, T. Le, S.H. Mahmoud, Simple HPLC-UV method for piperacillin/tazobactam assay in human plasma, Antibiotics 12 (2) (2023) 321, https://doi.org/10.3390/antibiotics12020321.
[31] A. Al-Attas, J.J. Nasr, N.N El-Enany, F. Belal, A green capillary zone electrophoresis method for simultaneous determination of piperacillin, tazobactam, and cefepime, Biomed. Chromatogr. 29 (12) (2015) 1811–1818, https://doi.org/10.1002/bmc.3500.
[32] A.C. Cartwright, The British Pharmacopoeia, 1864–2014: Medicines, International Standards and the State; Routledge, London, 2016.
[33] R.C. Moreton, United States Pharmacopeia–National Formulary, Int. J. Pharm. Excip. 6 (3) (2016).
[34] C.M. Perry, A. Markham, Piperacillin/tazobactam: an updated review of its use in the treatment of bacterial infections, Drugs 57 (1999) 805–843, https://doi.org/10.2165/00003495-199957050-00017.
[35] Y. Hayashi, J.A. Roberts, D.L. Paterson, J. Lipman, Pharmacokinetic evaluation of piperacillin–tazobactam, Expert Opin Drug Metab Toxicol 6 (8) (2010) 1017–1031, https://doi.org/10.1517/17425255.2010.506187.
[36] M. Rawlins, D. Henderson, A.J. Hijab, Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral administration, Eur. J. Clin. Pharmacol. 11 (4) (1977) 283–286, https://doi.org/10.1007/BF00607678.
[37] U.S. Food and Drug Administration. *Acetaminophen Injection, for intravenous use*; Prescribing Information, NDA 206968; U.S. Government Printing Office: Washington, DC, 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/206968s000lbl.pdf (accessed 1Feb 2026).
[38] F.R. Mansour, A. Bedair, M. Locatelli, Click analytical chemistry index as a novel framework, supported with open source software to assess analytical methods, Advan Sample Prep 14 (2025) 100164, https://doi.org/10.1016/j.sampre.2025.100164.
[39] N. Manousi, W. Wojnowski, J. Płotka-Wasylka, V. Samanidou, Blue applicability grade index (BAGI) and software: a new tool for the evaluation for method practicality, Anal. Methods 25 (19) (2023) 7598–7604, https://doi.org/10.1039/D3GC02347H.
[40] M.Z. Kamel, H.Z. Yamani, L.A. Hussein, Y.A. Trabik, First fluorescence method for monitoring the doping stimulant solriamfetol in plasma and urine. A pharmacokinetic study in volunteers'urine with greenness, whiteness, and blueness assessments, Microchem. J. 207 (2024) 111930, https://doi.org/10.1016/j.microc.2024.111930.
[41] K.M. Kelani, Y.M. Fayez, A.M. Abdel-Raoof, R.A. Fekry, Development of an eco-friendly HPLC method for ibuprofen and phenylephrine, BMC Chem. 17 (1) (2023) 141–152, https://doi.org/10.1186/s13065-023-01056-4.
[42] R.A. Fekry, K.M. Kelani, Y.M. Fayez, M.A. Tantawy, Comparative validated chromatographic methods for the simultaneous determination of caffeine, codeine, and paracetamol along with the related compound "p-aminophenol" in tablets, JPC-J. Planar Chromatogr– Mod. TLC 39 (1) (2022) 51–59, https://doi.org/10.1007/s00764-022-00150-y.
[43] H.Z. Yamani, L.A. Hussein, M.F. Abdel-Ghany, Microwave-assisted extraction of gallic acid from *Acacia arabica*, Arch. Pharm. Sci. ASU 3 (1) (2019) 78–89, https://doi.org/10.21608/aps.2019.20240.
[44] H.-A.H. Abd-ElSalam, E.S. Elzanfaly, Y.F.J. Bassuoni, Smartphones as optical detectors in pharmaceutical analysis, A review, Microchem. J. 212 (2025) 113316, https://doi.org/10.1016/j.microc.2025.113316.
[45] A. Petruczynik, K. Wróblewski, M. Waksmundzka-Hajnos, et al., Effect of chromatographic conditions on retention behavior and system efficiency for HPTLC of selected psychotropic drugs on chemically bonded stationary phases, Acta Pol. Pharm. 72 (1) (2015) 39–47.
[46] T. Kowalska, J. Sherma, in: Preparative layer chromatography, CRC Press, Boca Raton,, 2006.
[47] B. Spangenberg, C.F. Poole, C. Weins, Quantitative Thin-Layer Chromatography: A Practical Survey, Springer, Berlin, 2011.
[48] D.V. Baker, J. Bernal-Escalante, C. Traaseth, Y. Wang, et al., Smartphones as a platform for molecular analysis: concepts, methods, devices and future potential, Lab Chip 25 (2025) 884–955, https://doi.org/10.1039/D4LC00966E.
[49] M.S. Elsherbiny, K.M. Kelani, S.M. Eid, M.M. Abbas, et al., Simultaneous determination of favipiravir and molnupiravir using TLC with smartphone image processing: *applications in pharmaceuticals and environmental matrices*, Microchem. J. 215 (2025) 114225, https://doi.org/10.1016/j.microc.2025.114225.
[50] M. Wadie, E.M. Abdel-Moety, M.R. Rezk, H.M. Marzouk, A novel smartphone HPTLC assaying platform versus traditional densitometric method, RSC Advances 13 (2023) 11642–11651, https://doi.org/10.1039/D3RA01211E.
[51] International Conference on Harmonisation (ICH). *Validation of Analytical Procedures: Text and Methodology Q2(R2)*; ICH: Geneva, 2005.
[52] M.M.Z. Sharkawi, M.A. Ahmed, N.F. Farid, N.S. Abdelwahab, TLC densitometric approach for concurrent determination of a quinary mixture for treatment of migraine with appraisal to method greenness and whiteness, Sci. Rep. 14 (2024) 30477, https://doi.org/10.1038/s41598-024-79518-5.
[53] A.F. El-Yazbi, R.M. Youssef, An eco-friendly HPTLC method for eszopiclone in pharmaceutical preparation: investigation of its water-induced degradation kinetics, Anal. Methods 7 (18) (2015) 7590–7595, https://doi.org/10.1039/C5AY01809A.
[54] M.R. Rezk, E.M. Abdel-Moety, M. Wadie, M.A. Tantawy, Stability assessment of tamsulosin and tadalafil co-formulated in capsules by two validated chromatographic methods, J. Sep. Sci. 44 (2) (2021) 530–538., https://doi.org/10.1002/jssc.202000975.
[55] M. Yuwono, G. Indrayanto, Validation of chromatographic methods of analysis, Profiles Drug Subst. Excip. Relat. Methodol. 32 (2005) 243–259, https://doi.org/10.1016/S0099-5428(05)32009-0.