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Simultaneous determination of a mixture of beta-lactam drugs in their pure forms and pharmaceutical preparation using high performance liquid chromatography with an ultraviolet detector

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Abstract

A modern, rapid RP-HPLC-UV method was developed and validated in compliance with FDA and EMA guidelines for simultaneous quantification of 15 β-lactam antibiotics) Ampicillin, Amoxicillin, cephalexin, cefotaxime, cefoxitin, cefamandole, cephalothin, piperacillin, penicillin, oxacillin, cloxacillin, nafcillin Carbenicillin, Mezlocillin and Dicloxacillin) in pharmaceutical formulations and pure forms. The method employs Column NEUCLEODUR C-18 (4.0 mm x 100 mm, 5µm particle size), at a temperature of thirty degrees Celsius, and the mobile phase was acetonitrile and KH_2PO_4 using gradient elution with a total separation time of 13 minutes, a flow rate of 1.3 ml/min, at = pH 4.5 for the buffer solution and the λ max was 220 nm. The method demonstrated a linear calibration range of 0.2-20 µg/mL with coefficients of estimation of 0.9994 Recoveries were 100.4 - 90.86%. The chromatography approach in this study was applied to simultaneously determination of pharmaceutical compounds in commercial products.

Keywords: amoxicillin, beta lactam, determination, mezlocillin, P-HPLC.

التقدير المتزامن لمزيج من ادوية بيتالاكتام في اشكالها النقية والمستحضرات الصيدلانية باستخدام طريقة كروموتوغرافيا السائل عالي الاداء مع مكشاف الاشعة فوق البنفسجية

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الخلاصة

تم ابتكار طريقة كروماتوغرافيا سائلة عالي الاداء حديثة وسريعة والتحقق منها وفقا لمتطلبات ادارة الغذاء والدواء ووكاله الادوية الاوروبية للتقدير المتزامن للادوية باشكالها الصيدلانية والنقية ويتناول هذا البحث تحديد ادوية بيتالاكتام (الامبسلين، الاموكسيلين، سيفاماندول، سيفالوثين، بيبراسلين، اوكساسلين، كلوكساسلين، نافسيلين، كربنسلين، ميزلوسيلين ودايكلوكساسلين) وهي تقنية كروماتوغرافيا السائل عالي الاداء الطورالعكسي مع كاشف الاشعة فوق البنفسجية باستخدام العمود NEUCLEODUR C 18- (4ملم × 100ملم،5 ميكرو) عند درجة حرارة 30 درجة مئوية ويتكون الطور المتحرك من الاسيتونترايل KH_2PO_4 باستخدام الشطف متدرج مع وقت فصل اجمالي قدرة 13 دقيقة ومعدل تدفق 1.3 مل /دقيقة عند PH 4.5 = لمحلول

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البفر وكان الطول الموجي 220 نانومتر وتراوح مدى التركيز بين 0.2 الى 20 مايكروجرام/مل مع معاملات تحديد 0.9994 وكانت حالات الاسترداد 100.4%- 90.86% وتم تطبيق طريقه الكروموتوغرافيا في هذه الدراسة لتقدير الادوية في وقت واحد في اشكال الجرعات الصيدلانية.

1. Introduction

Accurate analytical quantification of active pharmaceutical ingredients (APIs), degradants, and related receptors is critical for medicinal formulations [1]. Analytical methods for analyzing pharmaceutical preparations are less complicated compared to analyzing drugs in serum specimens and biological compounds including blood, urine, and hair [2]. Monitoring medication in medicinal preparations is very necessary because they have a direct impact on patient health outcomes [3]. Chemical analysis plays a significant part in the control of medication preparations and drug development and a vital role in ensuring high integrity for patients. For this, correct tools for quality control are of great significance in medication manufacturing [4]. The development and research of new drugs lead to increasingly complex compounds and formulas in medicines and therefore require very sensitive analytical techniques and new techniques are required to separate and purify them [5]. As a result, advanced analytical approaches must be developed to control the quality of medication analysis. There are many techniques, such as spectrophotometry, electroanalytical methods, mainly voltammetry, and titration, fluorometric, and chromatographic techniques, including TLC, GC, and HPLC, for the identification and quantitative assessment of medicinal composites [6].

A widely used class of antibiotics for treating bacterial infections is characterized by the presence of a beta-lactam ring in its chemical structure [7]. Prevents the construction of the wall of bacterial cells, leading to their death. It works by inhibiting the enzyme transpeptidase [8], which plays a significant job in cell wall patterns. Effective against a wide range of bacteria [9]. It's used to treat upper and lower respiratory infections, Urinary tract infections, Gastrointestinal infections, and Bacterial infections in the blood [10]. However, there are some side effects such as It may cause allergies in some people, may cause diarrhoea and digestive disorders [11]. The literature review identified analytical approaches for determining beta-lactam antibiotics, most of which include the HPLC method and have been developed to analyze antibiotics [12,13] including HPLC–tandem mass spectrometry [14], FT-IR spectroscopy [15,16], ultraviolet (UV) spectrophotometry [17- 19], Electrical methods, [20,21], flow injection [22,23] and Nanotechnology analysis [24,25].

HPLC is a strong analytic technique broadly utilized in many scientific and industrial fields used to separate, identify and measure apparatuses of complex mixtures [26, 27]. This method depends on the change in interactions among the combinations to be separated and a fixed phase present inside a column [28]. This technique depends on the principle of interaction of molecules with two phases: first: fixed phase: a porous solid that fills the inside of the column, second: Mobile phase: a liquid that passes through the column, carrying the sample with it. Molecules vary in speed as they pass through the Column based on their interaction with the fixed phase. Some molecules prefer to remain attached to the fixed phase and move slowly, while others prefer to remain in the mobile phase and move more quickly. This difference in speed causes the components of the mixture to separate [29, 30]

This study developed an advanced chromatographic method for the simultaneous quantification and separation of 15 beta-lactam antibiotics in a single analytical run. This is a major challenge due to the similarity of the chemical composition of this type of medicine.

Also, this modern and developed chromatographic technique is minor, accurate, timesaving, repeatable, and can be applied in quality control laboratories. For the simultaneous measurement of these chosen drug compounds from different therapeutic classes, no single HPLC method has been reported to our knowledge. The suggested approach was designed and validated according to ICH guidelines for analysis. Creating an analytical method is more economical than adjusting parameters for each analyte when analyzing real samples. The developed approach is suitable for labs with less sophisticated, specialized equipment. Nevertheless, the method will produce a sufficiently accurate identification of the selected compounds.

2. Methods

2.1. Materials and reagents

Standards of 15 beta-lactam medications were secured from Sigma (St. Louis, Mo., USA). While buffer KH_2PO_4 (HPLC grade), acetonitrile (HPLC grade) and HPLC solutions were supplied from LabScan (Ireland). All other chemicals have been bought from Lachema (Brno, Czech Republic). Stock solutions (100 µg/mL) of each standard were formulated in acetonitrile, working solutions were diluted together with deionized water.

2.2. Instrumentation

Measurements were conducted using an HPLC device, model Shimadzu 2020A, with a UV detector and NEUCLEODUR C-18 (4.0 mm×100 mm, 5 µm).

2.3. Chromatographic conditions

The mobile phase comprised: solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4) using gradients program 10% A (2 min), and 50% A (10 min). The column was preheated to 30 °C, the flow rate was 1.3 mL/min, UV set wavelength was 220 nm. An aliquot of 20 µl of a combination of the chosen chemicals was added to the system. The cumulative duration was 13 minutes.

2.4. Preparation of pharmaceutical stock solutions (100 µg/mL)

0.01 g of the reference drug was accurately weighed, dissolved in a 10%:90% (V/V) mixture of acetonitrile (CAN), and KH_2PO_4 solution, transferred to a 100 mL volumetric flask, and subsequently diluted to the calibration mark together with the alike solvent. Further diluted solutions were produced by the simple mitigation of the stock solution of the medications. Utilizing the stock solution of 100 µg/mL, the subsequent dilutions were executed by transferring aliquots (0.02 – 2 mL) from the standard solution interested in a sequence of 10 mL calibrated volumetric flasks, which stayed subsequently filled to the mark together the mobile phase to produce working standard solutions of varying concentrations (0.2 – 20 µg/mL).

2.5. Optimization of method Parameter

Separation in HPLC is influenced by multiple factors, involving the mobile phase, solvent kind and composition, acidity function, buffer solution concentration, and flow rate of mobile phase. The solvents for the separation of 15 medication combinations were identified, and the impact of the mobile phase flow rate was checked at rates of 0.7, 0.9, 1.1, and 1.3 mL/min. The chromatographic characteristics were assessed by considering both the resolution and symmetry of the peaks.

2.6. Method validation

Linearity, quantitation limit (LOQ), detection limit (LOD), precision, recovery, and other parameters of each of the 15 drugs were verified in pursuance according to ICH guidelines for the validation of analytical approaches.

2.7. Protocol for drug test in pharmaceutical formulations

Individual samples were weighed and prepared separately for each beta-lactam antibiotic. After dissolving in the mobile phase, the materials were moved to a volumetric flask and adjusted to the sign using the same solvent. The linear equation was used to choose drug concentration once a predetermined volume containing the necessary number of medications was transferred, matching the desired calibration curve range.

3. Results and Discussion

3.1. Method Development

A multitude of studies were performed to achieve optimal separation of beta-lactam medicines across various mobile phases and differing ratios of aqueous and organic phases. The optimal mobile phase identified was acetonitrile and potassium hydrogen phosphate at pH 4.5, as this combination provided high accuracy. The retention period of the beta-lactam antibiotics on the separation column was determined. At a flow rate of 1.3 milliliters per minute. The injection volume was 20 microliters, the sample retention duration was 13 minutes, and the reference material for beta-lactam medicines exhibited high accuracy. This study concentrated on enhancing the settings for straightforward, economical, efficient, and swift analysis, encompassing the mobile phase assessment. To obtain optimal results, the solvent type, mobile phase solvent strength, and solution pH were modified. Identify the detection wavelength and flow rate to establish the optimal chromatographic conditions for superior separation. A mobile phase condition has been refined to exclude influence from solvents and excipients. The first optimal chromatographic settings are detailed in the table below, and the refined chromatogram for beta-lactam antibiotics is presented in Table 1.

Table1: The settings that are considered to be optimal for the HPLC process.

Mobile phase compassion CAN: KH_2PO_4 50: 50 pH = 4.5	Column type: NEUCLEODUR: 4.0mm,100 mm, 5µm
	Sample temperature: ambient
Flow rate 1.3 mL/min	Column temperature: 30 °C
Injection volume 20 µL	Run time min 13
Wavelength 220 nm	Retention time 13 min

3.2. Mobile phase selection

The mobile phase was chosen based on the chemical and physical properties of the drugs to provide the best separation. The separation in HPLC depends on the mobile phase, whether the kind or composition of the solvent. The different solvents, acetonitrile and KH_2PO_4, were determined from the formation of the mobile phase that was studied, as this produces the motional phase is the greatest separation among the analytical materials.

3.3. Optimization of mobile phase flow rate

The interaction of compounds together in the fixed and mobile phases influences the precision of the peaks, alongside the flow rate of the mobile phase. It was observed that augmenting the flow rate by 1.3 ml/min diminished the retention period of all analyzed constituents, alongside the emergence of distinctly separated peaks, indicating effective separation. Favourable: an accelerated mobile phase flow diminishes the interaction between the analyte and the fixed phase, whereas a decreased flow rate results in prolonged operating

durations and increased closeness of the peaks. Figure 1 illustrates the impact of flow rate on the detention time of beta-lactam antibiotic families.

Table 2: variation of retention times with different flow rate minute

		Retention time minutes			
No.	Subject	Flow rate 0.7 ml/min	Flow rate 0.9 ml/min	Flow rate 1.1 ml/min	**Flow rate 1.3 ml/min**
1	amoxicillin	1.79	1.61	1.52	**1.50**
2	ampicillin	3.93	3.61	2.93	**2.59**
3	Cephalexin	5.13	4.44	3.86	**3.27**
4	Cefotaxime	7.10	6.44	4.99	**3.99**
5	Cefoxitin	8.01	7.35	5.84	**4.83**
6	Cefamandole	9.10	8.44	6.63	**5.51**
7	Cephalothin	9.94	9.22	7.31	**6.20**
8	Piperacillin	11.11	10.40	8.22	**7.02**
9	penicillin	12.03	11.44	9.27	**7.84**
10	oxacillin	12.85	12.20	10.11	**8.92**
11	Cloxacillin	13.93	13.28	11.02	**9.84**
12	Nafcillin	15.11	14.44	11.84	**10.62**
13	Carbenicillin	16.28	15.63	12.76	**11.23**
14	mezlocillin	17.20	16.60	13.64	**12.11**
15	Dicloxacillin	18.09	17.45	14.54	**13.21**

3.4. Validation of the analytical method

3.4.1. Linear range

The linear range describes the concentration interval over which an analytical method demonstrates a direct proportional relationship between measured response and analyte concentration. This linear relationship is validated by evaluating the confidence interval of the calibration curve's slope, which serves as a statistical measure of method linearity [31]. The ICH Guidelines advocate for the smallest of five concentrations to ascertain linearity. The approach to analysis aims to accurately and linearly establish the temporal interval between the top and lower levels [32]. The linearity of the tested approach was decided by plotting peak areas against the respective medication concentrations, utilizing R^2 correlation coefficients as detailed in Table 4. For the establishment of the linearity concentration ranges of beta-lactam drugs, the samples were prepared at concentrations from 0.2-20 µg/mL and injected, and the chromatograms were recorded using the above-stated method. The plot of peak area vs concentration of drugs is shown in figures 6-10, the straight line represents that the developed method is linear and there is a direct relationship between peak area and concentration. The range of this analytical method lies between 0.2-20 µg/mL of the drug concentration. Normally, the acceptance criteria for an analytical method include the correlation coefficient being ≥0.997. However, it was observed that the linearity and range of the current method were within acceptable bounds. This was calculated using regression line analysis.

Table 3: Analytical performance of the developed method

No	Subject	Linearity range µg/ml	Regression equation	determination coefficients (R^2)	Limit of Detection LOD µg/ml	Limit of Quantitation LOQ µg/ml
1.	Amoxicillin	0.2-20	Y=19141x+7530.6	0.9987	0.14	**0.42**
2	ampicillin,	0.2-20	Y=19754x+13104	0.9962	0.13	**0.39**
3	Cephalexin	0.2-20	Y=19464x+11843	0.9984	0.13	**0.39**
4	Cefotaxime	0.2-20	Y=21586x+12192	0.9990	0.14	**0.42**
5	Cefoxitin	0.2-20	Y=18839x+11464	0.9990	0.14	**0.42**
6	Cefamandole	0.2-20	Y=20267x+22018	0.9992	0.14	**0.42**
7	Cephalothin	0.2-20	Y=20744x+19493	0.9994	0.14	**0.42**
8	Piperacillin	0.2-20	Y=22407x+43854	0.9993	0.13	**0.39**
9	Penicillin	0.2-20	Y=17738x+25998	0.9991	0.13	**0.39**
10	oxacillin	0.2-20	Y=18946x+23180	0.9989	0.14	**0.42**
11	Cloxacillin	0.2-20	Y=21349x+16501	0.9997	0.14	**0.42**
12	Nafcillin	0.2-20	Y=20334x+22671	0.9994	0.14	**0.42**
13	Carbenicillin	0.2-20	Y=19106x+0.9971	0.9988	0.14	**0.42**
14	mezlocillin	0.2-20	Y=21781x+20034	0.9991	0.13	**0.39**
15	Dicloxacillin	0.2-20	Y=21842x+25475	0.9994	0.13	**0.39**

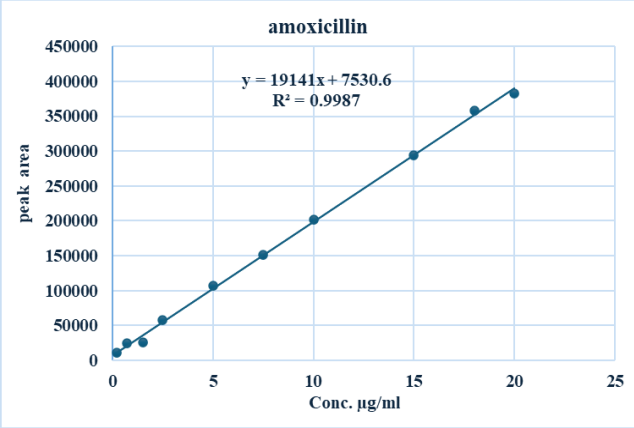

Figure 1: calibration curve of amoxicillin

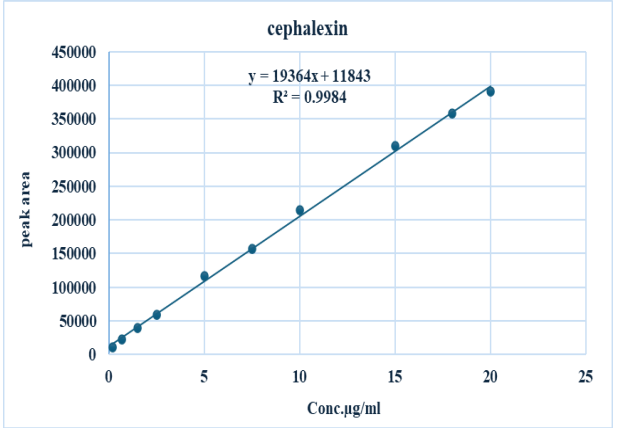

Figure 2: calibration curve of cephalexin

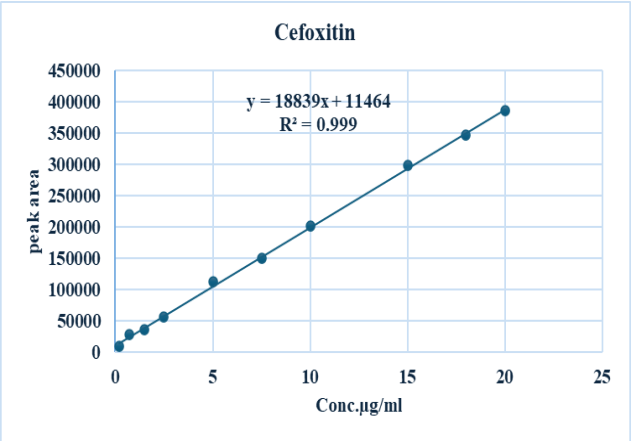

Figure 3: calibration curve of cefoxitin

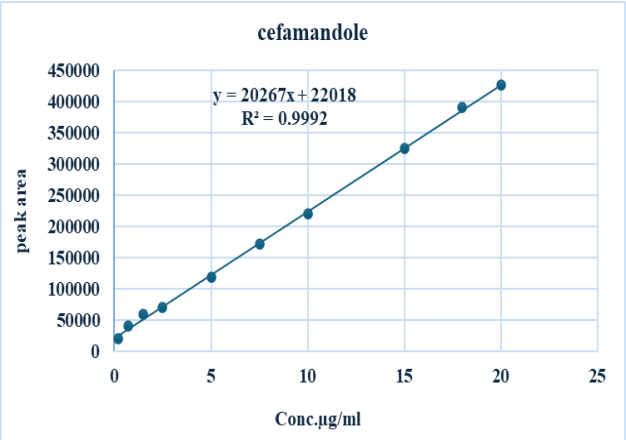

Figure 4: calibration curve of cefamandole

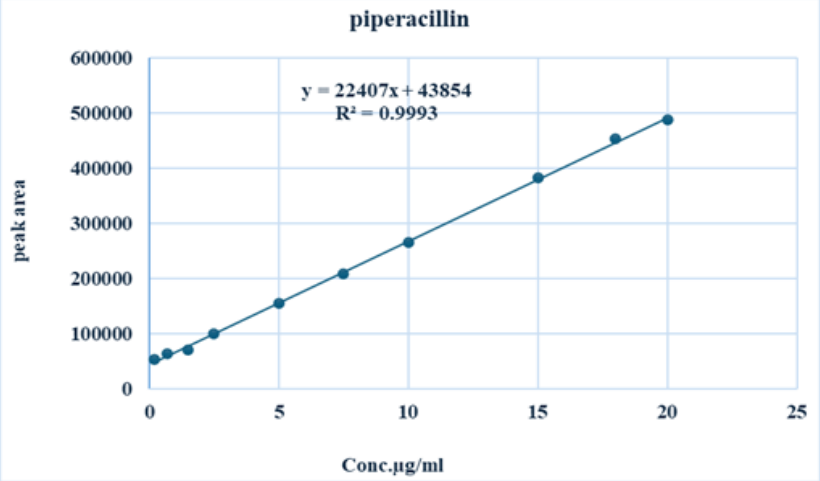

Figure 5: calibration curve of piperacillin

3.4.2. Sensitivity

The Limit of Detection (LOD) represents the lowest concentration or quantity of an analyte that can be reliably detected and differentiated from background noise in an analytical measurement [33]. The limit of quantification is a minimal quantity of analyte in a specimen that can be quantitatively assessed together with appropriate accuracy and precision [34 , 35]. The conclusions are shown in Table 4, which shows that the detection limits range 0.13-0.14 µg/ml and quantitation limits 0.39-0.42 µg/ml.

3.4.3. Precision

The precision study demonstrated suitability for analytical applications, as the percentage relative standard deviations (%RSD) ranged between 0.13% to 2.45%, which is significantly below the 15% standard [36, 37].

Table 4: The method's precision and accuracy were assessed at two spiking levels (the average of a minimum of three replicates).

No.	Subject	Spiked matrix µg/ml	Found µg/ml	Recovery %	RE%	RSD%
1	Amoxicillin	10	10.15	101.5	-1.5	**2.45**
		2.5	2.85	103.5	-3.2	**0.98**
2	ampicillin	10	10.45	104.5	-4.5	**1.86**
		2.5	2.41	96.49	3.51	**0.27**
3	Cephalexin	10	10.43	100.36	-0.36	**0.79**
		2.5	2.43	97.47	2.53	**0.13**
4	Cefotaxime	10	9.82	98.29	1.71	**1.09**
		2.5	2.35	94	6	**1.00**
5	Cefoxitin	10	10.12	101.21	-1.21	**1.87**
		2.5	2.52	100.98	-0.98	**0.72**
6	Cefamandole	10	9.8	98	2	**1.43**
		2.5	2.36	94.68	5.32	**0.54**
7	Cephalothin	10	10.15	101.15	-1.5	**0.67**
		2.5	2.33	93.4	6.6	**0.63**
8	Piperacillin	10	9.90	99	1	**0.99**
		2.5	2.5	100	0	**0.45**
9	Penicillin	10	9.91	99	1	**1.82**
		2.5	2.31	92	8	**0.41**
10	oxacillin	10	10.03	100.3	-0.3	**0.49**
		2.5	2.41	96.70	3.3	**1.27**
11	Cloxacillin	10	10.07	100.74	-0.74	**0.40**
		2.5	2.32	93.15	6.85	**0.71**
12	Nafcillin	10	9.97	99.7	1.3	**1.09**
		2.5	2.27	91	9	**0.56**
13	Carbenicillin	10	10	100	0	**0.38**
		2.5	2.58	103.3	-3.3	**0.40**
14	Mezlocillin	10	10.02	100.24	-0.24	**0.36**
		2.5	2.27	90.90	9.1	**0.47**
15	Dicloxacillin	10	9.93	99.3	0.7	**0.57**
		2.5	2.26	90.68	9.32	**0.25**

3.4.4. Accuracy

Accuracy is termed as the proximity of a computed rate to the actual or recognized amount. In practice, accuracy signifies the disparity between the observed average value and the real value. It is often articulated as a retrieval of specified, supplementary quantities of analyte through test [38]. The recovery percentages were computed as indicated in Table 5. Recoveries varied from 90.68% to 104.5% for pure compounds and from 91.05% to 104% for tablet dosage forms, all of which fall within the permitted range [39]. The formulation of the solution and the components aside from the active substances in the commercial tablet may account for the variation in recovery.

3.5. Assaying commercial samples is an application of the approach

The modern technique was to determine the prepared capsules of commercially available pharmaceutical drugs in the local markets. In addition, powder formulations of amoxicillin, ampicillin, cephalexin, penicillin and cloxacillin in the form of capsules, as well as cefotaxime and piperacillin in the form of injectable formulations, were examined. The results were summarized in the table and thus it was confirmed that they possess superior quality about the content of their active medicinal components.

Table 5: Analytical outcomes of commercial specimens

No.	subject	Commercial name, company	Dosage of drugs	Taken µg/ml	Found µg/ml	Recovery %	RE%	RSD %
1	amoxicillin	Veera Sandra ind. Area, India	Capsule 500 mg	2.5	2.57	103	3	1.21
				10	10.2	102	2	0.83
		Sino pharma China	Capsule, 500 mg	2.5	2.60	104	4	0.6
				10	10.17	101.78	1.78	0.7
2	ampicillin	Ajanta pharma limited, India	Capsule,50 0 mg	2.5	2.38	95.27	-4.73	0.33
				10	10.18	101.86	1.86	1.36
		Jiangsu kangbao pharma, China	Capsule, 500 mg	2.5	2.39	95.87	-4.13	0.44
				10	10.13	101.35	1.35	0.47
3	cephalexin	Pioneer, Iraq	Capsule, 500 mg	2.5	2.43	97.87	-2.13	0.17
				10	10.39	103.9	3.9	1.00
		parenteral, India	Capsule, 500 mg	2.5	2.52	101.17	1.17	0.60
				10	10.28	102.89	2.89	0.82
4	cefotaxime	Newbury, Switzerland	Vial for iv injection, 1 gm	2.5	2.30	92.2	-7.8	0.15
				10	9.86	98.29	-1.71	0.89
		Barcelona -Spain	Vial for iv injection, 0.5 gm	2.5	2.31	92.65	-7.35	0.60
				10	10.09	100.98	0.98	0.76
5	piperacillin	Cooper, Athena Greece	sterile powder injection, 4 gm	2.5	2.47	98.8	-1.2	0.40
				10	9.8	98	-2	1.21
		Aaversi-rational ltd, Georgia	sterile powder injection, 4gm	2.5	2.48	99.39	0.61	0.66
				10	9.84	98.47	-1.53	1.27
6	penicillin v	Pfizer, Switzerland	Capsule, 500 mg	2.5	2.32	92.66	-7.34	0.52
				10	10.27	102.7	2.7	1.02
		Bristol - Myer Squibb company	Capsule, 500 mg	2.5	2.43	97.44	-2.56	0.49
				10	9.89	98.95	-1.05	1.29
7	cloxacillin	Gsk, UK	Capsule,50 0 mg	2.5	2.29	91.8	-8.2	0.73
				10	10.14	101.4	1.4	0.28
		Novartis, Switzerland	Capsule, 500 mg	2.5	2.27	91.05	-8.95	1.00
				10	9.95	99.59	-0.41	0.92

Table 6: comparison of analytical approach with this study

Antibiotic	Mobile phase	Stationary phase	Flow rate	Rt.	Linearity	Detection limit	Ref.
Amoxicillin	acetonitrile and phosphate buffer containing methanol at pH 5.0	C18e (250 mm × 4.0 mm, 5 µm)	0.8 ml/min	17 min	480 - 1120 µg/mL	2.0 µg/mL	[12]
Amoxicillin	0.1% formic acid in ultrapure water (A) and ACN (B)	Zorbax SB column, 150 × 46 µm, 5 µm (Agilent, Santa Clara, CA, USA)	1.5 ml/min	1.72 min	10–100 µg/mL	0.2 µg/mL	[40]
***Amoxicillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	1.5 min	0.2-20 µg/mL	0.14 µg/mL	This work
Ampicillin	30 mM phosphate buffer, pH 4.0 (mobile phase A) and acetonitrile	C_{18} (250 × 4.6 mm, 5 µm)	1 ml/min	20 min	0.05–0.3 µg/mL	0.03 µg/mL	[41]

	(mobile phase B)						
Ampicillin	acetonitrile: water (60:40, v/v), pH adjusted to 4 with orthophosphoric acid	C18 column (25 cm × 4.6 mm I.D., 5 µm)	1 ml/min	2.033 min	5–40 µg /mL	1.53 µg/mL	[42]
***Ampicillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	2.59 min	0.2-20 µg/mL	0.13 µg/mL	This work
Cephalexin	water, methanol (MOH) and acetonitrile (ACN) in the ratio of (60:20:20 V/V/V), pH = 4	C18 column (250 × 4.6 mm, 5µm)	1 ml/min	3.412 min	1-75 mg/L	0.05 mg/L	[43]
Cephalexin	acidic water: acetonitrile (85:15, v/v) at pH 4.5 adjusted by phosphoric acid	hypersil BDS C18 column (250 × 4.6 mm, 5 µm)	2 ml/min	6.0 min	0.05–10 ppm	0.003 ppm	[44]
***Cephalexin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	3.27 min	0.2-20 µg/mL	0.13 µg/mL	This work
Cefotaxime	ACN: distilled water (70:30, v/v)	Thermo Scientific® Venusil XBP C 18 column (L) (5µm, 4.6 x 250 mm).	1 ml/min	1.79 min	2.5-100 µg/mL	4.2×10^{-5} µg/mL	[45]
Cefotaxime	water, tri-fluoroacetic acid (5 mM, pH 3) and acetonitrile (70:30, v/v)	C18 column (5 µm × 250 mm × 4.6 mm	1 ml/min	10 min	10–5000 µg/ kg	3.0 µg /kg	[46]
***Cefotaxime**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	3.99 min	0.2-20 µg/mL	0.14 µg/mL	This work
Cefoxitin	Solvents A and B were 10 mM formic acid in deionized water and 10 mM formic acid in methanol	C18 analytical column (50 mm × 4.6 mm, 1.8 µm; Agilent XDB-C18)	0.5 ml/min	10 min	1-100 µg/mL	0.25 µg/mL	[47]
Cefoxitin	acetonitrile in 0.02 M acetate buffer (pH 4.3) (15:85, v/v) v/v)	Corasil RP C18 (37-50 gin)	1 ml/min	12.2 min	1-100 µg/ml.	0.5 µg/ml	[48]
***Cefoxitin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	4.83 min	0.2-20 µg/mL	0.14 µg/mL	This work
Cefamandole	acetonitrile–methanol–100 mM monosodium phosphate (pH 5.0; 15:20:65, v/v)	C18, 150× 1 mm i.d.; particle size 5 mm)	0.05 ml/min	7.1 min	0.1–50 mg/mL	0.05 mg/mL	[49]

***Cefamandole**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	5.51 min	0.2-20 µg/mL	0.14 µg/mL	This work
Cephalothin	water with 0.7% of glacial acetic acid and ethanol (70:30 v/v)	C18 Agilent column M (150 × 4.6 mm; 5 µm)	1 ml/min	4.20 min	20 - 100 µg/mL	1.95 µg/mL	[16]
Cephalothin	0.1% formic acid and (b) 0.1% formic acid in methanol.	Phenomenex Kinetex C8, 2.1 × 50 mm, 1.7 µm, 100 Å	1 ml/min	4.1 min	0.2–100 µg/mL	Less than 0.46 µg/mL	[50]
***Cephalothin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5µm µm)	1.3 ml/min	6.20 min	0.2-20 µg/mL	0.14 µg/mL	This work
Piperacillin	acetonitrile: water mobile phase mixture with 0.1% trifluoracetic acid	C18, (5 µm, 250 × 4.6 mm)	0.8 ml/min	22.5 min	0.5–400 µg/mL	0.5 µg/mL	[51]
Piperacillin	mixture of potassium dihydrogen orthophosphate buffer (pH 3.5), acetonitrile in the ratio of 60:40 v/v	C18 250 x 4.6 x 5 µm analytical column	1 ml/min	2.5 min	5-15 µg/ml	0.81 µg/mL	[52]
***Piperacillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	7.02 min	0.2-20 µg/mL	0.13 µg/mL	This work
Penicillin	methanol/phosphate (0.1 M potassium dihydrogen phosphate, pH: 4.5)	C18 column (4.6 × 250 mm, 5 µm)	0.6 ml/min	4.2 min	1-300 µg/ L	0.493 µg/ L	[53]
Penicillin	ammonium acetate solution used as aqueous mobile phase was prepared by dissolving the appropriate amount of this salt	C18 analytical column (100 mm x 3.0 mm I.D., particle size 2.7 µm)	0.4 ml/min	4.5 min	100 - 10000 ng/ mL	44 - 51 ng /mL	[54]
***Penicillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	7.84 min	0.2-20 µg/mL	0.13 µg/mL	This work
Oxacillin	phosphoric acid (10 mM) and acetonitrile	a column packed with particles smaller than those used for regular HPLC (particle size for UHPLC, <2 µm), column (2.1 by 100 mm, 1.9 µm)	5 ml/min	13 min	2- 100 mg/L	2 mg/L	[55]
Oxacillin	0.1% TFA/ACN	C8 5 µm, 25064	1.1	4.3	25 – 300	73.6 µg/kg	[56]

	50:50 v/v	mm^2	ml/min	4 min	µg /kg		
***Oxacillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	8.92 min	0.2-20 µg/mL	0.14 µg/mL	This work
Cloxacillin	phosphoric acid (10 mM) and acetonitrile	a column packed with particles smaller than those used for regular HPLC (particle size for UHPLC, <2 µm), column (2.1 by 100 mm, 1.9 µm)	5 ml/min	11 min	2 -100 mg/L	2 mg/L	[57]
Cloxacillin	10 mM phosphoric acid solution, adjusted to pH 2 with hydrochloric acid, and acetonitrile	column (150 by 4.6 mm, 5 m; Waters, Saint Quentin, France), coupled with an Atlantis T3 guard column (20 by 4.6 mm, 5 m; Waters, Saint Quentin, France)	2 ml/min	16.5 min	10 - 50 µg/ml	2 µg/ml	[58]
***Cloxacillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	9.84 min	0.2-20 µg/mL	0.14 µg/mL	This work
Nafcillin	. The combination of acetonitrile (A) and 0.2% aqueous formic acid (B) was executed using a gradient method.	Phenomenex (Torrance, CA, USA) ASynergi 4 µm MAX-RP 80A column (100 × 2 mm)	300 μL/ min	15.15 min	0.2- 1.5 mg/L	7.4 μg/ kg	[58]
Nafcillin	Acetonitrile, 60mL methanol	C18 column (4 µm, 150 × 3.9 mm I. D.)	1 ml/min	12 min	20- 2000 ng/ mL	8 ng/mL	[59]
***Nafcillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	10.62 min	0.2-20 µg/mL	0.14 µg/mL	This work
Carbenicillin	0.05 M ammonium acetate and methanol	5C18-AR (250 × 4.6 mm I.D., 5/µm particle size, Nacalai Tesque Co., Kyoto, Japan)	1.2 ml/ min	18 and 24 min	5-10 mg/ml	10 µg/ml	[60]
Carbenicillin	35 or 37% methanol with the balance being 0.05 M KH2P04 (v/v).	150 X 4.6-mm i.d. column	1.3 ml/min	17 min	0.25 - 4.05 mg/ml	0,06 mg/ml	[61]
***Carbenicillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	11.23 min	0.2-20 µg/mL	0.14 µg/mL	This work
Mezlocillin	0.1 M, pH 6.00, sodium phosphate–0.02 M	C column (5 mm particle size) (Mach-18 2.5.	1 ml/min	3.6 min	1–500 mg/ ml	0 .2 mg /ml	[62]

	sodium thiosul-phate–acetonitrile solution (1:1:1, v/v)	Quantification erey-Nagel, Duren, Germany)					
Mezlocillin	acetonitrile and phosphate buffer mixture (24/76 [vol/vol]; 43 mM K2HPO4,	column (4 by 200 mm) was filled with reverse-phase material 5C18	1 ml/min	15.30 min	1 and 250 µ/ml	0.1 µg/ml	[63]
***Mezlocillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	12.11 min	0.2-20 µg/mL	0.13 µg/mL	This work
Dicloxacillin	acetonitrile: water (65: 35 by volume	C18 column (3.5 µm ps, 100 mm × 4.6 mm id)	0.5 ml/min	3.50 min	1–10 µg/ mL	0.539 µg/mL	[64]
Dicloxacillin	acetonitrile: water (60:40, v/v)	C18 column (25 cm × 4.6 mm I.D., 5 µm)	1 ml/min	3.809 min	5–40 µg/ mL	1.56 µg/ mL	[42]
***Dicloxacillin**	solvent A (acetonitrile) and solvent B (20 mm KH_2PO_4)	column NEUCLEODUR C-18 (4.0 mm × 100 mm, 5 µm)	1.3 ml/min	13.21 min	0.2-20 µg/mL	0.13 µg/mL	This work

Conclusion

A highly sensitive and reliable HPLC-UV method was successfully developed and validated for the simultaneous quantification of 15 β-lactam antibiotics (amoxicillin, ampicillin, cephalexin, cefotaxime, cefoxitin, cefamandole, cephalothin, penicillin G, oxacillin, nafcillin, carbenicillin, mezlocillin, and dicloxacillin) in both pure pharmaceutical standards and commercial formulations, demonstrating robust performance for quality control and regulatory compliance in pharmaceutical analysis. The drug samples were dissolved in the solvents used, acetonitrile and potassium buffer phosphate, then filtered and then poured over the stationary phase for separation. The analysis time was 13 minutes. The developed method provided good and acceptable performance and was easily applied to pharmaceutical preparations. Additionally, this study represents the first reported method for the simultaneous quantification of these 15 β-lactam antibiotics in a single analytical run. The aim of analyzing drugs in HPLC method is to determine the accuracy and precision of different drugs in addition to applying the proposed method to pharmaceutical preparations to ensure the effectiveness and safety of drugs. This method is also used in quality control measurements because it is a fast, accurate and inexpensive method used to analyze many drugs at the same time.

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Authors contributions

The authors have made equal contributions.

Conflict of interests

Declared none

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