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Simultaneous Determination of 12 β-Lactam Antibiotics in Human Plasma by High-Performance Liquid Chromatography with UV Detection: Application to Therapeutic Drug Monitoring▽

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A rapid and specific high-performance liquid chromatography method with UV detection (HPLC-UV) for the simultaneous determination of 12 beta-lactam antibiotics (amoxicillin, cefepime, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, imipenem, meropenem, oxacillin, penicillin G, piperacillin, and ticarcillin) in small samples of human plasma is described. Extraction consisted of protein precipitation by acetonitrile. An Atlantis T3 analytical column with a linear gradient of acetonitrile and a pH 2 phosphoric acid solution was used for separation. Wavelength photodiode array detection was set either at 210 nm, 230 nm, or 298 nm according to the compound. This method is accurate and reproducible (coefficient of variation [CV] < 8%), allowing quantification of beta-lactam plasma levels from 5 to 250 μg/ml without interference with other common drugs. This technique is easy to use in routine therapeutic drug monitoring of beta-lactam antibiotics.

The beta-lactam antibiotics constitute the most important family of antimicrobial agents, both in terms of the large number of compounds available and in terms of prescription volume. Until recently, beta-lactams were not considered strong candidates for therapeutic drug monitoring (TDM), as they are assumed to have a wide therapeutic index and the most frequent adverse effects involve non-dose-related allergic reactions. But concepts are changing, and several studies demonstrate the advantage of TDM for these antibiotics (4, 32). Clinicians and pharmacologists agree that certain adverse events like encephalopathy, seizure, and pseudolithiasis occur when doses are excessive (2, 8, 12, 15, 16, 19, 23, 27). Various infectious diseases, e.g., endocarditis, meningitis, and osteomyelitis, require administration of high-dose antibiotics via intermittent or continuous infusion (6, 36, 38). Knowledge of the antibiotic plasma concentrations, combined with bacterial susceptibility evaluated in terms of the MIC, would enable treatment efficacy to be optimized while limiting the risk of dose-related adverse effects and avoiding suboptimal concentrations (32). The pharmacokinetics of beta-lactams can be highly variable depending on the clinical situation, leading to unpredictable plasma concentrations. Critically ill patients with severe infection demonstrate unusual volumes of distribution and half-lives of drugs (3, 26). Similarly, pharmacokinetic properties are altered in severely burned patients, who have a high risk of infection (20, 28). Plasma concentrations are also difficult to predict in patients with renal failure who require dose adjustments (22).

There are several proposed methods for assaying beta-lactams in biological fluids, either for a particular compound (5, 11, 24, 30) or for a group of compounds (9, 10, 13, 17, 18, 21, 29, 33, 34). Many other methods were developed for food industry and agriculture applications (1). The method previously published by McWhinney et al. (25) has been validated for 12 compounds but used three different mobile phases to separate the compounds. A beta-lactam assay for TDM must be robust and rapid, providing reliable results that can be reported to the clinical ward within a few hours. A single process simplifies the implementation of TDM for the therapeutic group of beta-lactam antibiotics, which includes a large number of compounds. In this context, the simultaneous determination of plasma concentrations of the beta-lactams most commonly administered intravenously at high doses in patients with severe infections would be most welcome.

The aim of this work was to develop a precise, reproducible, and rapid high-performance liquid chromatography (HPLC) method applicable for TDM of the most commonly prescribed beta-lactams: six penicillins (amoxicillin, cloxacillin, oxacillin, penicillin G, piperacillin, and ticarcillin), four expanded-spectrum cephalosporins (cefepime, cefotaxime, ceftazidime, and ceftriaxone), and two carbapenems (imipenem and meropenem).

MATERIALS AND METHODS

Chemicals. Amoxicillin, ceftriaxone, cloxacillin sodium salt, and oxacillin sodium salt were purchased from Sigma (Saint-Quentin, France). Cefotaxime sodium salt, penicillin G potassium salt, and piperacillin sodium salt were purchased from ICN Biomedicals (Orsay, France). Imipenem was kindly provided by Merck and Co. (Paris, France). Ceftazidime and ticarcillin were supplied by GlaxoSmithKline (Marly-Le-Roi, France), cefepime was supplied by Bristol-Myers Squibb (Rueil-Malmaison, France), and meropenem was supplied by AstraZeneca (Rueil-Malmaison, France). Acetonitrile, from Carlo Erba Reagenti (Val de Reuil, France), was of HPLC grade. Phosphoric acid (85%) was purchased from Merck (Nogent-sur-Marne, France). Milli-Q water (Saint-Quentin, France) was used throughout the experimentations.

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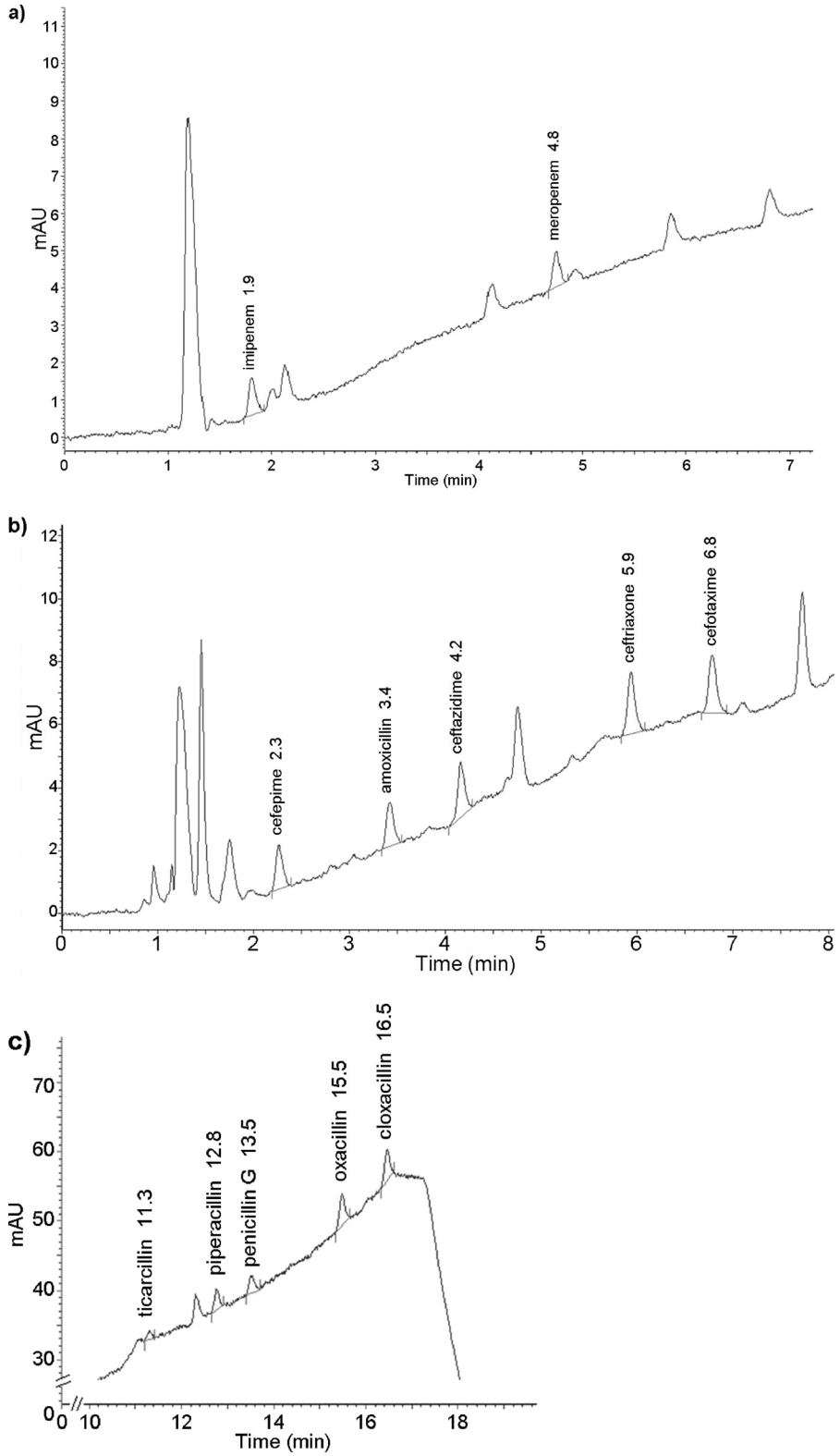

FIG. 1. HPLC chromatograms of an internal quality control with 10 μg/ml of each antibiotic at 298 nm (a), 230 nm (b), and 210 nm (c). Values after the antibiotic names are retention times.

Instrumentation and chromatographic conditions. The HPLC system included a P1000XR pump equipped with a degasser, an AS3000 refrigerated autosampler, and a UV microarray spectrophotometer detector (UV6000LP; ThermoFisher Scientific, Villebon sur Yvette, France). The compounds were separated on an Atlantis T3 analytical column (150 by 4.6 mm, 5 μm; Waters, Saint Quentin, France), coupled with an Atlantis T3 guard column (20 by 4.6 mm, 5 μm; Waters, Saint Quentin, France). The mobile phase, filtered and degassed, consisted of a 10 mM phosphoric acid solution, adjusted to pH 2 with hydrochloric acid, and acetonitrile. A linear gradient from 7% to 19% acetonitrile in 6 min and 19% to 49% from 6 to 16 min was used with a flow rate of 2 ml/min. Run time was prolonged to 22 min to return to initial conditions. The effluent was monitored at 210 nm for cloxacillin, oxacillin, penicillin G, piperacillin, and ticarcillin, at 230 nm for amoxicillin, cefepime, ceftazidime, cefotaxime, and ceftriaxone, and at 298 nm for imipenem and meropenem. Data were recorded with ChromQuest software (version 2.5.1). Quantification was based on the peak height of each compound; baselines were inspected visually and adjusted manually when necessary.

Standard solutions and QC materials. Stock solutions of each beta-lactam were prepared at 50 or 100 mg/ml in water and stored at −80°C. Under these conditions they are stable for a few months (9, 18, 25). They were further mixed in water to obtain a 1-mg/ml solution, which was used to produce standard solutions at 250, 125, 40, and 10 μg/ml. Three working solutions of beta-lactams at 50, 1,000, and 2,000 μg/ml were prepared from stock solutions and diluted 10-fold in blank plasma to obtain quality control (QC) materials at 5 μg/ml, 100 μg/ml, and 200 μg/ml.

Sample preparation. Venous blood samples were collected in heparinized tubes, immediately transported on ice to the laboratory, and centrifuged for 10 min at 3,000 × *g* and 4°C. If necessary, the plasma samples were stored at −20°C until analysis. For the extraction procedure, 200 μl of water and 700 μl of acetonitrile were added to 100 μl of control or patient plasma samples. For the calibration curve, 100 μl of corresponding working solution, 100 μl of water, and 700 μl of acetonitrile were mixed with 100 μl of blank plasma. Each sample was vortex mixed and centrifuged at 3,000 × *g* for 10 min at 4°C. One hundred microliters of the supernatant was diluted with 900 μl of water, and 20 μl was injected into the HPLC system. This dilution provides the same percentage of acetonitrile in the injected sample as the initial conditions of the linear gradient.

Method validation. The method was validated according to the acceptance criteria of industrial guidance for bioanalytical method validation (14).

Specificity and selectivity. Specificity and selectivity were measured by analyzing six different blank plasma samples. Different plasma samples containing 39 frequently prescribed drugs were analyzed to investigate potential interferences. The drugs were acetaminophen, amikacin, amiodarone, amlodipine, amphotericin B, amprenavir, carbamazepine, ciprofloxacin, clonazepam, digoxin, disopyramide, doripenem, doxapram, efavirenz, ertapenem, flunitrazepam, gentamicin, indinavir, itraconazole, lopinavir, mycophenolic acid, nelfinavir-M8, nevirapine, phenobarbital, phenytoin, propranolol, ritonavir, saquinavir, quinine, ofloxacin, ramipril, ranitidine, salicylic acid, sulfamethoxazole, tacrolimus, theophylline, trimethoprim, valproic acid, and vancomycin.

Precision and accuracy. The recommended value for precision and accuracy is ±15%, except for the lower limit of quantification (LOQ), for which a limit of ±20% is acceptable. The limit of detection (LOD) is defined as the first concentration with a peak height of at least three times the baseline value. To test within-day and between-day reproducibility, six aliquots of each QC sample were tested the same day to evaluate within-day reproducibility and once a day for 3 days to evaluate between-day reproducibility. Mean and standard deviation (SD) were calculated. The coefficient of variation (CV) was used to calculate precision, and the bias was used to express accuracy (7).

Recovery. The recovery of each compound was calculated at three concentrations, 10, 100, and 250 μg/ml, by comparing the beta-lactam peak heights of extracted QC samples to the beta-lactam peak heights of nonextracted standard solutions at the same concentration.

Stability. The stability of the different antibiotics was tested on the same extraction dilution at the beginning and at the end of the injection sequence, i.e., an interval of 15 h, at 9°C. The stability of beta-lactams was also determined in patients' plasma preserved at 4°C or room temperature for 12 h.

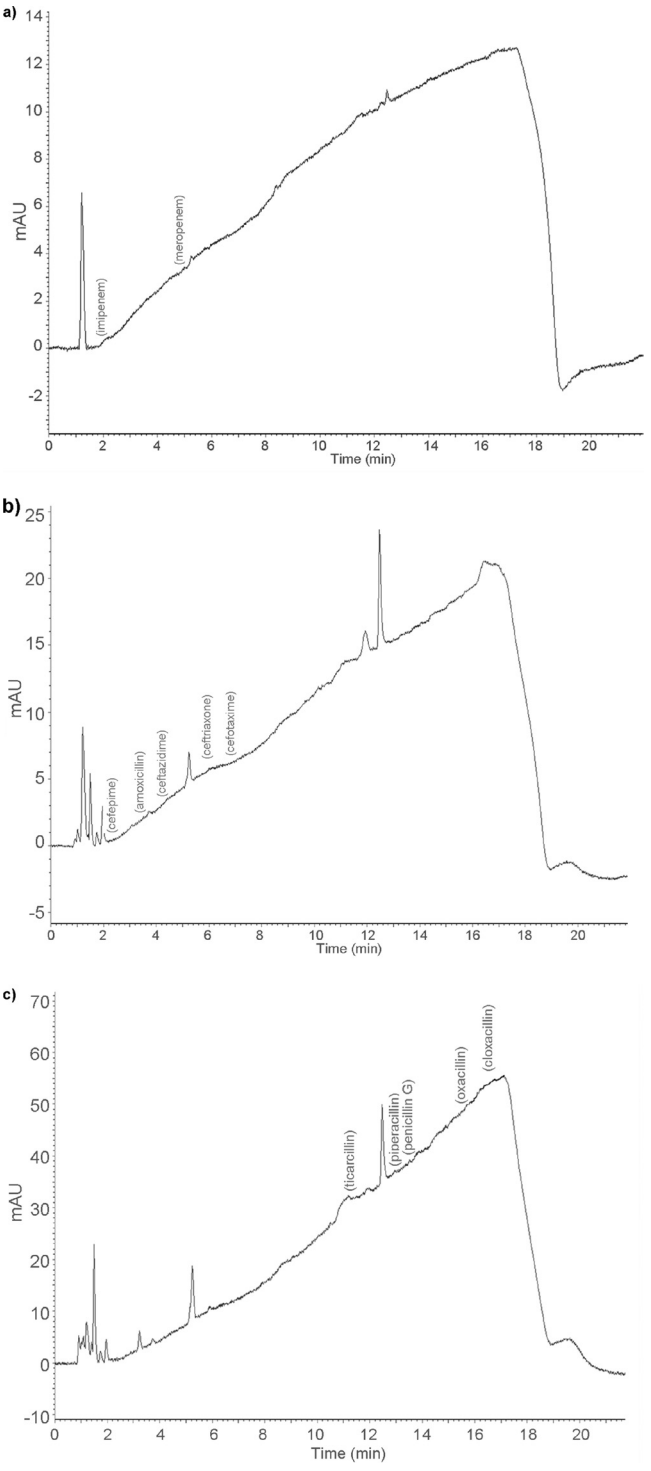

FIG. 2. HPLC chromatograms of a blank plasma sample at 298 nm (a), 230 nm (b), and 210 nm (c). Retention times for the different compounds are indicated by the positions of the names.

RESULTS AND DISCUSSION

Chromatography. Separation of the 12 compounds was satisfactory, as shown in Fig. 1. No interference with endogenous compounds was observed, allowing use of specific wavelengths of 210, 230, and 298 nm, as shown in Fig. 2 (blank plasma sample) and Fig. 3 (patient plasma samples). Deviation from the baseline is related to the gradient of acetonitrile, which absorbs UV. So the baseline absorbance was higher when the acetonitrile amount increased. The Atlantis T3 column en-

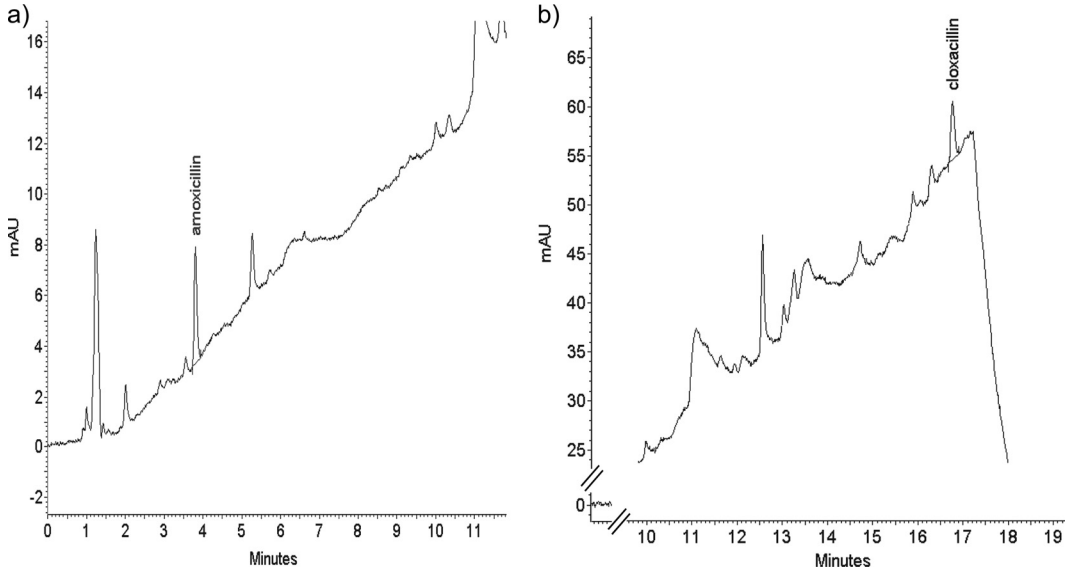

FIG. 3. Chromatograms of extracted plasma samples from patients treated with amoxicillin (residual concentration, 33.5 mg/liter) (a) and cloxacillin (residual concentration, 15.3 mg/liter) (b).

abled a simplified HPLC method allowing good retention of polar drugs. A gradient of acetonitrile enabled elution of less-polar compounds. This was the only column we know allowing good separation of all compounds and supporting this extreme pH. With other C_{18} columns, retention times ranged from 1 min (passage through the column without retention) to above 1 h (cloxacillin) and separation was not optimal. The pH of the mobile phase was another parameter that modified retention time. Changes in retention times of the different compounds according to pH of the mobile phase are shown in Fig. 4. The curve shows that extreme pH (2) provided the best separation while maintaining retention times under 17 min. For most of the compounds, retention time decreased as the pH increased, with the risk of elution with the solvent front or coelution of several compounds (penicillin G plus piperacillin at pH 5 or amoxicillin plus ceftazidime plus ceftriaxone at pH 7). We found the best compromise between stationary phase and pH. In this way, the 12 antibiotics were assayed within 22 min. We recommend the use of a guard column to extend the lifetime of the column subjected to acid pH. At this pH, imipenem pre-

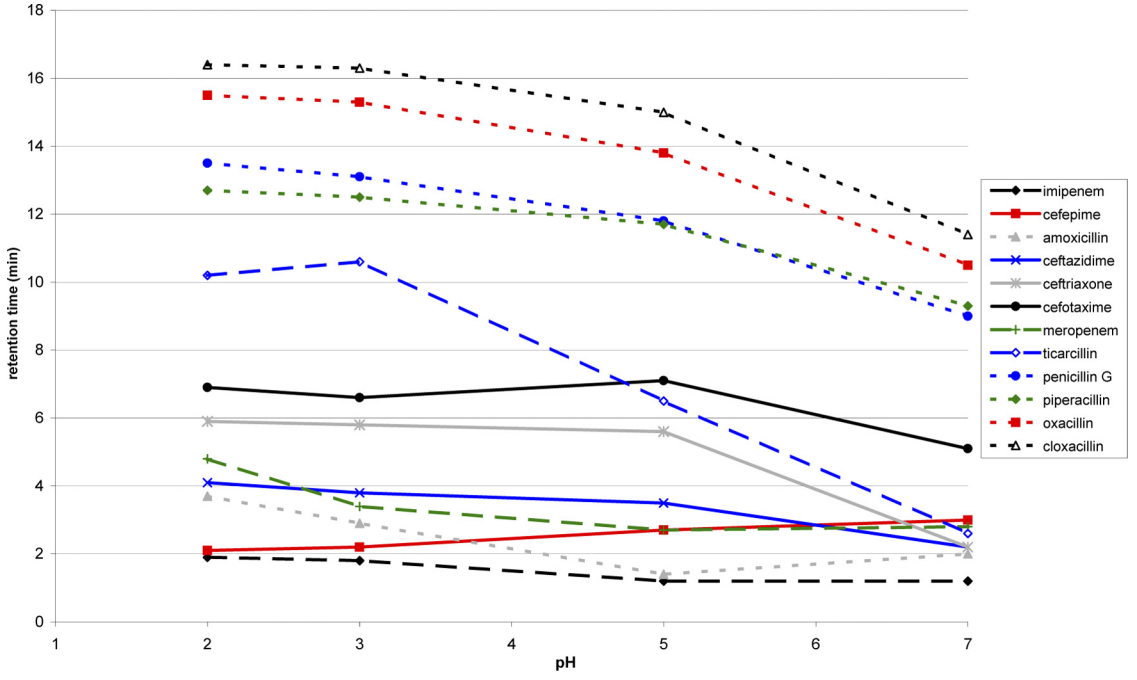

FIG. 4. Retention times of beta-lactam antibiotics by pH of the mobile phase.

TABLE 1. Chromatographic data and extraction recovery for the different compounds

Compound	Wavelength (nm)	Retention time (min)	Linear regression ($y = ax + b$) value for:		R^2	Recovery (%) at concn (μg/ml) of:		
			a	*b*		10	100	250
Imipenem	298	1.9	0.01085	−0.10	0.9998	87.3	93.4	95.6
Cefepime	230	2.3	0.00205	0.02	0.9930	118.4	98.3	98.5
Amoxicillin	230	3.4	0.00249	−0.03	0.9945	99.3	100.2	101.1
Ceftazidime	230	4.2	0.00198	0.17	0.9991	98.2	96.3	97.4
Meropenem	298	4.8	0.00186	0.46	0.9999	114.7	101.7	100.3
Ceftriaxone	230	5.9	0.00129	0.23	0.9917	98.7	98.6	97.2
Cefotaxime	230	6.8	0.00207	−0.07	0.9943	100.0	99.3	100.3
Ticarcillin	210	11.3	0.00960	0.36	0.9988	104.5	102.7	100.3
Piperacillin	210	12.8	0.00407	−0.40	0.9969	102.9	102.7	102.7
Penicillin G	210	13.5	0.02605	−0.39	0.9952	96.9	100.9	102.2
Oxacillin	210	15.5	0.00205	0.05	0.9995	104.9	101.9	102.4
Cloxacillin	210	16.5	0.00021	0.33	0.9979	103.2	102.9	103.3

sented 2 peaks because of its tautomeric form (31). We attempted to stabilize our samples and the stock solutions by diluting with MES (morpholineethanesulfonic acid) buffer at pH 7.4 or phosphate buffer at pH 10 but were unable to avoid this double peak. However, we noticed that this tautomeric form was stable, and the accuracy and precision assays confirmed that we could determine the plasma concentration of imipenem under these conditions by considering the first of the two peaks.

Sample preparation. Different types of sample preparation were tested, solid phase extraction (SPE) with three different stationary phases (SCX, NH2, and OASIS HLB; Waters, St Quentin, France) and protein precipitation. SPE was not effective for the 12 compounds. Protein precipitation was the most reproducible and allowed the best recovery. As performed by McWhinney et al. (25), we removed lipid-soluble components by a chloroform or hexane wash after protein precipitation. The procedure with chloroform increased the LOQ while concentrating the compounds in the aqueous phase, but it increased the signal-to-noise ratio and induced some high interfering peaks. The procedure with hexane did not change anything. This wash was not added to the extraction procedure, so our method is extremely rapid.

Method validation. Values for retention time, detection wavelength, linear regression, correlation coefficient, and extraction recovery for the different compounds are given in Table 1.

The calibration curves, fitted using linear regression between the beta-lactam peak heights and the concentrations of the respective compounds, appeared linear from 2 to 250 μg/ml for amoxicillin, cefepime, cefotaxime, ceftazidime, imipenem, and meropenem and from 5 to 250 μg/ml for ceftriaxone, cloxacillin, oxacillin, penicillin G, piperacillin, and ticarcillin. All calibration curves were well described using a weighted least-squares regression (weighting factor: 1/concentration) to achieve homogeneity of variances. The coefficients of determination (R^2) were all above 0.99.

Specificity and selectivity. There was no evidence of interference with the different compounds tested. Not all the beta-lactam metabolites were available and could be evaluated. Therefore, few metabolites were verified, and one of them, desacetyl-cefotaxime, was determined in another run because of a risk of interference with amoxicillin or ceftazidime (retention time = 3.8 min) (34). This interference was a problem for standard solutions and QC but not in patients treated with cefotaxime, amoxicillin, or ceftazidime. Beta-lactams are antibiotics which are very rarely prescribed simultaneously. Patients treated with cefotaxime present detectable concentrations of desacetyl-cefotaxime but are never simultaneously treated with amoxicillin or ceftazidime. Conversely, patients treated with amoxicillin or ceftazidime are never treated with cefotaxime at the same time. Currently, determination of desacetyl-cefotaxime is not routinely prescribed in our hospital.

Accuracy and precision. The LODs were <1 μg/ml for amoxicillin, cefepime, cefotaxime, ceftazidime, imipenem, and meropenem and 2 μg/ml for the other compounds. The LOQ was 2 or 5 μg/ml for the different compounds. Data on the accuracy and precision analysis are summarized in Table 2. All the repeatability and reproducibility tests give a variation of less than 8%.

Target residual concentrations were within the 10 to 50 μg/ml range and were 30 to 100 μg/ml for piperacillin and ticarcillin (13, 17, 20, 28, 32). The efficacy of beta-lactams was proved for plasma concentrations over 4 to 5 times greater than the MIC more than 50% of the time between two injections. Clinicians asked for the determination in severe infections, for which they request concentrations above 10 times the MIC 100% of the time. MICs for strains sensitive to beta-lactams are between <0.5 and <8 μg/ml. When the residual concentration is lower than 4 times this value, the antibiotic is no longer sufficiently effective. We concluded that it was not necessary to determine concentrations lower than 2 or 5 μg/ml for this application.

Recovery. Mean recovery values were greater than 90% for the different compounds.

Stability. The stability study, performed on extracts preserved in a refrigerated automatic injector for 15 h at 9°C, demonstrated a reduction in spike height from 3 to 18% depending on the antibiotic. Ceftazidime exhibited the most pronounced degradation pre- and postextraction: in plasma preserved at 4°C or room temperature, concentrations declined 3.7 and 7%, respectively, after 12 h.

Therapeutic drug monitoring. Every day we receive 1 or more requests for TDM of several beta-lactams, hence the

TABLE 2. Intraday and interday precision and accuracy values for the different compounds

Antibiotic	Theoretical concn (μg/ml)	Intraday validation (n = 6)			Interday validation (n = 6)		
		Mean concn (μg/ml)	CV (%)	Accuracy (%)	Mean concn (μg/ml)	CV (%)	Accuracy (%)
Imipenem	5	4.7	3.1	94.0	4.9	7.2	98.0
	100	97.4	3.1	97.4	99.5	5.9	99.5
	200	197.7	2.9	98.9	197.6	3.8	98.8
Cefepime	5	4.8	4.3	96.0	4.6	5.6	92.0
	100	101.2	3.7	101.2	98.4	2.7	98.4
	200	189.0	4.8	94.5	193.2	2.7	96.6
Amoxicillin	5	5.1	3.1	102.0	4.3	2.7	86.0
	100	104.1	3.1	104.1	98.2	1.6	98.2
	200	210.5	1.8	105.3	197.7	1.3	98.9
Ceftazidime	5	5.4	2.7	108.0	4.9	1.8	98.0
	100	100.0	2.4	100.0	100.2	1.9	100.2
	200	197.0	1.9	98.5	199.3	1.5	99.7
Ceftriaxone	5	4.9	2.6	98.0	4.4	2.4	88.0
	100	88.0	3.5	88.0	95.1	2.3	95.1
	200	207.5	1.1	103.8	201.3	1.6	100.7
Meropenem	5	4.9	3.9	98.0	4.7	3.4	94.0
	100	102.1	2.6	102.1	94.5	1.4	94.5
	200	197.4	1.7	98.7	196.1	1.2	98.1
Cefotaxime	5	4.5	5.6	90.0	4.1	1.4	82.0
	100	97.4	2.0	97.4	97.5	2.1	97.5
	200	191.9	1.8	96.0	196.8	1.3	98.4
Ticarcillin	5	4.5	3.9	90.0	4.0	2.8	80.0
	100	106.7	3.2	106.7	99.3	2.1	99.3
	200	208.1	0.9	104.1	199.8	1.4	99.9
Piperacillin	5	4.8	2.7	96.0	4.2	3.3	84.0
	100	101.3	3.8	101.3	100.1	2.2	100.1
	200	193.2	4.1	96.6	199.5	2.0	99.8
Penicillin G	5	4.6	1.7	92.0	4.3	4.6	86.0
	100	89.4	3.3	89.4	99.7	2.9	99.7
	200	198.5	1.7	99.3	200.0	1.8	100.0
Oxacillin	5	4.7	3.2	94.0	4.9	3.1	98.0
	100	102.0	2.7	102.0	99.5	1.7	99.5
	200	198.7	1.8	99.4	201.3	1.3	100.7
Cloxacillin	5	4.3	5.1	86.0	4.2	5.5	84.0
	100	103.8	2.6	103.8	99.2	1.8	99.2
	200	198.7	1.9	99.4	202.4	1.4	101.2

importance of having a unique technique to be able to meet these requests whatever the compound. During the past 18 months, 2,196 measurements (30 per week) have been performed in our laboratory on these 12 drugs for 761 patients. Amoxicillin and cloxacillin were the two beta-lactam agents most determined. Samples were drawn at steady state to determine trough concentration for intermittent administrations or plateau concentration for continuous infusions. Analyses performed in response to the first requests for plasma concentrations (n = 746) showed that 15.3% of patients had inadequate concentrations, either <10 μg/ml or >100 μg/ml, for all compounds included. The limit of 10 μg/ml was established based on MIC breakpoints of most strains involved (35), and the limit of 100 μg/ml was set as the threshold for toxicity. Based on our clinical experience, effective and nontoxic concentrations are mostly between 10 and 50 μg/ml and up to 100 μg/ml for piperacillin and ticarcillin, whose MIC breakpoints are higher. Considering this range, 35.0% of patients showed inadequate plasma concentrations, whatever the administration schedule and despite doses adapted according to weight and renal function. Target concentrations have to be precisely determined for each patient, depending on the strain and MIC, consistent with the recommendations of the French Society for Microbiology (35). Because plasma concentrations of beta-lactams remain largely unpredictable, TDM of these antibiotics is now an everyday tool, in addition to antimicrobial susceptibility testing of each strain, for the proper management of patients receiving high intravenous doses.

Conclusion. A rapid, robust, and specific HPLC method with UV detection for the simultaneous determination of 12 beta-lactams in small samples of human plasma is described. Daily, this method can be applied to meet all requests in a single sequence run. Compared with other analytical methods published previously, the method presented here offers the advantage of a rapid simultaneous assay of commonly prescribed antibiotics using one rapid extraction protocol and satisfying all validation criteria. It is complementary to the method published by McWhinney et al. (25) because it does not concern the same compounds.

In clinical practice, this technique is already in routine use for TDM (37). Around 30 assays of beta-lactam concentrations have been performed each week on patient plasma since the validation. In addition, this method will be applied to more-recent beta-lactams such as ertapenem and doripenem.

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REFERENCES

1. **Bailón-Pérez, M. I., A. M. García-Campaña, M. del Olmo-Iruela, L. Gámiz-Gracia, and C. Cruces-Blanco.** 2009. Trace determination of 10 beta-lactam antibiotics in environmental and food samples by capillary liquid chromatography. J. Chromatogr. A **1216:**8355–8361.
2. **Basto, E., J. P. Sollet, G. Bleichner, and B. Eurin.** 1998. Fatal encephalopathy induced by ceftazidime. Therapie **53:**608–610.
3. **Belzberg, H., et al.** 2004. Imipenem levels are not predictable in the critically ill patient. J. Trauma **56:**111–117.
4. **Blondiaux, N., et al.** 2010. Daily serum piperacillin monitoring is advisable in critically ill patients. Int. J. Antimicrob. Agents **35:**500–503.
5. **Bowman, D. B., M. K. Aravind, J. N. Miceli, and R. E. Kauffman.** 1984. Reversed-phase high-performance liquid chromatographic method to determine ceftriaxone in biological fluids. J. Chromatogr. **309:**209–213.
6. **Burgess, D. S., R. W. Hastings, and T. C. Hardin.** 2000. Pharmacokinetics and pharmacodynamics of cefepime administered by intermittent and continuous infusion. Clin. Ther. **22:**66–75.
7. **Causon, R.** 1997. Validation of chromatographic methods in biomedical analysis. Viewpoint and discussion. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **689:**175–180.
8. **Chatellier, D., et al.** 2002. Cefepime-induced neurotoxicity: an underestimated complication of antibiotherapy in patients with acute renal failure. Intensive Care Med. **28:**214–217.
9. **Dailly, E., R. Bouquié, G. Deslandes, P. Jolliet, and R. Le Floch.** 2011. A liquid chromatography assay for a quantification of doripenem, ertapenem, imipenem, meropenem concentrations in human plasma: application to a clinical pharmacokinetic study. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **879:**1137–1142.
10. **Denooz, R., and C. Charlier.** 2008. Simultaneous determination of five beta-lactam antibiotics (cefepime, ceftazidime, cefuroxime, meropenem and pip-

eracillin) in human plasma by high-performance liquid chromatography with UV detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **864:**161–167.
11. **De Pourcq, P., J. Hoebus, E. Roets, J. Hoogmartens, and H. Vanderhaeghe.** 1985. Quantitative determination of amoxicillin and its decomposition products by high-performance liquid chromatography. J. Chromatogr. **321:**441–449.
12. **Dupuis, A., et al.** 2001. Pharmacokinetic-pharmacodynamic modeling of electroencephalogram effect of imipenem in rats with acute renal failure. Antimicrob. Agents Chemother. **45:**3607–3609.
13. **Ehrlich, M., F. D. Daschner, and K. Kümmerer.** 2001. Rapid antibiotic drug monitoring: meropenem and ceftazidime determination in serum and bronchial secretions by high-performance liquid chromatography-integrated sample preparation. J. Chromatogr. **751:**357–363.
14. **FDA.** 2001. Guidance for industry, bioanalytical method validation. FDA, Washington, DC.
15. **Grill, M. F., and R. Maganti.** 2008. Cephalosporin-induced neurotoxicity: clinical manifestations, potential pathogenic mechanisms, and the role of electroencephalographic monitoring. Ann. Pharmacother. **42:**1843–1850.
16. **Huang, W. T., Y. J. Hsu, P. L. Chu, and S. H. Lin.** 2009. Neurotoxicity associated with standard doses of piperacillin in an elderly patient with renal failure. Infection **7:**374–376.
17. **Jehl, F., P. Birckel, and H. Monteil.** 1987. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography. J. Chromatogr. **413:**109–119.
18. **Jehl, F., H. Monteil, and R. Minck.** 1983. Focus and value of the assay of 5 beta-lactams using high-performance liquid chromatography. Pathol. Biol. **31:**370–374.
19. **Lamoth, F., et al.** 2009. High imipenem blood concentrations associated with toxic encephalopathy in a patient with mild renal dysfunction. Int. J. Antimicrob. Agents **34:**386–388.
20. **Le Floch, R., J. F. Arnould, A. Pilorget, E. Dailly, and E. Naux.** 2010. Antimicrobial blood concentrations in burns. A five years' retrospective survey. Pathol. Biol. **58:**137–143.
21. **Legrand, T., et al.** 2008. Simultaneous determination of three carbapenem antibiotics in plasma by HPLC with UV detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **875:**551–556.
22. **Lemaire-Hurtel, A. S., et al.** 2009. Recommended dosage adaptation based on renal function is not always sufficient to avoid betalactam antibiotics side effects. Nephrol. Ther. **5:**144–148.
23. **Martínez-Rodríguez, J. E., et al.** 2001. Nonconvulsive status epilepticus associated with cephalosporins in patients with renal failure. Am. J. Med. **111:**115–119.
24. **Mascher, H. J., and C. Kikuta.** 1998. Determination of amoxicillin in human serum and plasma by high-performance liquid chromatography and on-line postcolumn derivatisation. J. Chromatogr. A **812:**221–226.
25. **McWhinney, B. C., et al.** 2010. Analysis of 12 beta-lactam antibiotics in human plasma by HPLC with UV detection. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **878:**2039–2043.
26. **Mehrotra, R., R. De Gaudio, and M. Palazzo.** 2004. Antibiotic pharmacokinetic and pharmacodynamic considerations in critical illness. Intensive Care Med. **30:**2145–2156.
27. **Mohkam, M., et al.** 2007. Ceftriaxone associated nephrolithiasis: a prospective study in 284 children. Pediatr. Nephrol. **22:**690–694.
28. **Pea, F., P. Viale, and M. Furlanut.** 2005. Antimicrobial therapy in critically ill patients: a review of pathophysiological conditions responsible for altered disposition and pharmacokinetic variability. Clin. Pharmacokinet. **44:**1009–1034.
29. **Pehourcq, F., and C. Jarry.** 1998. Determination of third-generation cephalosporins by high-performance liquid chromatography in connection with pharmacokinetic studies. J. Chromatogr. A **812:**159–178.
30. **Perez, B., C. Prats, E. Castells, and M. Arboix.** 1997. Determination of cloxacillin in milk and blood of dairy cows by high-performance liquid chromatography. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. **698:**155–160.
31. **Ratcliffe, R. W., et al.** 1989. Studies on the structures of imipenem, dehydropeptidase I-hydrolyzed imipenem, and related analogs. J. Org. Chem. **54:**653–660.
32. **Roberts, J. A., et al.** 2010. Therapeutic drug monitoring of beta-lactams in critically ill patients: proof of concept. Int. J. Antimicrob. Agents **36:**332–339.
33. **Samanidou, V. F., E. N. Evaggelopoulou, and I. N. Papadoyannis.** 2006. Development of a validated HPLC method for the determination of four penicillin antibiotics in pharmaceuticals and human biological fluids. J. Sep. Sci. **29:**1150–1160.
34. **Seguin, P., et al.** 2009. Plasma and peritoneal concentration following continuous infusion of cefotaxime in patients with secondary peritonitis. J. Antimicrob. Chemother. **63:**564–567.
35. **Société Française de Microbiologie.** 2010. Comité de l'antibiogramme—recommandations. Société Française de Microbiologie, Paris, France. http://www.sfm-microbiologie.org/.
36. **Tattevin, P., et al.** 2005. Use of high-performance liquid chromatography (HPLC) to monitor beta-lactam plasma concentrations during the treatment of endocarditis. Clin. Microbiol. Infect. **11:**76–79.
37. **Verdier, M. C., O. Tribut, P. Tattevin, C. Michelet, and D. Bentué-Ferrer.** Assessment of interindividual variability of plasma concentrations after administration of high doses of intravenous amoxicillin or cloxacillin in critically ill patients. J. Chemother., in press.
38. **Visser, L. G., P. Arnouts, R. van Furth, H. Mattie, and P. J. van den Broek.** 1993. Clinical pharmacokinetics of continuous intravenous administration of penicillins. Clin. Infect. Dis. **17:**491–495.