Research Results in Pharmacology

Research Results in Pharmacology 9(2): 55–59
UDC: 615.074
DOI 10.18413/rrpharmacology.9.10027

Research Article

Development and validation of a quantitative HPLC/MS/MS method for the determination of piperacillin in blood plasma

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Academic editor: Tatyana Avtina ♦ **Received** 11 February 2023 ♦ **Accepted** 21 April 2023 ♦ **Published** 01 June 2023

Citation: Petrov VI, Anikeev IS, Zayachnikova TE, Strygin AV, Dotsenko AM (2023) Development and validation of a quantitative HPLC/MS/MS method for the determination of piperacillin in blood plasma. Research Results in Pharmacology 9(2): 55–59. https://10.18413/rrpharmacology.9.10027

Abstract

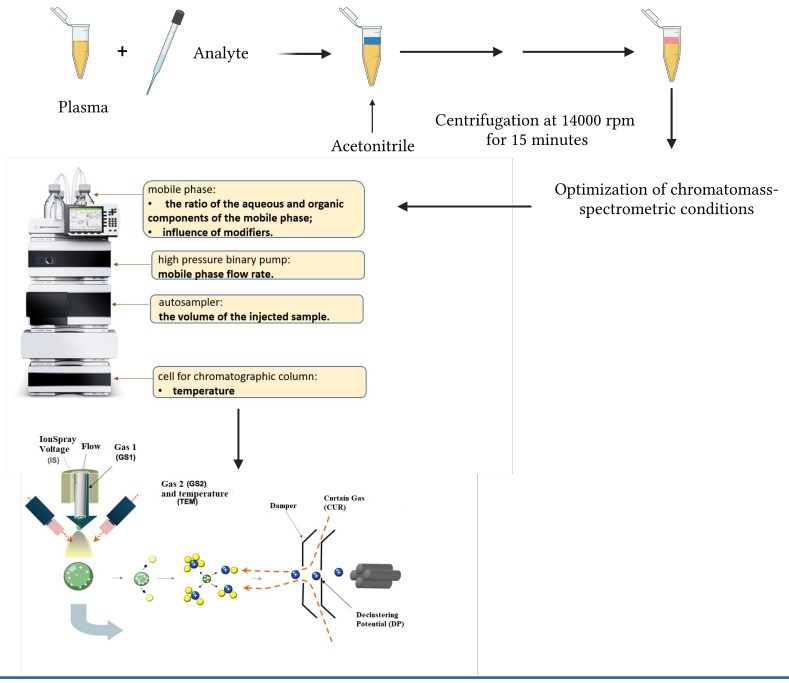
Introduction: Reduced mortality in patients with sepsis taking piperacillin is possible when they receive a long-term infusion, which improves the effect of antimicrobials. However, such piperacillin therapy requires therapeutic drug monitoring, the use of the latest analytical equipment and developed methods for the quantitative determination of piperacillin.

Materials and Methods: Dry samples of the appropriate certified piperacillin standards were used to prepare stock and standard solutions of piperacillin. Separation of the components was performed using an Agilent 1260 HPLC system with a binary pump and a temperature controlled autosampler. Analyses were detected using a Sciex QTRAP 5500 hybrid mass spectrometric system. Validation of the developed method was carried out in accordance with the rules for conducting bioequivalence studies of drugs within the framework of the Eurasian Economic Union; 2016, in Astana.

Results and Discussion: Piperacillin ions-"precursors" corresponded to particles m/z 518.2. The most intense ions-"products" registered during the fragmentation of protonated molecules in the collision cell were particles m/z 143.1 and m/z 115.0. During the validation of the developed method, the main validation parameters were established: linearity, accuracy, accuracy, and sensitivity (lower limit of quantitation).

Conclusion: The validated analytical range of the method was 0.5–100 μg/mL in plasma. The resulting analytical range makes it possible to apply the developed method for conducting the analytical part of studies of the pharmacokinetics of piperacillin.

Graphical abstract:



Keywords

HPLC/MS, validation, quantitation, piperacillin, bioanalytics

Introduction

A reduction in mortality in patients with sepsis taking piperacillin is possible when they receive a long-term infusion, which improves the effect of antimicrobials. However, such piperacillin therapy requires therapeutic drug monitoring (TDM), which is a promising strategy for individualization and increasing the effectiveness of antibiotic therapy. Therapeutic drug monitoring with dosing adapted to the altered pharmacokinetics of the individual patient will avoid underdose or overdose. (Barco et al. 2015; Schmitt et al. 2017; Verhoven et al. 2018; Capiau et al. 2019).

However, for the correct application of therapeutic drug monitoring and the study of a sufficiently large number of samples, it is necessary to use the latest analytical equipment and developed methods for the quantitative determination of piperacillin (Cohen-Wolkowiez et al. 2014; Berm et al. 2016; Anikeev et al. 2018; Hagel et al. 2019).

Therefore, our goal was to develop and validate a highly sensitive and selective HPLC-MS/MS method for the determination of piperacillin in human plasma.

Aim of the study. Finding the optimal conditions for the quantitative determination of piperacillin in blood plasma using HPLC-MS/MS and validation of the method.

Materials and Methods

Chemicals and reagents

For the preparation of stock and standard solutions of piperacillin and prazosin as an internal standard, dry weights of the corresponding certified standard of piperacillin (Sigma Aldrich, Milan, Italy), special highly purified water (Milli-Q), formic acid for HPLC-MS, acetonitrile, (Scharlab, Spain) were used. The matrix for the preparation of calibration standards and quality control samples was human blood plasma obtained from healthy volunteers.

Equipment

Dry substances were weighed using an Ohaus Explorer EX225/AD semi-microanalytical balance (Ohaus, USA). Separation of the components was performed using an Agilent 1260 HPLC system with a binary pump and a temperature controlled autosampler (USA). Analyzed substances were detected using a hybrid mass spectrometric system Sciex QTRAP 5500 (Singapore), Shaker (Biosan, Latvia), Special analytical software Analyst 1.6.

Preparation of stock and standard solutions

To prepare a standard solution of piperacillin and an internal standard with a concentration of 1.00 mg/mL, dry samples were weighed and then placed in a 25-mL volumetric flask and made up to the mark with ultrapure water. The final concentration of working solutions was: 5; 10; 25; 50; 100; 200. and 1000 mcg/mL.

Preparation of calibration and quality control samples

Using a dispenser, 100 µl of plasma were transferred into 1.5 ml microtubes, 10 µl of a working solution of the appropriate concentration was added with a dispenser until calibration solutions of 0.5; 1; 2.5; 5; 10; 20 and 100 µg/ml for piperacillin.

Quality control (QC) samples were prepared at the following four concentration levels: 0.5 µg/mL (LQQ), 1.5 µg/mL (low QC), 50 µg/mL (medium QC), and 75 µg/mL (high QC) for piperacillin.

Chromatographic and Mass Spectrometric Conditions

Chromatographic separation of the components was carried out on a Poroshell 120 C18 column (4.6x50 mm, 2.7 µm). When developing the conditions for the mass spectrometric detection of the desired substances by the method of multiple reaction monitoring (MRM), ions-" precursors" and the corresponding ions – "products" were determined. Piperacillin "precursor" ions corresponded to particles m/z 518.2. The most intense ions-"products" registered during the fragmentation of protonated molecules in the collision cell were particles m/z 143.1, m/z 115.0 (Li et al. 2012, 2013; Keçeli et al. 2014; Barco et al. 2015; D'Cunha et al. 2018).

Validation

Validation of the developed method was carried out in accordance with the rules for conducting bioequivalence studies of drugs within the framework of the Eurasian Economic Union; 2016, in Astana (Council of the Eurasian Economic Commission 2016). During the validation of the developed method, the main validation parameters will be established: linearity, accuracy, correctness, sensitivity (lower limit of quantitation), and linearity.

To validate the linearity, the calibration samples were examined, which corresponded to the concentrations: 1; 2; 5; 10; 20; 50 and 100 µg/mL for piperacillin. Calibration curves were constructed by plotting peak area ratios versus plasma concentrations using a weighted 1/ x^2 linear regression model. The lower limit of quantitation was defined as the lowest concentration on the calibration curve, which was 1 µg/mL.

Statistical analysis

For the detection of analytes, a Sciex QTRAP 5500 hybrid mass spectrometric system was used. Chromatography–mass spectra were recorded using the Analyst 1.6 software. Peak integration, calculation of the quantitative content of the studied compounds, and statistical data processing were carried out using the MultiQuant 2.4 program.

For quantitative determination, a calibration curve method with a weighting factor of 1/x2 was used. The ratio of the peak areas of the analyte and the internal standard was taken as a parameter (Timmerman et al. 2011; Jager et al. 2014; Fan et al. 2019; Li et al. 2013; Mu 2019).

Results and Discussion

Piperacillin ions-"precursors" corresponded to particles m/z 518.2. The most intense ions – "products" registered during the fragmentation of protonated molecules in the collision cell were particles m/z 143.1, m/z 115.0 (Fig. 1). In the course of optimizing the conditions for chromatographic separation, an isocratic elution mode was chosen.

The mobile phase was 80/20 acetonitrile/water at a flow rate of 0.3 mL/min. The mobile phase modifier was 0.1% formic acid, which was added to both the aqueous and organic components of the mobile phase. Under these conditions, the retention time of piperacillin was 1.86 min (Fig. 2).

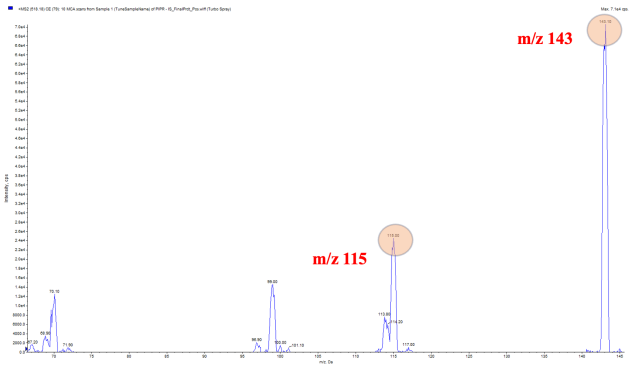


Figure 1. Mass spectrum of piperacillin in blood plasma: along the abscissa axis – the ratio of mass to charge m/z (Da), along the ordinate axis – the signal intensity.

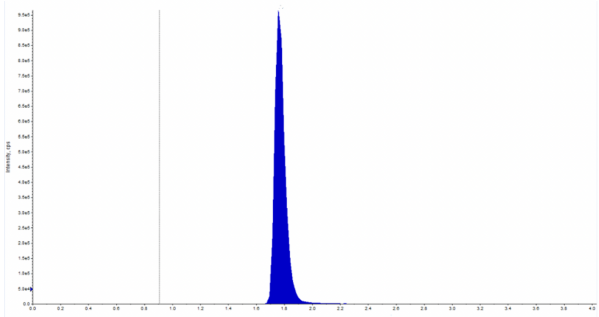


Figure 2. Chromatogram of piperacillin in blood plasma: along the abscissa axis – time (min); along the ordinate axis – signal intensity.

During the validation of the developed method, the main validation parameters were established: linearity, precision, correctness, sensitivity (lower limit of quantitation), stability, and matrix effect. The developed method confirmed its linearity in the concentration range from 0.5 to 100 µg/mL using a weighted coefficient 1/ x^2, with r^2 > 0.99. The coefficient of variation (%) calculated when determining the inter- and intraday accuracy did not exceed 15% for the main range of concentrations (Table 1).

Successful TDM requires the use of specific validated methods, knowledge of new physicochemical approaches to sample preparation of biosamples, and the availability of modern equipment.

We have developed and validated methods for the quantitative HPLC-MS/MS determination of: piperacillin in blood plasma using standard sample preparation methods (protein precipitation, etc.).

When developing the conditions for the mass spectrometric detection of the desired substances by the method of multiple reaction monitoring (MRM), ions-" precursors" and the corresponding ions-"products" were determined. The "precursor" ion of piperacillin was the ion m/z 518.2. The most intense ions-"products" registered during the fragmentation of protonated molecules in the collision cell were particles m/z 143.1 and m/z 115.0.

The developed methods confirmed their linearity in the selected concentration ranges using the weighted

Table 1. Table of validation parameters for the quantitative determination of piperacillin for the method using protein precipitation as a sample preparation

Parameter		Value			
		LLOQ (0.5 µg/mL)	LQC (1.5 µg/mL)	MQC (50 µg/mL)	HQC (75 µg/mL)
Precision (CV %)	Within run	4.1	11.2	3.70	5.5
	Between cycles	14.8	14.2	7.1	6.7
Correctness (%)	Within the cycle	113.5	98.8	92.4	102.1
	Between cycles	92.1	95.8	97.5	105.2
Stability (%)		-	96.8	-	107.9
Selectivity (%)		5.1	-	-	-
Correlation coefficient		0.99			

Note: LLOQ – lower limit of quantification (lower limit of quantification); LQC – Low level (lower concentration level); MQC – Medium level (medium concentration level); HQC – High level (upper concentration level).

coefficient 1/x^2, with r^2>0.99. The coefficient of variation (%) calculated in determining the inter- and intraday accuracy did not exceed 15% for the main range of concentrations for all drugs.

The lower limit of quantification of the procedure was determined based on linearity, precision, and precision data.

This analytical method for the determination of piperacillin in blood plasma using HPLC-MS/MS will make it possible to use it in a wide routine practice, especially for the correct use of therapeutic drug monitoring and the study of a sufficiently large number of samples in the determination of piperacillin.

Conclusion

In this study, the optimal conditions for the quantitative determination of piperacillin in human plasma were established using chromatographic and mass spectrometric equipment.

The method has been validated according to all the requirements of the Eurasian Economic Union and the European Medicines Agency and meets all the requirements for bioanalytical methods. The validated analytical range of the method was 0.5–100 µg/mL in plasma. The resulting analytical range makes it possible to apply the developed method for conducting the analytical part of studies of the pharmacokinetics of piperacillin.

Conflict of Interests

The authors declare no conflict of interests.

Funding

The study was supported by the Russian Science Foundation (Project no. 22-25-20207).

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