Quantification of β-lactam antibiotics in urine and wipe samples from environmental and biological monitoring by SPE and LC-MS/MS

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Introduction

The occurrence of pharmaceuticals - especially antibiotics and hormones - in the environment, in food as well as the occupational exposure of farm workers causes considerable attention. In case of health care personnel only the occupational exposure against cytotoxic drugs has been studied intensively [1-3] and resulted in improved safety standards for handling of these substances. In contrast to the carcinogenic and teratogenic effects of antineoplastic drugs, long term exposure against antimicrobial agents has been associated with an increased risk of development and spread of antibiotic resistance, which has been considered as one of the biggest health problems of the 21st century by the World Health Organisation (WHO).

The aim of this project was to develop efficient analytical procedures to be applied in biological and environmental monitoring of the most important β-lactam antibiotics in health care facilities.

Methods

Wipe samples were extracted with deionized water and directly analyzed. For sample enrichment and clean up of water and urine samples we developed a solid phase extraction method using Strata X (Phenomenex) and Oasis HLB 6cc (Waters) polymer cartridges. Final analysis was carried out by RP HPLC and triple quadrupol mass spectrometry detection.

High performance liquid chromatography

The separation was performed on a 125 x 2 mm Nucleodur 100-5 C18 EC column (Macherey-Nagel) with a binary gradient of 0.1 % formic acid in water (v/v) (phase A) and 0.1% formic acid (v/v) in pure acetonitrile (phase B) at a flow-rate of 0.35 ml/min at 30°C.

Tandem mass spectrometry

API 3000 triple quadrupol mass spectrometer (Applied Biosystems) equipped with Turbolonspray™ interface operating at 500°C in positive and negative mode, using nitrogen as nebulizer, auxilary and collision gas. Orifice and focusing ring voltage were optimized by continuous flow experiments (table 1). The analytes were detected by multiple reaction monitoring (MRM).

Table 1 MS/MS-detection parameters

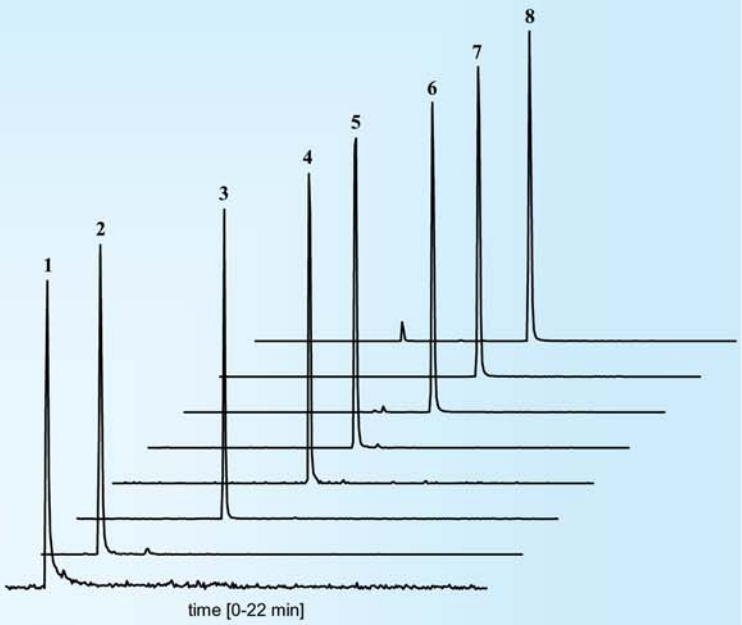
	Orifice voltage [V]	Ring voltage [V]	Precursor ion [*m/z*]	Product ion I [*m/z*]	Product ion II [*m/z*]
Cefotiame (**1**)	31	240	526.1	174.0	141.0
Amoxicillin (**2**)	-26	-130	364.1	223.1	206.1
Ampicillin (**3**)	36	240	350.1	106.0	74.0
Cefazoline (**4**)	-31	-260	453.0	321.0	166.9
Cefuroxime (**5**)	-51	-320	423.0	174.0	318.0
Piperacillin (**6**)	-31	-220	516.2	330.1	233.1
Penicillin G (**7**)	-31	-200	333.1	192.1	289.1
Penicillin V (**8**)	-31	-180	349.1	208.0	93.0

Table 2 Retention times (RT), recoveries and limits of detection (LOD) for wipe sample extracts (water) and urine samples

	RT [min]	Recovery Strata X [%][a]	Recovery Oasis HLB [%][b]	LOD water [µg/L]	LOD urine [µg/L]
Cefotiame (**1**)	2.02	75	130 ± 20	27	5.0
Amoxicillin (**2**)	2.81	78	31 ± 4	0.8	20
Ampicillin (**3**)	7.02	74	54 ± 3	0.5	0.4
Cefazoline (**4**)	9.32	71	72 ± 7	0.9	11
Cefuroxime (**5**)	9.87	80	63 ± 6	0.3	2.2
Piperacillin (**6**)	11.79	63	57 ± 2	0.2	2.8
Penicillin G (**7**)	12.26	81	72 ± 7	0.2	0.7
Penicillin V (**8**)	13.02	67	56 ± 3	0.3	0.4

[a] n = 1; [b] n = 3

Figure 1 LC-MS/MS-Chromatogram of a spiked sample (100 µg/L) after solid phase extraction on a Strata X cartridge.



Results

Sample enrichment and clean up for urine was carried out with solid phase extraction on Strata X and Oasis HLB 6cc cartridges. Mean recoveries rates are shown together with the limits of detection for wipe and urine samples in table 2. Because of matrix effects the determination of urine samples is less sensitive as for wipe sample extracts. Excellent baseline separation was achieved for all compounds (figure 1). Calibration curves were linear over three orders of magnitude. Wipe sample extracts could be analyzed without further sample clean up. First results from different hospitals showed the occurrence of antibiotics in 66 % of the wipe samples (n = 35).

Conclusions

We developed a sensitive method for 8 β-lactame antibiotics from environmental and biological monitoring. After first experiments it seems that Strata X SPE cartridges are more suitable for sample clean up than Oasis HLB cartridges. This method will be applied for further investigations to quantify contamination of workplace and uptake by exposed personnel in order to assess and reduce possible health risks.

References

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