Samenvatting proefschrift

Development and application of LC-MS/MS multi-methods for the quantification of antibiotics and cytostatic drugs at occupational safety and environmental investigations

Jochen Tuerk[1]

Apart from patient's wellbeing, the potential health risk for persons coming into contact with highly effective drugs such as antibiotics and antineoplastics as well as the environmental protection while handling these drugs must be considered.
The aim of this thesis is to develop and apply liquid chromatographic multi methods for analysis of occupational exposure and environmental protection-relevant antibiotics and cytostatic drugs in pharmacies and hospitals.

For analysis of occupational exposure, a multi method was developed for eight structurally different antibiotics by means of HPLC-UV, -MS and -MS/MS. The comparison of different types of detection showed that HPLC-UV and -MS were suitable for measurements of wipe samples with high concentrations, e. g. for validation of cleaning procedures or analysis after unintentional substance release. For environmental and biological monitoring purposes, tandem mass spectrometry should be used due to low concentrations in the ng/mL range and matrix effects from urine samples. The results of first wipe samples from four European hospitals showed that 89 % of the surfaces were contaminated during the preparation of cefuroxime.
In the first investigation of surface contaminations as well as internal and outside human exposure to antibiotics, the developed LC-MS/MS method was further extended to seven active substances. A combined environmental and biological monitoring of amoxicillin, ampicillin, cefazoline, cefotaxime, cefotiame, cefuroxime, chloramphenicol, ciprofloxacin, ofloxacin, penicillin G, penicillin V, piperacillin, trimethoprim, sulfamethoxazole and vancomycin was conducted. It was found that 98 % of the work surfaces and 100 % of the work clothes were contaminated with antibiotics under the examined conditions. A correlation between contaminations and preparation of the antibiotics was observed. During the investigation period, used substances were detected frequently and found in relatively high concentrations of up to 248 ng/cm^2. In addition to the prepared compounds, a variety of other antibiotics in particular on surfaces and textiles could also be determined.
An internal exposition was detected at 48 % of the total number of employees. However, the detected concentrations were in the lower ng/mL range and thus substantially lower than the surfaces and textile samples. Beside the smaller number of different antibiotics no exposure with several substances could be determined contrary to the surface and textile contaminations when biological monitoring was performed. Figure 1 gives a summary of the contaminated samples.

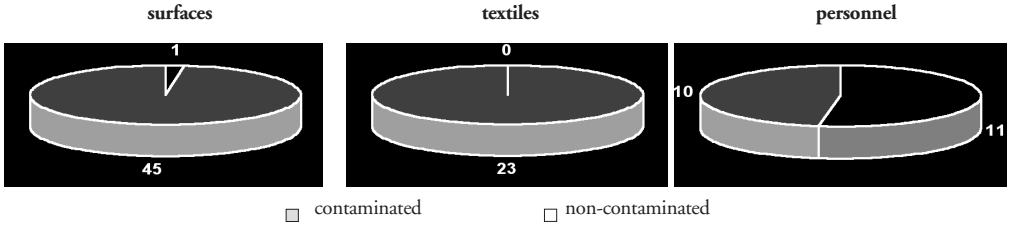

Figure 1: Summary of the antibiotic contaminations with the environmental and biological monitoring in a North Rhine-Westphalian hospital.

In addition to the direct contamination pathways, indirect contamination sources and mechanisms should be investigated in the future work. Moreover, the effectiveness of the current surface cleaning procedures should be analysed. However, a toxicological evaluation of these results and estimation of possible health risks is not possible. For this reason occupational exposure of the personnel has to be prevented. Apart from the resistance problem while handling antibiotics, in particular allergies represent a substantial hazard potential for the employees in the health care system.

As a replacement for the substance specific cytostatic drug sampling and GC-MS single compound analysis methods accomplished so far in the context of this thesis a substantially simpler LC-MS/MS multi method was developed for the simultaneous sampling and analysis of the cytostatic drugs 5-fluorouracil, chlorambucil, cyclophosphamide, cytarabin, etoposide, ifosfamide and methotrexate. The

[1] *Institute of Energy and Environmental Technology (IUTA e.V.), Bliersheimer Str. 60, D-47229 Duisburg, Germany; email: tuerk@iuta.de*

validation data obtained confirmed that sampling on different surfaces had a large influence on the recovery rates. Results obtained from the novel LC-MS/MS multi compound analysis in this thesis were comparable to those of antineoplastic analysis from the literature. Contamination levels at different sampling points within and outside of the pharmacy differ over several orders of magnitude (ranges from < 0.001 ng/cm^2 to 796 ng/cm^2). During total evaluation of a pharmacy or ward, measurement uncertainty resulting from surface influence can be neglected due to a large difference between clean and contaminated surfaces. During evaluation of single results, however, it is problematic that according to the German regulations for handling hazardous substances (GefStoffV) for CMR compounds it is a requirement for no occupational exposure. Therefore, limit values for handling of these substances do not exist. Due to missing toxicological and statistical data, there are also no threshold values. Altogether exposure can be minimized by consistent application of the state-of-the-art preparation and handling of cytostatic drugs using technical and personal protection equipment, as well as intensive training of the personnel. Therefore, the risk during handling of CMR substances could be substantially lowered. Analysis of the working situation and success of the met measures can be achieved in a simple and economic way by using the LC-MS/MS multi methods developed in this thesis. In case of unintentional substance release or accident, biological monitoring of the affected employee to evaluate the substance uptake and environmental monitoring to control the cleaning procedures are recommended.
Substance independent statistic calculations of 2644 measured cytostatic drugs gave a value of 0.028 ng/cm^2 for the 90th percentile. For the formation of an orientation value on the basis of a statistic data analysis the values in this thesis must be extended by the other German values like platinum and GC-MS data from IUTA, results from the LMU Munich as well as the expected results from the MEWIP study. On the basis of this broad database, the formation of orientation values can be made possible via discussion between pharmacy associations and government safety organizations.

Next to occupational safety in this thesis, aspects of environmental protection were also considered while handling highly effective medicaments in hospitals. Pharmaceuticals and their metabolites excreted by patients may reach the sewage system and wastewater treatment plants. Since many of these highly effective and toxic compounds are not biologically degradable, these substances are released directly into surface waters. Immediate elimination of these substances at the inlet points could contribute to the reduction of environmental loads of highly active ingredients. Advanced oxidation processes (AOP) were developed for the treatment of highly loaded part streams of hospitals and evaluated in a semiworks. For substance specific process analysis for control and optimisation of the AOP procedure a LC-MS/MS multi method was developed and applied. Limits of detection for eight antibiotics and seven cytostatic drugs were between 0.1 and 5 µg/L. A weighted matrix calibration for accurate quantification was necessary for this purpose as well as for the analysis of urine samples from biological monitoring. In addition to direct analysis of hospital part streams, analysis of substantially lower concentration of sewage treatment plant effluents and surface waters is also possible by adding a suitable sample preparation (clean up and enrichment) by means of solid phase extraction.

Elimination efficiency of selected cytostatic drugs and antibiotics were investigated in dependence of UV radiation, type and quantity of the oxidation agent, period of treatment and temperature as well as the influence of different reaction volumes in laboratory experiments. The results showed that degradation of the compounds (> 99 %) as well as a reduction of ecotoxicological characteristics (> 95 %) can be achieved with ozone and also UV oxidation processes. Treatment periods between 10 and 90 minutes per litre for primary compound degradation were necessary depending on the type of oxidation agent (ozone or hydrogen peroxide) and on whether low or medium pressure mercury lamps were used. Therefore, AOPs are possible for direct elimination of antibiotics and cytostatic drugs from part streams of hospital wastewaters. Experiments conducted in this thesis are the basis for the construction of a pilot plant. Further investigations for process optimisation in terms of efficiency and economy will be conducted in the future work.