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The influence of the sample matrix on LC-MS/MS method development and analytical performance

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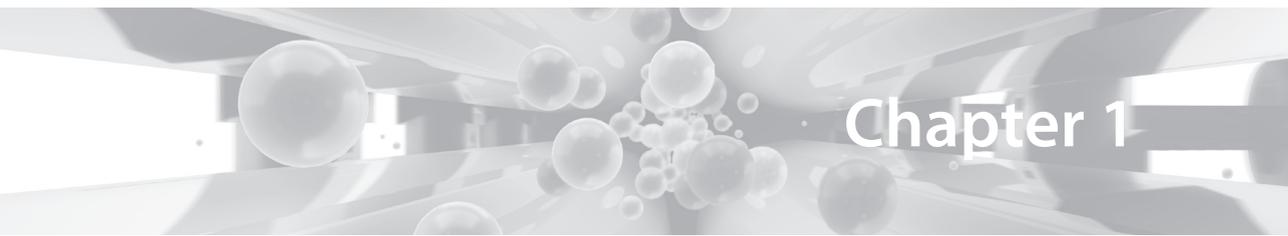
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Chapter 1

General introduction, scope and outline of the thesis

BACKGROUND

High Performance Liquid Chromatography coupled with tandem mass spectrometry (LC-MS/MS) has nowadays established itself as the primary analytical technique to support therapeutic drug monitoring (TDM), clinical and forensic toxicology and drugs of abuse analysis. Because of the sensitivity and selectivity of the LC-MS/MS, elaborate sample extraction techniques like solid phase extraction and liquid liquid extraction may often be unnecessary. The application of fast and simple extraction techniques like protein precipitation or sample dilution is therefore feasible. On the turn side, matrix effects are therefore observed more frequently and the LC-MS/MS method should then be optimized to overcome these matrix effects. Other types of matrix effects could originate from substance interaction with the matrix.

The matrices whole blood, plasma, serum and urine samples are common in TDM and toxicological analysis. More recently, dried blood spots (DBS), saliva and hair have been introduced in an increasing number of clinical laboratories. The monitoring of drugs of abuse in psychiatry, workplace, detention and child abuse drug testing can also be performed in urine, hair, sweat (patches) and saliva. Each human matrix has its clinical and analytical advantages and disadvantages, and the performed analysis and interpretation of the analysis results strongly depend on this matrix.

Each matrix has its specific application to measure drug concentrations in relation to intake of that particular drug. For example, blood, serum, plasma and saliva may be used to monitor drug use on the day of ingestion. While urine is a suitable matrix for testing the previous two days and sweat for a maximum of the previous seven days. Information about drug use over a period of several months can be provided by segmental analysis of hair strands (hair growth about 1 cm per month), which may distinguish single exposure from long-term exposure [1]. Hair and sweat patches are a non-invasive alternative for blood samples and less inconvenient and time-consuming than supervised urine collection. In addition, urine samples are easily diluted in vivo by excessive drinking or in vitro adulterated by additives. The use of urine, sweat and hair imply that the obtained concentrations are very difficult to relate to the amount of ingested drug. Instead, the analysis of abused drugs in these matrices may provide the physician or health-care professional with information about drug abuse, and could be used for patient-specific therapy.

During the last several years, DBS analysis is gaining popularity for TDM [2-4]. For DBS, just a single drop of blood from a finger is used to create a blood spot on a special spotting card.

This spot is dried and sent to the laboratory. Because of the small blood volumes that are needed for DBS, pharmacokinetic studies and TDM will be less burdensome for the patient. Patients who use drugs for long periods of time or live in remote areas could benefit greatly by DBS analysis. DBS sampling can be performed at home by self-sampling, saving travelling costs and improving the effectiveness of patient treatment [2]. While conventional plasma sampling is often not feasible in resource limited areas due to lack of equipment or cooled transportation. Moreover, DBS sampling has many potential advantages such as prolonged sample stability, lower risk of infections and transport at ambient temperature [2-5]. These advantages may facilitate the application and implementation of TDM in many different settings and even in resource limited areas.

New matrices will challenge investigators to identify important parameters that may negatively influence the analytical results. The impact and source of influence of these parameters need to be evaluated in order to provide a framework in which the analytical results are reliable and valid.

OBJECTIVES OF THE THESIS

The aim of this thesis is to identify, evaluate and overcome issues caused by the effect of different matrices on the performance of analytical procedures.

The main objectives of this thesis are gaining insight in:

- Efficient extraction procedures for a variety of matrices.
- Developing (multi analyte) LC-MS/MS methods.
- Critical parameters that influence analytical results in DBS analysis.
- Evaluation of drug instability in the particular matrices and procedures to overcome stability issues.
- Improving analytical method validation for specific matrices.

OUTLINE OF THE THESIS

Chapter 2: Whole blood analysis

The analysis of whole blood is common for substances that reside in red blood cells. Whole blood is blood that is mixed with an anticoagulant (e.g. heparine, EDTA or citrate) directly

after sampling. Whole blood is more difficult to process than plasma because of the viscous red blood cells, which tend to clot during sample preparation. Although the tandem mass spectrometer is a highly selective detection technique, a chromatographically selective method needs to be developed with care for possible interfering peaks, memory effects and ion suppression or ion enhancement.

The development of a whole blood analysis method was performed for the immunosuppressants tacrolimus, sirolimus, everolimus, cyclosporin A and focused on the following issues:

- The development of an efficient, reproducible and robust sample preparation for whole blood by testing various extraction solvents with and without zinc sulphate.
- The development of a fast multi-analyte chromatographic gradient, which can still separate interfering peaks.
- Achieving best LC-MS/MS sensitivity by optimizing two MS/MS methods where sirolimus and everolimus were measured separately from tacrolimus and cyclosporin A.

Chapter 3: Dried blood spot analysis

In this chapter we studied the critical parameters concerning DBS sample preparation, extraction and analysis. For DBS analysis, substances may be difficult to extract from the spotting card matrix or they may form complexes with the endogenous components present in the DBS matrix. The extraction efficiency may be influenced by different aspects like extraction conditions, blood hematocrit and concentration of the substance. The blood hematocrit also affects the viscosity of the blood and that in turn influences the formation of a blood spot, which affects the analytical results. The analysis of a partial DBS (fixed area) should relate to a certain volume of blood but it is no guarantee that the volume will be reproducible with an acceptable precision under all circumstances. While the analysis of a fixed volume (whole spot analysis) is unpractical for patient sampling because the patient would have to accurately sample the intended blood volume, which is prone for errors. It will be important to investigate the level of impact of these influences before routine patient analysis can be implemented [6]. Although DBS analysis will benefit the service towards the patient, the development and validation of analytical DBS methods will become more complex compared to plasma or serum analysis. On top of classical parameters for method validation of liquid whole blood or plasma, additional parameters like the effect of

the hematocrit and blood spot volume should be validated. The performed research should result in improved procedures concerning DBS analysis.

This chapter focuses on the following issues for analytical DBS research:

- The correct preparation of the target hematocrit values for standards and quality control samples.
- Finding optimal DBS extraction procedures in order to improve recoveries and minimize matrix effects.
- The relation of the number of hydrogen bond acceptors of the analyte with the analyte recovery.
- The influence of the drying time of the DBS on the recovery of the analytes.
- The influence of the hematocrit value of the blood and the concentration of the analyte on the recovery and blood spot formation.
- The performance of the various types of dried blood spot cards.
- The use of calibration standards and quality control samples for the measurement of the endogenous creatinine.

Chapter 4: Plasma analysis

Remifentanyl is used in anesthesia and intensive care medicine and is rapidly metabolized in both blood and tissues, which results in a very short duration of action. The *in vivo* half-life of remifentanyl is approximately 3 minutes, independent of the duration of infusion [7]. Even after blood sampling remifentanyl is unstable in whole blood and plasma because of endogenous esterases and chemical hydrolysis.

Chapter 4 focussed on the following matrix related issues regarding the stability of remifentanyl:

- Citric acid, ascorbic acid and formic acid are tested to improve the stability of remifentanyl by decreasing the pH of the plasma.
- The stability of remifentanyl is investigated in whole blood, EDTA plasma and acidified EDTA plasma at various temperature conditions.

Chapter 5: Hair and sweat patches

Stability issues for the analysis of drugs of abuse in hair or sweat are different compared to matrices like blood or plasma. During the hair growth and sweat excretion into patches, the sample is not yet in a controlled environment and stability may be an issue. In that case, the (additional) analysis of the metabolite may be an option. However, when drugs are incorporated into the hair or the sweat patch, the human metabolism can no longer affect the drug. The amount of drug in hair can be affected by external factors, such as washing, bleaching and drugs deposited on the hair. The amount of drug in hair can also depend on the personal properties of the hair, like natural colour and race. For the analysis of drugs of abuse, the analytical result needs to exceed a pre-set concentration, the so called cut-off value, in order to report drug abuse. These cut-off values depend on several factors, like analytical performance, used matrix, presence of a metabolite, and age of the patient, but also political or clinical insights. Concentrations found above this cut-off value are considered “positive” and drug use can be concluded. In the case of the analysis of drugs of abuse, an analytical method is required to be very selective. For this, international guidelines can be followed [8-11]. However, when these are not available or incomplete, the researcher will have to propose and substantiate new guidelines.

We aimed to develop two analytical methods for a number of drugs of abuse for hair and sweat patches while focusing on the following issues:

- The development of a procedure for washing external contamination from the hair.
- Assessing the last wash step for external contamination of the hair sample.
- The development of a procedure to efficiently extract the drugs from the hair.
- To set cut-off values which are specific to the analytical capabilities of the developed analytical method.
- The development of validation rules for the validation of a qualifier mass transition.

Chapter 6: General discussion and future perspectives

In the final chapter, several important aspects of the various human matrices and the developed analytical procedures will be discussed along with the investigated parameters

that are critical for a robust, selective, sensitive, accurate and precise method. The impact of the developed analytical procedures on the laboratory, the personalized treatment of the patient and the future perspectives are discussed as well.

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