CHAPTER 2

LC-MS/MS FOR THERAPEUTIC DRUG MONITORING OF ANTI-INFECTIVE DRUGS

* Anette Veringa
  * Marieke G.G. Sturkenboom
  Bart G.J. Dekkers
  Remco A. Koster
  Jason A. Roberts
  Charles A. Peloquin
  Daan J. Touw
  Jan-Willem C. Alffenaar

* Both authors contributed equally to the manuscript

Accepted in Trends in Analytical Chemistry 2016
Abstract

Therapeutic drug monitoring (TDM) is a tool used to integrate pharmacokinetic and pharmacodynamic knowledge to optimise and personalize drug therapy. TDM is of specific interest for anti-infectives: to assure adequate drug exposure and reduce adverse events, to increase patient compliance and to prevent antimicrobial resistance. For TDM, drug blood concentrations are determined to bring and keep the concentration within the targeted therapeutic range. Currently, LC-MS/MS is the primary analytical technique for fast and accurate quantification of anti-infective drug concentrations. In addition to blood, several alternative matrices (cerebrospinal fluid, inflammatory fluids, specific cells and tissue) and alternative sampling strategies (dried blood spot and saliva) are currently being explored and introduced to support TDM. Here, we review the current challenges in the bioanalysis of anti-infective drugs and give insight in the pre- and postanalytical issues surrounding TDM.
2.1 Introduction

Traditionally, therapeutic drug monitoring (TDM) was restricted to anti-epileptic drugs and aminoglycosides, but also now covers - amongst others - immunosuppressant drugs, drugs acting on the cardiovascular system, anti-HIV drugs and antifungal drugs. For some classes of drugs, TDM has not only proven to be beneficial for patient outcome, but also to be cost-effective [1, 2].

With increasing pathogen resistance to anti-infective drugs, there is a clear need for new agents. However, the development of new anti-infectives is time consuming and expensive. Therefore, treatment optimization of the current anti-infectives should be a focus of contemporary treatment. Due to its urgency, development of antimicrobial resistance has a high priority for many organizations and even entered the political agendas. Treatment optimization can be realized by selecting the appropriate antimicrobial drug, assuring adequate drug exposure in relation to the susceptibility of the micro-organism and reducing adverse events in order to increase patient's compliance with treatment.

For many years, immunoassays and traditional high performance liquid chromatography (HPLC) methods were the major techniques used to determine concentrations of anti-infective drugs in human specimens. However, immunoassay techniques are only available for a limited number of drugs and cross-reactivity, for instance with drugs and their metabolites, is a problem. HPLC coupled with UV detection (HPLC-UV) often requires extensive sample preparation and is therefore labour intensive. In addition, long runtimes are often required in order to obtain a selective analysis method. In addition, both immunoassays and HPLC-UV methods often lack sensitivity. Nowadays, analytical challenges like these have been overcome with the introduction of HPLC coupled with tandem mass spectrometry (LC-MS/MS). With the use of LC-MS/MS, sensitivity and selectivity has significantly improved, allowing simple and fast sample preparations and short runtimes. This review will focus on the bioanalytical hurdles related to the measurement of anti-infective drugs, but also will give insight in pre- and post-analytical issues in order to help clinical chemists, clinical pharmacologists and analytical technicians to raise their standards.

2.2 TDM

For over 30 years, TDM has been used as a tool to integrate pharmacokinetic and pharmacodynamic knowledge to optimise drug therapy at the individual patient level [3]. Pharmacokinetics describe the behaviour of a drug in the patient's body, including absorption, distribution, metabolism and excretion, whereas pharmaco-
dynamics describe the biochemical or pharmacological effect of a drug on the patient's body or micro-organism within the body. Together, both parameters determine the pharmacological profile of the drug.

TDM uses drug blood concentrations to personalise drug therapy in order to bring and keep the concentration within the targeted therapeutic range \[4,5\]. Below this range the drug concentration is subtherapeutic or ineffective, whereas high concentrations may result in adverse events or toxicity.

TDM is used when it is impossible to measure the pharmacodynamic effect of the drug faster or in a more direct way, or it is used to optimise dosing in patients with severely altered pharmacokinetic parameters (e.g. critically ill patients in ICU \[1,4\]). For anti-infectives, it is both difficult and time-consuming to observe whether the infection is being treated adequately. If the infection is not treated adequately, it may be too late to turn the tide of illness, resulting in treatment failure including patient morbidity or mortality or the emergence of antimicrobial resistance.

Before TDM can be performed, several prerequisites have to be fulfilled. First, a concentration effect relationship or therapeutic range should be established \[4\]. Secondly, large interindividual (e.g. sex, age or genetic variations) or intraindividual variability (e.g. drug-drug interactions, decreased renal function or liver failure) in pharmacokinetics should be observed, resulting in a large variation in blood concentrations \[4\]. The final obvious prerequisite is that a sensitive and specific assay must be available to determine the drug in blood or other biological matrices \[4,5\].

2.2.1 Pharmacokinetic/pharmacodynamic relationships

For anti-infectives, the minimum inhibitory concentration (MIC), a measure of potency of the drug for the micro-organism, is central to pharmacodynamics \[6\]. The MIC is the lowest concentration at which an antibiotic inhibits visible growth of the micro-organism after 18 to 24 hours incubation \[7\]. Unlike antibiotics, there is no simple standard pharmacodynamic parameter, such as the MIC, that tests antiviral susceptibility \[7\]. Although not applied in clinical practice, the half maximum inhibitory concentration (IC\textsubscript{50}) could be used to establish efficacy in an appropriate \textit{in vitro} or animal model \[7\].

The efficacy of anti-infective drugs not only is dependent on the pathogen's MIC, but also on the exposure of the drug in the patient. This exposure is commonly described by the area under the concentration-time curve (AUC) \[6\]. For many drugs, the AUC/MIC ratio is the most relevant pharmacokinetic/pharmacodynamic (PK/PD) index (Figure 2.1) \[6\].
In addition to the AUC/MIC ratio, other PK/PD indices also may be relevant. An overview of the effective PK/PD indices of many antibiotics was previously provided by Roberts et al. \cite{1}. For instance, beta-lactam antibiotics, such as penicillins and carbapenems, display time-dependent pharmacodynamics, meaning that the time of the unbound (or free) drug concentration exceeds the MIC ($fT_{>\text{MIC}}$) is the most relevant PK/PD index \cite{8}. For these drugs, both frequency of dosing and duration of infusion are important \cite{6}. Constant drug concentrations rather than high peak concentrations result in more effective treatment \cite{9}. Moreover, for these drugs higher concentrations do not result in greater effectiveness. For these reasons, continuous administration, preceded by a loading dose to quickly attain steady state, has been suggested as an potentially improved strategy to conventional intermittent dosing \cite{9}.

The peak level or maximum concentration of a drug ($C_{\text{max}}$) also may be important. For instance aminoglycosides, exert their effectiveness and prevent from drug resistance by the $C_{\text{max}}$/MIC \cite{1}.

\textbf{Figure 2.1:} The effective pharmacokinetic/pharmacodynamic (PK/PD) parameters in anti-infective drugs. AUC, area under the concentration-time curve; $C_{\text{min}}$, minimum concentration; $C_{\text{max}}$, maximum concentration; MIC, minimum inhibitory concentration; $T_{>\text{MIC}}$, time of the drug blood concentration above the MIC.
Depending on the effective PK/PD index and the pharmacokinetics of the drug one or more sampling times are usually chosen for TDM.

### 2.2.2 Multidisciplinary team

Although TDM is routinely performed for several anti-infective agents, optimal treatment of the patient also depends on effective communication and cooperation between many healthcare professionals (Figure 2.2). In general, drug treatment of infectious diseases is selected based on clinically suspected pathogens. Adjustment of the treatment is required after antimicrobial susceptibility testing results become available. Since resistance to anti-infective drugs is a problem of increasing magnitude, narrowing the anti-infective treatment is recommended based on the susceptibility of the pathogen. Where antimicrobial resistance is observed, therapy should be changed to a more effective regimen. Subsequently TDM can be performed, if a sensitive and accurate analytical method is available.

![Figure 2.2: The multidisciplinary team involved in the infectious disease treatment.](image)

Antimicrobial stewardship (AMS) programmes have been developed to optimise clinical outcomes and minimize unintended negative consequences of antimicrobial use. An infectious disease physician and a clinical pharmacist with infectious
disease training are the core members of the AMS team \cite{10,11}. Among other factors, AMS is involved in appropriate treatment initiation and modification where appropriate. Furthermore, dose optimization is a part of AMS, in which TDM plays an important role for an increasing number of anti-infectives \cite{10,11}. Therefore, good collaboration between the infectious disease physician and clinical pharmacist is necessary for the correct diagnosis and treatment of the infection, and the correct interpretation and implementation of the TDM results. Additionally, a clinical microbiologist can provide surveillance data on the susceptibility of the pathogen and potential emergence of antimicrobial resistance. For implementation of recommendations, computer support is necessary and an information system specialist also may play an important role in AMS. Thus, to optimise clinical outcome for the patient, good cooperation between these professionals plays a crucial role in AMS and is cost-effective in many cases \cite{10,12}.

### 2.3 LC-MS/MS in TDM

LC-MS/MS has nowadays established itself as the primary analytical technique to support TDM \cite{13}. The commonly used matrices for TDM are blood, plasma, and serum. More recently, dried blood spots (DBS) and saliva have been introduced for TDM. Matrices like cerebrospinal fluid (CSF), inflammatory fluids, specific cells and tissue are not routinely used for TDM, but may be relevant in specific cases \cite{8}. However, each matrix has its analytical advantages and disadvantages and the clinical interpretation of the results strongly depends on this matrix. A number of guidelines on bioanalytical and clinical method validation have been published in order to improve and ensure the quality of analytical method validation and the generated analytical results. Among these are the Food and Drug Administration (FDA) with the ‘bioanalytical method validation’, European Medicines Agency Committee (EMEA) with the ‘guideline on bioanalytical validation’, and the Clinical and Laboratory Standards Institute (CLSI) with the ‘C62-A, Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline’ \cite{14,17}.

LC-MS/MS has replaced HPLC-UV in many clinical laboratories in high income countries. Unfortunately, the required broad repertoire of antimicrobial drug assays necessary for an anti-infective TDM program will reduce the number of tests per LC-MS/MS instrument annually, resulting in a relatively high price per test. Although less attractive from a laboratory perspective, costs resulting from inadequate antimicrobial treatment are much higher. If cheap, first-line anti-infectives fail and have to be switched to salvage therapy with second-line anti-infective drugs, costs will rise substantially. Before a hospital makes investments in an LC-MS/MS to service a TDM program for antimicrobial drugs, one should make
an business case. In general 20,000-50,000 tests annually are considered to be an acceptable justification of the investment [18]. For small hospitals, combining an LC-MS/MS for other TDM programs as well (e.g. antidepressants, antipsychotics or immunosuppressants), could result in cost-effective operation of an LC-MS/MS. Another alternative could be sending a sample to a nearby reference center, if turnaround time is acceptable. For low income countries, HPLC-UV still is an alternative as long as sensitivity is not an issue. Hopefully, increased use of LC-MS/MS in clinical laboratories will result in lower investments costs enabling broader implementation of LC-MS/MS.

2.3.1 Sample preparation

Because of the sensitivity and selectivity of the LC-MS/MS, extensive sample extraction techniques like solid-phase extraction (SPE) and liquid-liquid extraction (LLE) are often unnecessary. Therefore, fast and simple extraction techniques, like protein precipitation or sample dilution, are feasible. However, due to the limited sample preparation, endogenous compounds including lipids, phospholipids, and fatty acids are not sufficiently removed from the sample with protein precipitation. These compounds can interfere with the ionisation process resulting in ionisation suppression. These so-called matrix effects are observed frequently and should be solved for a reliable assay. Other types of matrix effects can originate from substance interaction with the matrix. For example, the drug can form chelate complexes with ferric ions, bind with heme groups, or can bind with the sampling matrix [19–23]. Isotopically labelled internal standards may correct for matrix effects better than structural analogues, but are unfortunately more expensive.

Ionisation suppression during the LC gradient can be visualized by continous infusion of a high concentration of stock solution via a T-piece connection to the mobile phase flow. Injection of a blank processed sample followed by the LC gradient shows lowered substance response at periods of ionisation suppression in a normally stable, but elevated baseline. By comparing the substance response of a spiked neat sample with a spiked processed blank sample, the relative ionisation suppression can be calculated.

A structural analogue as internal standard is preferred to elute at the same retention time and to have comparable ionisation characteristics. Since this is often not possible, ionisation suppression should also be evaluated for the internal standard. When ionisation suppression is present at the retention time of the substance, the gradient should first be optimized in order to chromatographically separate the ionisation suppression from the substance retention time. Dilution of the processed sample or the use of another ionisation method, like atmospheric pressure chemical
ionisation (APCI), may also be used to avoid ionisation suppression. Ultimately, an extensive sample preparation like SPE or LLE could be performed, which will eliminate most of the ionisation suppression effects.

In some patient groups, especially newborns, it is difficult to collect a sufficiently large blood volume for HPLC-UV analysis. Due to its high selectivity and sensitivity, sample volumes of 10 μL of plasma or serum are sufficient for LC-MS/MS analysis. Multiple analyses can be performed with LC-MS/MS using a single blood sample or even a sample which was taken for other routine laboratory measurements.

For analytical procedures used to analyse multiple compounds in a single sample, it may be more efficient to apply protein precipitation instead of LLE or SPE. The variation in physical and chemical properties of the different compounds to be analysed complicates the development of a suitable LLE or SPE extraction method. An LLE or SPE extraction method with acceptable recoveries for multiple compounds will per definition be far less selective than an extraction method for a single compound. If the use of protein precipitation allows the quantification of the compound at the desired concentrations without ionisation suppression, it is the first choice of sample preparation for LC-MS/MS.

Although protein precipitation easily allows the simultaneous analysis of multiple compounds in one LC-MS/MS method, differences in chemical and physical properties might still complicate chromatographic separation. Alternatively, another analytical column (with the use of a column switch) and/or mobile phase (with the use of a quaternary pump) can be selected and reinjection of the samples can be performed automatically.

### 2.3.2 LC-MS/MS turnaround time

Use of the LC-MS/MS analysis technique has significantly improved the turnaround times for TDM samples. HPLC-UV and HPLC coupled with diode array detection (HPLC-DAD) or often require extensive sample preparation to clean up and/or to concentrate the sample. In addition, the chromatographic runtimes of these techniques often exceed ten minutes. Runtimes of approximately five minutes are often feasible and use of an ultra performance liquid chromatography (UPLC) method can even reduce runtimes to less than two minutes.

In order to ensure short turnaround times, it also is useful to minimize overhead injections. Bioanalytical method validation guidelines state that a sufficient number of standards should be used to adequately define the relationship between concentrations and response. According to the guidelines for bioanalytical studies a calibration curve of six to eight standards and quality control (QC) samples should
be incorporated in each analytical run. However for linear regression, multiple concentration levels are unnecessary for reliable and accurate calibration. Instead, two calibration concentrations (at the lower limit of quantification and at the higher limit of quantification) are sufficient and proved to provide equal quality in analysis results with QC samples at concentrations throughout the linear range \[26\]. A two-point calibration curve could be impaired when the curve becomes non-linear, possibly due to changing ionization characteristics or overdue maintenance. An isotopically labelled internal standard can compensate for changing ionization characteristics. In addition, with the use of QC samples throughout the linear range, linearity issues would result in unacceptable biases for the QC samples and run rejection. Overhead samples put great pressure on the sample turnaround time, especially when a run could consist of approximately 16 overhead samples and only one patient sample. Minimizing overhead samples can be realized by validating a two-point calibration curve in addition to an eight-point calibration curve, resulting in a large reduction of injections. Subsequently, for the analysis of just one patient sample, a QC sample before and after the patient sample may be sufficient. Reduction in the turnaround time can make TDM more efficient.

\subsection*{2.4 Free drug concentration}

Regularly, blood concentrations for TDM are determined as total drug concentrations, i.e. the sum of the unbound and plasma protein bound fraction of the drug. However, only the unbound, free drug can diffuse through biological membranes to the site of action and exert its pharmacological and/or toxicological effects \[27, 28\]. Therefore for highly protein bound drugs, a small change in the extent of protein binding may result in a major change in free fraction of highly protein bound drugs \[28, 29\].

In clinical practice, unbound drug concentrations of highly protein bound drugs may be relevant for specific conditions, for instance in critically ill patients suffering from hypoalbuminemia. This results in a higher free fraction of that particular drug with subsequently several effects (Figure \[2.3\]). Initially the unbound drug concentration increases. Since only the unbound drug can be removed from the blood, the amount of drug cleared from the blood increases. Furthermore, the distribution of the unbound drug from the blood to peripheral tissues is increased. As a result, the unbound drug concentration decreases to the original value, while the total drug concentration is decreased. Therefore, total drug concentrations may not be representative for the effective PK/PD index and the unbound drug concentration should be measured instead of total drug concentration, in particular for highly bound drugs \[8, 28, 30\].
2.4. Free drug concentration

2.4.1 Methods of separation

There are several methods to separate the sample into unbound and bound portions. The most commonly used methods are equilibrium dialysis, ultracentrifugation, and ultrafiltration.

Due to its robustness, equilibrium dialysis is the reference method for determining unbound drug concentrations. However, this method is less suitable in clinical practice because of the long time to reach equilibrium. Another method to separate bound and unbound drug concentration is ultracentrifugation. An important advantage of ultracentrifugation, compared with equilibrium dialysis and ultrafiltration, is the elimination of the possible interaction of the compound to the filter membrane, since no filter membrane is used in ultracentrifugation. However, the equipment used for ultracentrifugation is more expensive than the equipment used for equilibrium dialysis and ultrafiltration [31]. Consequently, one of the
most commonly used methods in clinical practice is ultrafiltration, because of its simple and rapid performance. Furthermore, with ultrafiltration all the proteins are filtered out and further sample pre-treatment may not be necessary for LC-MS/MS analysis. With ultrafiltration, blood samples are centrifuged in systems that contain a membrane with a certain molecular weight cut-off. The duration of centrifugation differs for ultrafiltration, but is significantly shorter than equilibrium dialysis, which can be more than 24 hours. Subsequently, the free drug concentration is measured in the ultrafiltrate. For several anti-infective drugs, free drug concentrations are determined using ultrafiltration. However, during method development, the possible interaction of the compound to the filter membrane should be evaluated as well as the influence of temperature, centrifugation time and centrifugal forces on protein binding of the drug [27][29][31][33].

2.5 Site of infection (alternative matrices)

For TDM blood samples are predominantly used, while the site of infection is located elsewhere. If there are no significant barriers, influx or efflux mechanisms at the site of infection, it is expected that equilibrium is rapidly reached between the drug concentration in tissue fluid and blood [34]. However, it is more accurately to measure the drug concentration at the site of infection.

2.5.1 CSF

For central nervous system infections, the penetration of drugs from blood to the site of infection may be variable. Due to inflammation associated with infection, the blood brain barrier may initially be permeable for drugs, with the barrier then being restored when the infection subsides. This results in reduced drug concentrations in the central nervous system before the infection has been completely resolved [34]. Therefore, it may be necessary to determine the concentration of the drug in the CSF. The LC-MS/MS analysis of CSF is comparable to the analysis of ultrafiltrate. CSF contains very little proteins and is therefore relatively clean. For the proteins that are present, a protein precipitation procedure is sufficient as sample preparation. Obtaining blank CSF for method validation is manageable, provided that institutional guidelines allow the use of left-over materials. The use of an isotopically labelled internal standard is highly recommended when different matrices are used between patient samples and standards and QC samples. Although CSF normally contains very low amounts of protein, central nervous system infections and intracranial bleeding may significantly increase the protein content in the patient sample. This may result in haemolytic CSF and matrix effects, which affects the analysis results. This variation in protein concentration between patient
samples and standards and QC samples should be incorporated in the analytical method validation.

2.5.2 Pulmonary epithelial lining fluids and alveolar macrophages

Anti-infectives are frequently used in pulmonary infections. For extracellular and intracellular respiratory pathogens, drug concentrations have been measured in respectively pulmonary epithelial lining fluid (ELF) and alveolar macrophages or bronchoalveolar lavage (BAL) fluid [8, 35, 36]. These studies are helpful as they show whether a drug may be suitable for the treatment of pulmonary infections. In clinical practice, ELF and alveolar macrophages concentrations, however, are rarely measured due to the poor availability of assays and/or the invasive nature of sample collection. Sometimes it is important to know whether the drug is present at sufficient concentrations at the site of infection. In the absence of a validated assay, one may use a standard addition method to obtain a semi-quantitative result.

2.5.3 Intracellular

It may be of interest to measure intracellular concentrations for some drugs. For example, for antiretroviral drugs since HIV replicates within the cells of the immune system. Moreover, some of these drugs are administrated as prodrugs and are converted intracellularly into an active form. Subsequently, several studies have shown that the efficacy and toxicity of some antiretroviral drugs depend on intracellular concentrations [37]. In clinical practice, intracellular concentrations are not routinely measured for antiretroviral drugs, because for most antiretroviral drugs like non-nucleoside reverse transcriptase inhibitors and protease inhibitors a clear relation exists between the plasma and intracellular concentration [37]. However, this does not apply for nucleoside reverse transcriptase inhibitors and therefore intracellular drug concentrations should be monitored for these. Together with the isolation and counting of peripheral blood mononuclear cells, the analysis of intracellular concentrations is still a major technical challenge. Intracellular drug molecules are bound to membranes or proteins and therefore it will be difficult to approximate the actual intracellular free drug concentration. Again, obtaining blank matrix consisting of peripheral blood mononuclear cells is difficult and laborious. Moreover, it could require additional sample preparation and concentration to accurately quantify the very low intracellular concentrations with LC-MS/MS [37].
2.5.4 Tissue

In some situations, it may be helpful to quantify the drug concentration in infected tissue material which has been obtained during operation. In addition to the blood concentration, drug concentrations in tissue-homogenate may provide information on the exposure of the tissue to the drug. The sample processing of the tissue material includes weighing and homogenization of the sample. After weighing, the extraction solvent containing the internal standard can be added to the sample and this will be centrifuged. The obtained supernatant can be analysed by LC-MS/MS. This method is still in its infancy and exposure-response relations are not described for the drug concentration in tissue-homogenate \([38]\). In addition, one should realise, that drugs may be distributed unequally throughout the tissue, for example during ischemia or when the drug is actively taken up by specific cells. In summary, tissue homogenates are unlikely to be useful for drugs without equal interstitial fluid and intracellular distribution and is likely to under represent concentrations of drugs that do not penetrate intracellularly (e.g. beta-lactams).

A less invasive and more accurate sampling technique for measuring drug tissue concentrations is microdialysis, which is increasingly being used in clinical pharmacokinetic studies but is not commonly used in clinical practice. In contrast to tissue biopsy, with microdialysis unbound drug tissue concentrations can be measured directly and continuously in the interstitial space fluid in various tissues. Therefore, microdialysis may provide extra information for patients with complicated infections and where blood concentrations appear to be sufficient, but anti-infective therapy is failing \([39]\).

2.6 Proficiency testing programme

A variety of analytical methods has been published for the quantification of anti-infective drugs in human serum or plasma. The reliability of these analytical methods is essential to provide information on the drug concentration to the antimicrobial stewardship that hopefully translates in the best outcome for our patients. Intralaboratory (internal) method validation and intralaboratory QC procedures, such as validation of equipment and qualification of technicians, should ensure that these methods have sufficient accuracy, precision and specificity \([14,15]\). Participation in an interlaboratory (external) QC or proficiency testing (PT) programme is an essential component of quality assurance and also provides evidence of laboratory competence for clinicians, researchers, accrediting bodies and regulatory agencies \([40]\).

A PT programme is essential to verify whether the analytical method used for TDM
complies with the quality required for patient care. Many PT programmes exist in the field of HIV, antifungal and antituberculosis drugs and have indeed led to analytical improvement [40–42]. For instance, in a PT programme for the measurement of antifungal drug concentrations, the results showed that one out of five measurements was inaccurate. The performing laboratory was the main determining factor for these inaccuracies, which probably means that intralaboratory method validation was inaccurate [41]. In addition, the results of a PT programme for antiretroviral drugs showed that the measurement of low antiretroviral concentrations also was problematic and led to inappropriate dosing recommendations [42]. These examples illustrate and emphasize the importance of PT programmes for analytical methods used for TDM in clinical practice.

### 2.7 Outpatient monitoring

Routinely, blood samples are used for TDM which are often collected by venepuncture [43, 44]. However, this sampling strategy has several disadvantages. First, venous sampling is difficult in some populations, such as neonates and patients suffering from venous damage [43]. Second, there may be logistical setbacks. For venous sampling the patient needs to travel to the hospital or a designated laboratory. This may not always be possible, for instance in resource-limited and remote areas [43]. Another problem, especially in (sub)tropical areas, is sample stability. Many drugs are not stable in serum or plasma at room temperature and have to be stored and transported at -20 °C or lower [44]. To resolve these stability problems, alternative sampling strategies have been developed, such as DBS, dried plasma spots and microsampling [45–47].

DBS sampling is increasingly applied for optimizing drug dosages for many drugs [43, 44, 48]. DBS is popular for its advantages like minimal invasive sampling, sample stability and small blood volume required for analysis. In general, a DBS sample consists of a peripheral blood sample obtained by a finger prick. With clear instructions and after training, patients will be able to perform the procedure themselves at home [44]. DBS methods have been published for several antibacterial, antifungal and antiretroviral drugs [44, 49]. Reference values for TDM are traditionally based on serum or plasma drug concentrations and not on whole blood concentrations. Therefore, clinical validation is required to translate capillary blood-to-serum or -plasma concentration [44, 48, 50]. Another possible important factor may be the interaction of the drug with the blood matrix or the DBS card matrix. Rifampicin has demonstrated to interact with endogenous blood components, like ferric ions from the red blood cells causing complex formation [22]. This causes low recoveries from DBS extracts which can be improved by the
addition of chelating agents, such as EDTA and deferoxamine, to the extraction procedure. Also direct binding of the drug by hydrogen bonding with the DBS card matrix may have an effect on recovery \cite{19, 20}. Recovery also is influenced by haematocrit value, substance concentration and drying time of the DBS card \cite{20}. This interaction is inherent to the current cellulose based card matrices \cite{21}. An advantage of the dried plasma spot technique over DBS is that it is not influenced by haematocrit value. Quantification of anti-infective drugs using the dried plasma spot technique has been described for fosfomycin, daptomycin, linezolid, triazole antifungal drugs and antiretroviral drugs \cite{45, 47}. Although the use of DBS and dried plasma spot techniques is not yet widely spread, both are a promising alternative for venous blood sampling and in some cases (i.e. low resource and remote areas) the only viable options.

Another patient friendly method of sampling is the use of saliva \cite{43, 51}. Compared to blood sampling, saliva is easy to collect and non-invasively with a negligible chance of infections \cite{52}. Furthermore, it is cheap and causes less stress and discomfort to the patients \cite{52}. As saliva is a very low protein matrix (\textasciitilde0.3\%), the measured concentration represents the unbound concentration of the drug. This may require a very sensitive LC-MS/MS analysis method or an extensive sample preparation procedure like SPE or LLE to concentrate the sample for drugs with high protein binding. As there are many other determinants of the salivary drug concentration, such as salivary flow rate, stability of the drug and its metabolites, time of sample collection and ingestion of food or beverages \cite{52}, target concentrations in saliva should be established on a drug-to-drug basis \cite{43}. Saliva methods using LC-MS/MS have been published for a few anti-infective drugs (doxycycline, fluconazole, linezolid, lopinavir and oseltamivir) \cite{52, 54}.

### 2.8 Conclusion

In conclusion, TDM plays an important role in the optimisation of treatment with anti-infective drugs. To perform TDM adequately, it is essential to design assays with a rapid turnaround time, enabling the antimicrobial stewardship to quickly adjust and optimise treatment if necessary. LC-MS/MS is a fast and accurate technique for quantification of anti-infective drugs. If an analytical method is developed and validated, interlaboratory quality control is an important component of quality assurance.

In clinical practice blood is the most commonly used matrix for TDM since it serves as a good surrogate for the site of infection. In general, it is easily obtained, in contrast to other matrices. However, in complex infectious cases other matrices could be used to optimise anti-infective treatment.
References


