ELGA VEOLIA

White paper

Reducing risk in HPLC / LC-MS therapeutic drug treatment and monitoring

Why water purity matters

WATER TECHNOLOGIES

Inside

“90% of all difficulties with high performance liquid chromatography are caused by column problems, most of which come from contaminated water.”

The above quote comes from LC/MS: A Practical User’s Guide, by M.C McMaster, and reflects the importance of using water of the highest quality for HPLC analysis.[1]

Intro

This paper will help you to understand just how important ultrapure water is for HPLC and LC-MS/MS. We will examine the challenges associated with their optimisation in different applications, challenges that are all too frequently directly related to the quality of the water used, and discover some key ways to overcome these challenges.

The applications we look at are wide-ranging, and include (i) ISD monitoring and treatment, (ii) monitoring of anti-psychotic drugs, (iii) drugs testing in forensic toxicology, and (iv) investigating the circulating levels of antimicrobials in severe infection.

Water purity is already recognised by researchers as critical to the success of HPLC. In fact, in a recent survey on HPLC applications, 89.7% of respondents recognise that water purity is critical to the success of their HPLC applications, and when the quality of the HPLC results was called into question, particulates and organic contaminants in the water were cited as the most likely potential major sources of error (n=229, internal data, ELGA 2019).

Since water is so important as part of the so-called mobile, or liquid, phase in HPLC and LC-MS/MS, it follows that water purity is key to any diagnostic or pharmaceutical applications that involve these techniques. This has also been previously discussed in the ELGA paper: "How to get the most accurate and reliable data from HPLC using ultrapure water", which highlights the influence of specific water contaminants on HPLC results, and emphasises the need for ultrapure (Type I) water in HPLC, with a resistivity of 18.2 MΩ.cm, total organic carbon (TOC) of <10ppb, and bacterial count of <1 CFU/ml.[2]

ELGA EDITORIAL TEAM

i.

LC-MS/MS FOR THERAPEUTIC DRUG MONITORING (TDM) AND TREATMENT

Challenge: inaccurate drug dosing, which can lead to death

The global increase in organ transplant activities is driving demand for more accurate, rapid and cost-efficient ways to monitor immunosuppressive drugs.

Despite the intrinsic risks of immunosuppressive drugs (ISDs), their widespread use facilitates the transplantation of tens of thousands of allografts per year, and consequently they have massive potential to decrease patient morbidity and mortality.

Therapeutic drug monitoring (TDM) of ISDs with a narrow therapeutic index is an increasingly popular tool for minimising drug toxicity while maximising the prevention of graft loss and organ rejection. Regular and accurate TDM in transplant patients is essential to reduce the risks associated with their use, which include:

- Narrow therapeutic range of drug efficacy, with a significant chance of under or over dosing the patient, who may also have an individual and variable response, depending on their current state of health and phase of therapy
- Variable pharmacokinetics, meaning that a suboptimal amount of the ISD may be available in the patient's system at any given time

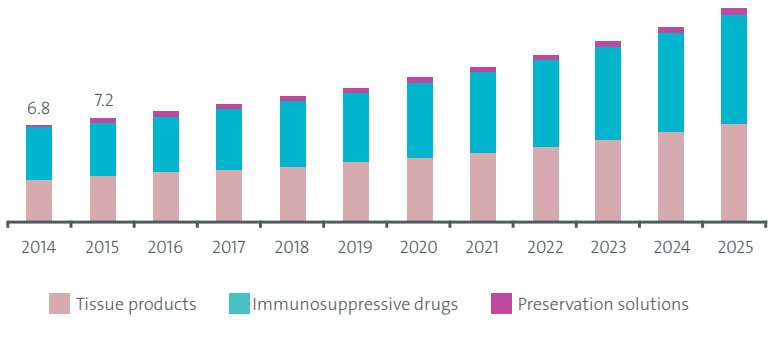
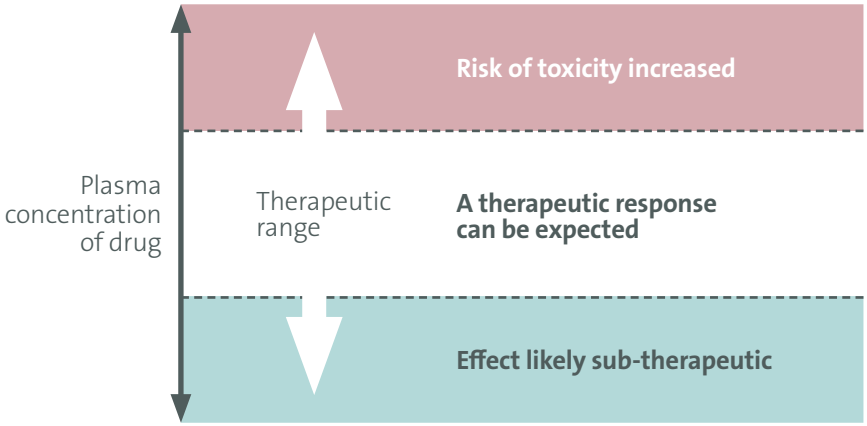


Figure 1. Increase in organ transplants in the US 2014-2025 (USD billion).[3]

- Potential toxicities if dosage is too high: to other organs (e.g. kidney) via acceleration of diseases like atherosclerosis, diabetes, hypertension, as well as increased susceptibility to opportunistic infections and malignancies
- Safety and regulatory compliance: strict guidelines must be adhered to for each individual patient

Fig 2 The importance of accurate measurement of ISDs



Immunoassays have been the workhorse and technology of choice for TDM of all types for several decades. However, immunoassays come with many disadvantages, including cross-reactivity with metabolites, **which can lead to inaccurate drug dosing, false positives and negatives, which can in turn have dire consequences**, leading in the worst cases to patient death.[4]

To this end, researchers are now replacing immunoassays with mass spectrometry (MS) methods such as LC-MS/MS, which offers increased specificity, sensitivity and accuracy over immunoassays. LC-MS/MS is fast, flexible, adaptable, and allows the simultaneous quantification of multiple analytes.[5]

The differences in drug concentrations that are being determined are so small (ng/ml), that successful implementation of LC-MS/MS for drug monitoring raises challenges, both in the pre-analytical stages of sample preparation, and in the running of the HPLC process itself.

Immunosuppressant drugs are also used to treat autoimmune diseases. These drugs weaken the immune system, thus suppressing the impact of potentially harmful autoimmune responses in the body. Autoimmune diseases that have been successfully treated with immunosuppressant drugs include, but are not limited to, psoriasis, lupus, rheumatoid arthritis, Crohn's disease, multiple sclerosis and alopecia areata.[6]

The five most commonly prescribed immunosuppressive medications are: cyclosporine A, tacrolimus, sirolimus (rapamycin), everolimus, and mycophenolic acid. Recent developments indicate that liquid chromatography-tandem mass spectrometry (LC-MS/MS) is now sufficiently standardised for TDM of the major ISDs that have been studied, and can now replace immunoassays.[7]

Tacrolimus has immunosuppressive effects *in vitro* through the inhibition of mixed lymphocyte reactivity and the generation of cytotoxic T cells. An interesting study on the evaluation of the concordance between immunoassays (ELISA) and LC-MS/MS methods for measurement of tacrolimus serum trough levels showed that the inter-subject variance coefficient for the ELISA method was higher than inter-subject variance coefficient for LC-MS/MS, concluding that lower inter-subject variance of LC-MS/MS could result in fewer dose changes and an overall better immunosuppression control in paediatric kidney-transplanted patients.

The challenges related to the accurate monitoring of ISDs revolve around sample type and quality, and the relative stability of the drug in question throughout the LC-MS/MS process. **In particular, the water used in the sample preparation process must be ultrapure: any bacteria or ions that are not intrinsic to the patient sample are likely to destroy its integrity,** leading to skewed results and false positive or negatives and inaccurate dosing.[1]
Fresh blood is probably the most common sample type used in ISD analysis, since it is the actual circulating concentration of a specific ISD that is important: its bioavailability.[8] However, recent studies using dried blood spots show that they can also be used as a sample type for monitoring ISDs ahead of LC-MS/MS.[9]

Whatever the sample type however, researchers and QA scientists are frequently dealing with threshold quantities of ISDs, over an extremely narrow therapeutic range. **In order to adequately solve this challenge, the water used in preparing patient TDM samples, and in the HPLC analysis ahead of MS, must be ultrapure, to avoid problems such as ghost peaks that could be mistaken for active pharmaceutical ingredients.**[2]

i.

LC-MS/MS FOR THERAPEUTIC DRUG MONITORING (TDM) AND TREATMENT

Solution: ensure an ultrapure water supply for your TDM assays

In effect, sufficiently pure water must be used for these clinical assays, of a standardised, audited and traceable composition, such as that provided by an in-house water purification system. The lower the variability introduced from an unreliable water source, the more likely the LC-MS/MS TDM assays developed can then be standardised and reproduced globally, and the methods hence approved more readily by the appropriate organisations, such as the FDA.

The far-reaching consequences of the standardisation of this whole workflow, **including the standardisation of the quality of the water used in HPLC and LC-MS, are that many more samples and more patient data can be meaningfully pooled, compared, and tests can be refined**. This will ultimately lead to more successful patient outcomes and make a significant contribution to human healthcare globally.

ii.

UHPLC-MS/MS METHOD FOR TDM OF ANTI-PSYCHOTIC DRUGS

Challenge: lower limits of detection

Another evocative area for therapeutic drug monitoring (TDM) is the development of methods to measure the levels of specific antipsychotic drugs. Such methods could potentially be used to optimise drug delivery, and improve patient outcome, given the individuality of patient responses to such drugs.[10] **Given the miniscule (ng/ml) concentrations of antipsychotic drugs detected by this method, the purity of the water used for the preanalytical stages and for HPLC is key.**[10]

To this end, a **selective and sensitive method based on UHPLC-MS/MS was developed for the simultaneous quantification of seven typical antipsychotic drugs** (cis-chlorprothixene, flupentixol, haloperidol, levomepromazine, pipamperone, promazine and zuclopenthixol) in human plasma. A simple protein precipitation procedure with acetonitrile was used for sample preparation. The use of stable isotope-labeled internal standards for all analytes kept internal standard-normalised matrix effects within the range of 92–108%.

The method was fully validated to cover large concentration ranges for haloperidol, flupentixol, levomepromazine, promazine, zuclopenthixol, cis-chlorprothixene and pipamperone. Trueness (89.1–114.8%), repeatability (1.8–9.9%), intermediate precision (1.9–16.3%) and accuracy profiles (<30%) were in accordance with the latest international recommendations.

ii.

LC-MS/MS FOR THERAPEUTIC DRUG MONITORING (TDM) AND TREATMENT

Solution: ensure an ultrapure water supply for your antipsychotic drug assays

Over 500 patient plasma samples were subsequently monitored. Water purity is key to the successful development and validation of assays where lower limits of detection are of the order of ng/ml, particularly when trying to reproduce results between labs.[10]

iii.

SAMPLE PREPARATION AND PROCESSING IN FORENSIC TOXICOLOGY

Challenge: find the sources of variability in forensic toxicology tests

Forensic toxicology encompasses the measurement of alcohol, drugs, and other toxic substances in biological specimens and the interpretation of such results in a medico-legal context. Here, we consider the measurement of drugs in hair, as a specific example, in which it was demonstrated that the quality of the water has a direct impact on the accuracy of the result.

Testing hair samples for drugs or pharmaceuticals is now a well-established technique in clinical and forensic toxicology, but quantitative results can vary between laboratories. A new study makes recommendations for improving reproducibility, which is particularly important for borderline test results.

Establishing a person's exposure to drugs of abuse or pharmaceuticals is important for many situations including forensics, clinical applications or in doping control. Drug analysis is usually carried out on body fluids, such as urine or blood samples. But in recent years, remarkable advances in sensitive analytical techniques has expanded opportunities for using drugs in less conventional samples, including hair.

Hair analysis offers many advantages as samples can be collected easily and non-invasively and under close supervision to prevent any potential adulteration or substitution. It also can dramatically extend the window for drug detection – to weeks, months or even years after a substance was taken.

For these reasons, hair analysis is becoming more widely used for retrospective drug monitoring.

But quantitative results can vary considerably between laboratories, mainly due to a lack of standardised protocols.

This can be of critical importance when the drug concentration is close to the cut-off value for a positive result, such as those defined by external organisations which include the European Workplace Drug Testing Society (EWDTS).

PUTTING THE SPOTLIGHT ON SOLVENTS

In a new study, researchers at the University of Zurich in Switzerland assessed the effect of using different solvents on the outcomes of hair analysis of samples from drug users.[11]

The team carried out extraction on pooled hair samples collected from known drug users using seven different solvents in one or two-step protocols. They then used liquid-chromatography-tandem mass spectrometry (LC-MS/MS) to detect and quantify different drugs or pharmaceuticals, including opiates, ketamine, antidepressants and antihistamines.

To prepare the mobile phase used for the liquid chromatography stage of the process, they used water processed by a PURELAB system from ELGA LabWater.

iii.

SAMPLE PREPARATION AND PROCESSING IN FORENSIC TOXICOLOGY

Solution: use ultrapure water to remove a major source of variability

The researchers found that drug yields depended on the specific substance and the solvent extraction method. For many drugs, a two-step extraction with methanol acidified with hydrochloric acid gave the highest extraction efficiencies, while in other cases the choice of solvent had little impact. However, using acetonitrile as the solvent in a one-step protocol gave universally low extraction efficiencies.

This important new study demonstrates that the choice of extraction solvent is an important factor for the quantification of drugs in hair samples. It highlights that extraction protocols should be harmonised across testing laboratories, particularly in cases whether the interpretation of a person's result is based on the same cut-off value.

The authors also strongly recommend that laboratories should use authentic hair samples collected from known drug users, rather than artificially spiked samples, to validate the accuracy and precision of their analytical methods.

iv.

MEASURING ANTIBIOTICS IN SEVERE INFECTIONS

Challenge: find the correct dose of antibiotics to cure severe infections

Accurate measurement of serum concentrations of antimicrobials may be important when treatment includes agents that have a narrow margin between their therapeutic and their toxic levels such as the aminoglycosides (especially gentamicin) or in patients with renal failure, who may accumulate unusually high levels of antimicrobials normally excreted by the kidneys. This is especially important if patients are immunocompromised.

Here we summarise a study where simple high performance liquid chromatography-mass spectrometry methods have been put in place for the TDM of antimicrobials.

To perform HPLC and LC-MS analyses under optimum conditions when dealing with low concentrations of analytes (ng/ml), the water used for mobile phase preparation needs to be highly purified: water quality has a direct impact on the achievable detection limits.[12]

The study looked at the simultaneous quantification of nine antimicrobials by LC-MS/MS for therapeutic drug monitoring in critically ill patients, and could also be useful when considering the general use (or overuse) of antibiotics.[13]

Adequate antibiotic treatment is a prerequisite for the successful treatment of systemic infections. Based on accumulating scientific evidence, a fixed dosage regimen can lead to insufficient and ineffective

antibiotic therapy. The aim of the study was thus to develop and validate a simplified, but sensitive method for the simultaneous quantification of antimicrobials by using LC-MS/MS for the development of personalised therapy regimens using therapeutic drug monitoring.

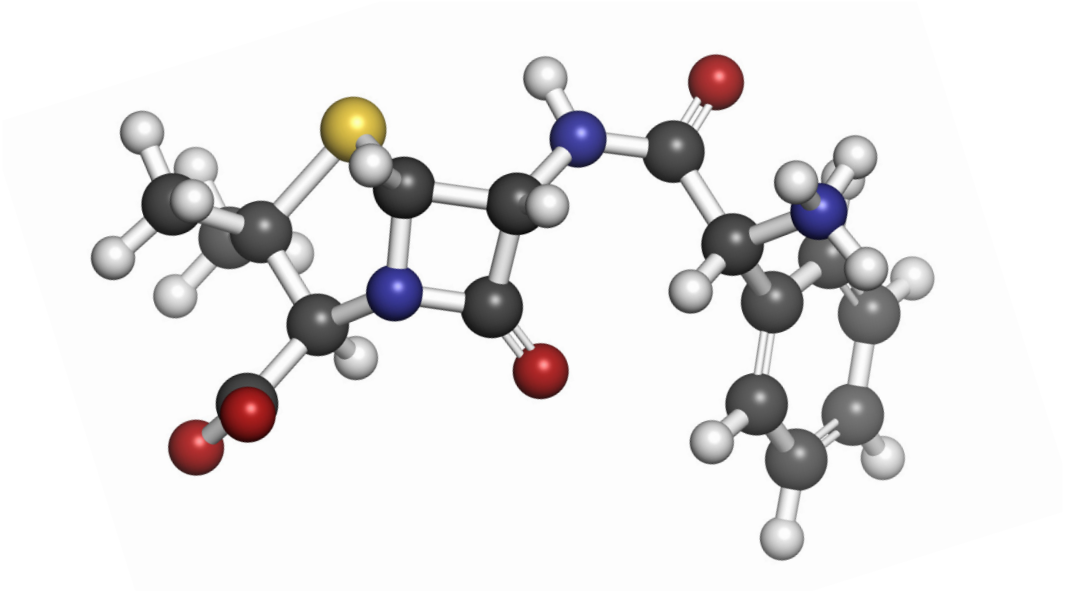
The nine antimicrobials monitored in this study were: aciclovir, ampicillin, cefuroxime, ciprofloxacin, meropenem, metronidazole, piperacillin, rifampicin, and tazobactam, all sampled from lithium–heparin plasma. A simple sample preparation method and a chromatographic run time of 10 minutes enabled the quick processing of the samples. The method was validated according to the guidelines for bioanalytical method validation of the European Medicines Agency and addressed sensitivity, specificity, linearity, accuracy, precision, dilution integrity, carry-over, recovery, matrix effects, and stability. With a chromatographic run time of 10 minutes, the antimicrobials eluted at retention times ranging from 1.1 to 2.2 minutes. Calibration curve for all antimicrobials was linear over a range of 1–100 mg/L, and a 2-fold or 5-fold dilution of the samples was possible. No interferences and carry-over were observed, and the samples were stable for at least 5 hours at room temperature or in the autosampler. The authors conclude that the LC-MS/MS method developed in this study is appropriate and practical for the therapeutic drug monitoring of antimicrobials in daily clinical laboratory practice thanks to its short analysis time, the need for a small amount of plasma, and its high specificity and accuracy.

iv.

MEASURING ANTIBIOTICS IN SEVERE INFECTIONS

Solution: use ultrapure water in antimicrobial assays

To perform HPLC and LC-MS analyses under optimum conditions when dealing with low concentrations of analytes (ng/ml), the water used for mobile phase preparation needs to be highly purified: water quality has a direct impact on the achievable detection limits.[12]



Conclusion

Through these specific examples, from the therapeutic drug monitoring in immunosuppressant treatment, to the precise measurement of illicit substances amongst drug users, it is clear that the purity of the water used in sample preparation and HPLC ahead of MS is critical, in order to drive accurate and successful patient and medico-legal outcomes.

In these HPLC-related assays, we are most often looking at lower limits of detection of analytes of ng/ml, and constantly seeking to standardise tests and hence improve reproducibility in results across different labs. Installing an in-house water purification system is the best way to minimise the risk associated with variable water quality in such sensitive clinical applications.

To explore how you can optimise the quality of the water in your HPLC-related clinical applications, and discuss your needs further, visit https://www.elgalabwater.com/products

References

1. McMaster, M.C. (2005) HPLC and Column Maintenance (Chapter 4), LC/MS: A Practical User's Guide, John Wiley & Sons, Hoboken, N.J., Page 33.

2. ELGA LabWater, How to Get the Most Accurate and Reliable Data from HPLC using Ultrapure Water https://www.elgalabwater.com/primer-hplc-ultrapure-water. Accessed 6 Nov 2019.

3. https://www.grandviewresearch.com/industry-analysis/transplantation-market. Accessed 6 November 2019

4. Dasgupta, A. (2016) Personalized Immunosuppression in Transplantation: Role of Biomarker Monitoring and Therapeutic Drug Monitoring. Chapter 2 - Limitations of immunoassays used for therapeutic drug monitoring of immunosuppressants, 29-56.

5. Hétu, P.-O., Robitaille, R. and Vinet, B. (2012) Successful and cost-efficient replacement of immunoassays by tandem mass spectrometry for the quantification of immunosuppressants in the clinical laboratory. Journal of Chromatography B, Volumes 883–884, 95-101.

6. Kant V, Verma P K and Kumar P. (2009). Immunosuppressive drug therapy: An overview. J. Immunol.Immunopathol. 11(2): 21-32.

7. Paniagua-González, L., et al. (2019) A multidrug LC–MS/MS method for the determination of five immunosuppressants in oral fluid, Bioanalysis 11(6), pp1509-1521.

8. Tsipotis, E., Gupta, N. et al. (2016) Bioavailability, Efficacy and Safety of Generic Immunosuppressive Drugs for Kidney Transplantation: A Systematic Review and Meta-Analysis. American Journal of Nephrology 44(3):206-218

9. Vermeersch, P. et al., (2019) Preanalytical considerations in therapeutic drug monitoring of immunosuppressants with dried blood spots Diagnosis v6, pp57–68.

10. Gradinarua, J., et al., (2014) Quantification of typical antipsychotics in human plasma by ultra-high performance liquid chromatography tandem mass spectrometry for therapeutic drug monitoring. Journal of Pharmaceutical and Biomedical Analysis, Volume 88, 25 January 2014, Pages 36-44 https://doi.org/10.1016/j.jpba.2013.07.041

11. Madry, M. M., Kraemer, T. and Baumgartner, M. (2018) Systematic assessment of different solvents for the extraction of drugs of abuse and pharmaceuticals from an authentic hair pool. Forensic Science International 282, 137-143.

12. Regnault C., et al., (2004) Ultrapure water for liquid chromatography-mass spectrometry studies. J Chromatogr A. 1030(1-2), 289-95.

13. Neugebauer, S., et al., (2019) Simultaneous quantification of nine antimicrobials by LC-MS/MS for therapeutic drug monitoring in critically ill patients. Therapeutic Drug Monitoring 41, 29-37.

ELGA VEOLIA

Dedicated to Discovery

info@elgalabwater.com / www.elgalabwater.com

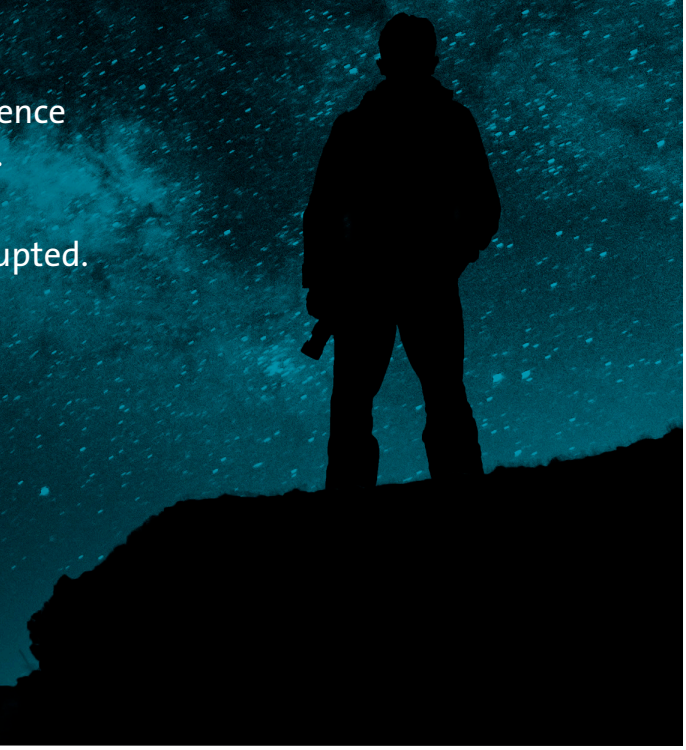
ELGA Labwater are specialists in the engineering, service & support of water purification systems.

Unrivalled product design has achieved international recognition and awards.

Worldwide technical service teams support science & healthcare globally with specialist expertise.

Global digital performance monitoring from Hubgrade ensures laboratory work is uninterrupted.

A global supply chain supports clients from regional centres on every continent.



To find your nearest ELGA representative, go to *www.elgalabwater.com* and select your country for contact details.

Elga Global Operations Centre.
tel: +44 (0) 203 567 7300
fax: +44 (0) 203 567 7205

GIT INNOVATIONS AWARD 2010

reddot design award winner 2011

GOOD DESIGN AWARD 2014

Design Award Gold 2015 IBO

Hubgrade

OVER 70 INTERNATIONAL PATENTS