Dissertation

zum Erwerb des Doktorgrades der Humanbiologie

an der Medizinischen Fakultät der

Ludwig-Maximilians-Universität München

Approaches for optimizing biomarker profiling using liquid chromatography tandem mass spectrometry

Anna Catharina Suhr

Melle

2017

Aus dem Institut für Laboratoriumsmedizin
der Ludwig-Maximilians-Universität München

Direktor: Univ.-Prof. Dr. med. Daniel Teupser

Approaches for optimizing biomarker profiling using liquid chromatography tandem mass spectrometry

Dissertation
zum Erwerb des Doktorgrades der Humanbiologie
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität München

vorgelegt von
Anna Catharina Suhr
aus
Melle

2017

Mit Genehmigung der Medizinischen Fakultät

der Universität München

Berichterstatter:	Prof. Dr. med. Michael Vogeser
Mitberichterstatter:	PD Dr. med. Dr. rer. nat. Harald Mückter
	Prof. Dr. med. Oliver Peschel
	PD Dr. rer. nat. Elisabeth Deindl
Dekan:	Prof. Dr. med. dent. Reinhard Hickel

Tag der mündlichen Prüfung: 29.06.2017

Eidesstattliche Versicherung

Suhr, Anna Catharina

Ich erkläre hiermit an Eides statt,

dass ich die vorliegende Dissertation mit dem Thema

“Approaches for optimizing biomarker profiling using liquid chromatography tandem mass spectrometry“

selbstständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, den 27.09.2016

Kumulative Dissertation

Keep calm and mass spec on.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Background

1.1.1. Definition

Biological markers, or biomarkers, play an important role in nearly all medical disciplines since they are '*objective indications of medical state observed from outside the patient – which can be measured accurately and reproducibly,*' as described by Strimbu and Tavel [1].

As objective medical indicators, biomarkers are used in screening procedures (e.g. blood glucose to assess patient risk of diabetes mellitus), diagnosis (e.g. troponin to evaluate suspected myocardial infarction) and patient follow-up (e.g. prostate-specific antigen to determine the success of therapy against prostate cancer).

Biomarkers can take very different forms, including small molecules, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, whole cells, measurements of physiological functions (e.g. blood pressure), histologic specimens and imaging [1]. Moreover, in addition to medical science, biomarkers play an important role in other life sciences.

1.1.2. Metabolomics and biomarker discovery

The discovery of new biomarkers to better diagnose disease states has long been important to clinical researchers. The critical challenge in this field of research is how to identify promising biomarker candidates.

Recently, technical advances in analytics and informatics have enabled the emergence of the so-called '-omics' disciplines: genomics (DNA), transcriptomics (RNA), proteomics (proteins) and metabolomics (metabolites). Based on their comprehensive approaches, all four have huge potential for the discovery of new biomarkers [2].

Since changes due to disease or environmental conditions are usually well reflected by alterations in cell metabolite patterns, the metabolomics discipline is of particular importance in the search for new biomarkers. Consequently, in this study, the main focus is on small molecules and their potential to serve as biomarkers.

The two strategies for biomarker discovery depend on the information currently available regarding the pathogenesis of a given disease: untargeted hypothesis-free

metabolomics and targeted metabolomics, which are based on previously known facts. Both strategies can result in a set of interesting metabolites [3].

This predefined set is then quantified for two sample groups – patients and healthy controls – and statistical analyses are performed to determine which analyte panel is most appropriate for differentiating these two groups. If a promising biomarker (panel) candidate is identified, it is validated in a clinical study with larger cohorts of patients and controls to assess its sensitivity and specificity as a diagnostic test [4].

Biomarker panels can be composed of both closely related compounds and, at the same time, compounds from a broad variety of chemical classes. Consequently, in metabolomics as well as in subsequent biomarker validation, there is a need for analytical techniques that are both capable of discriminating between very similar substances in one analytical run as well as facilitating the analysis of compounds with different physiochemical characteristics. Current techniques include nuclear magnetic resonance (NMR) spectroscopy, gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) [5].

For various reasons that will be detailed below, an increasing number of publications in recent years indicate that LC-MS is the technique of choice in metabolomics [6].

Mass spectrometers identify previously ionized analytes by their *mass-to-charge ratio* (m/z). Depending on the type of mass spectrometer, they are either suited for identification and elucidating structure or for absolute quantification.

When using tandem MS (MS/MS), analyte ions (*precursor ion*) are further fragmented into *product ions*. Since these *product ions* are somewhat specific to the structure of a molecule, selectivity is further enhanced by tandem MS such that more analytes (even those with the same precursor m/z) can be quantified in parallel. Since biomarker profiling is mainly based on the quantification of numerous analytes in one run, triple quadrupole tandem mass spectrometers (‘triple quads’) are widely used in biomarker profiling [5].

The GC-MS and LC-MS techniques share the unique advantage of *stable isotope dilution* (SID), i.e. the application of stable isotope-labelled (SIL) analyte counterparts as internal standards. The analyte and the respective SIL internal standard have different masses (different m/z) but behave very much alike since they share nearly identical physicochemical properties. Hence, the use of SID allows for the correction of analyte loss during sample preparation as well as for the differing *matrix effects* of different samples. The so-called *matrix effect* describes the influence of co-eluting

matrix ingredients that suppress or enhance the ionization of the analyte. Furthermore, the use of SIL internal standards allows for the identification of chemical background peaks of interfering substances that are similar to those of the analyte [4]. As a consequence, SID clearly increases the robustness of assays with respect to the diversity of matrices of human samples in biomarker profiling.

Frequently, analytes must be derivatized to make them detectable in GC-MS analysis, and this is especially so for non-volatile or thermolabile analytes. Since ionization in LC-MS takes place at ambient pressure and does not depend on high temperature, these compounds are much more accessible with LC-MS [4]. Additionally, LC is very versatile due to the availability of different types of stationary and mobile phases [3].

1.1.3. Biomarker profiling in clinical diagnostics

Parallel and selective quantification, as discussed above, becomes even more important if a whole panel is addressed rather than a single biomarker. A very good example of the use of biomarker panels for differential diagnosis is given by the work of Arlt et al. [7]. The authors used GC-MS to establish a panel of steroids in urine for detecting malignancy in adrenal tumours. If ‘by chance’ during a diagnostic imaging procedure for an unrelated clinical purpose an adrenal mass is discovered, differentiation between adrenocortical carcinoma and adrenocortical adenoma is difficult when relying only on imaging and tumour size alone; nor does histology always deliver a decisive result. Arlt et al. demonstrated the extraordinary sensitivity and specificity of a biomarker panel based on steroid metabolomics [7].

The group of Mapstone [8] used (un-)targeted lipidomics based on LC-MS/MS to search for biomarkers of Alzheimer’s disease (AD) in peripheral blood. The authors discovered a profile of ten metabolites that identify people who will experience the onset of AD (or mild cognitive impairment) within two to three years. This blood-derived metabolic profiling technique may be very helpful in the search for disease-modifying therapies for AD [8].

A third interesting example is the use of metabolic profiling to enable the early diagnosis of Parkinson’s disease before decisive clinical symptoms become apparent. Luan et al. were able to discriminate with quite high sensitivity and specificity between patients at early, mid and advanced disease states and those in

control groups by analysing a panel of 18 metabolites (among them e.g. amino acids, steroids) via MS techniques [9].

As these examples demonstrate, diagnoses based on biomarker profiling - rather than only on one or two markers - are becoming more common. This may be especially true for diseases that have to date lacked the identification of any decisive medical parameters.

Today, the majority of assays in laboratory medicine employ automated analysers based on techniques such as photometry and immunochemistry. However, these techniques are not generally applicable for the analysis of biomarker panels since they are capable of quantifying only one analyte at a time. Furthermore, immunoanalysers are vulnerable to interferences caused by cross-reactions with similar substances or heterophilic antibodies [10].

In contrast, as described above, LC-MS/MS techniques offer specific and parallel quantification. In addition, an assay can usually be developed quickly and individually since, unlike immunoanalysers, LC-MS/MS systems are accessible to laboratory personnel and also very versatile, as noted above. Reagents and consumables can also be ordered independently from commercial kits, which allow greater flexibility in respect of novel insights in clinical research [11, 12]. Consequently, the use of LC-MS/MS in laboratory medicine is expected to increase.

1.1.4. Challenges of LC-MS/MS assays

Despite these encouraging aspects, the employment of LC-MS/MS in biomarker profiling faces several challenges: There is a lack of authentic blank matrices (e.g. plasma, serum, urine, saliva) that are free of the biomarkers of interest. Since most of these biomarkers also occur in healthy people – at different concentration levels – hardly any analyte-free authentic matrices are available. This hampers the convenient preparation of calibrators and controls with defined amounts of analytes in an authentic matrix. Several techniques have been employed to deplete matrices of analytes (e.g. charcoal-stripped serum for steroid analysis), but these typically considerably affect the composition of the matrix. As a consequence, it is doubtful that the resulting artificial products accurately characterise the authentic matrix. Therefore, these depleted matrices must be assigned as *surrogate matrices*, which can also consist of solvents such as water or methanol or a solution of serum albumin.

Other strategies that can be employed for calibrating without an authentic blank matrix are standard addition or a calibration based on a surrogate analyte [13, 14]. However, the most widely employed approach is the use of a surrogate matrix for the preparation of calibrators and controls.

In this context, SID plays a crucial role since the composition and resulting matrix effect of a surrogate matrix differs significantly from that of the authentic matrix of the unknown samples. Unfortunately, SIL counterparts are not always commercially available, especially not for endogenous compounds, which may be an issue with respect to accurate and robust quantification in biomarker profiling.

Furthermore, regarding biomarkers, it is quite likely that isobaric compounds occur, possibly as other analytes in the panel, or as disturbances present in the matrix. Isobaric compounds not only share precursor ions with the same m/z, but also product ions with same m/z. Consequently, even MS/MS cannot discriminate between them. A chromatographic separation of potentially interfering isobaric compounds is of utmost importance in such cases [3, 6]. Additionally, selectivity can be enhanced by monitoring at least two different product ions per analyte.

Another challenge is the lack of guidelines for assay development and validation of biomarkers as endogenous compounds, in contrast to the well-known guidelines for xenobiotica provided, for example, by the European Medicines Agency (EMA), the Food and Drug Administration (FDA), and the Clinical & Laboratory Standards Institute (CLSI) [15-17]. Thus, existing validation protocols are typically ‘home-brewed,’ which clearly hampers the standardization and comparability of assay quality. Several authors have recommended ‘fit-for-purpose validation protocols’ for endogenous substances. In particular, these protocols address critical validation aspects for endogenous compounds, such as the measurement of matrix effects, the assessment of selectivity or the correct determination of the lower limit of quantification (see e.g. [13, 14, 18, 19]). Thus far, there is no consensus paper or compulsory guideline for validating biomarker assay methods. As such, if biomarker profiling is used as a diagnostic procedure, considerable difficulty arises concerning accreditation etc.

As discussed above, the matrix effect from co-eluting compounds can hamper accurate quantification. This is an important issue in biomarker profiling since the individual composition of samples of human material can vary substantially. A carefully chosen sample preparation can help to reduce the number of disturbing

matrix compounds in the extract. Additionally, the sample preparation for biomarker panels must be suitable for a group of analytes with varying physicochemical properties.

However, in contrast to fully-automated immunoanalysers, LC-MS/MS involves laborious sample preparation (batch mode), long run times per sample and complex instrument maintenance that requires specially trained personnel and limits sample throughput [20]. Recent advances such as the development of ultra-high performance liquid chromatography (UHPLC), the integration of online solid phase extraction (SPE) and two-dimensional LC into MS platforms, as well as sound progress in liquid handling systems, has reduced the required hands-on time and improved the routine applicability of LC-MS/MS in clinical laboratories. Additionally, the development of multiplexing has enabled a better occupancy rate of expensive mass spectrometers since, in this case, one MS instrument can provide read outs for several LC instruments with staggered analysis time slots [12]. However, completely automated LC-MS/MS analysers are as yet unavailable – and this is a general drawback for the wider use of LC-MS/MS in laboratory medicine.

1.1.5. Aim and Scope

The aim of this research project was to develop solutions to address some of the abovementioned challenges in LC-MS/MS biomarker profiling and to improve the potential for using biomarker profiling in routine laboratory medicine, i.e. in clinical diagnostics.

In the first project, the lack of an authentic blank matrix – as a typical challenge arising in the development of methods for the quantification of endogenous compounds – was addressed. The goal was to fill a gap in the validation process for LC-MS/MS assays of biomarker panels which use a surrogate matrix for calibration.

The objective in the second project was to develop and evaluate a novel, automatable sample preparation technique for demanding endogenous biomarker panels.

In both projects, a key step was the development of LC-MS/MS assays for biomarker profiling of clinically relevant small lipophilic compounds, and these two multi-analyte assays served as representative applications.

1.2. Development of a validation experiment for surrogate matrix calibrated LC-MS/MS biomarker assays

Although the use of SID should compensate for differences between the surrogate matrix used for calibration and the authentic matrix of the unknown samples (see section 1.1.2), for assay validation proof is needed regarding the suitability of the chosen surrogate matrix. Certified, i.e. metrologically traceable, controls or proficiency testing samples can be analysed to indirectly demonstrate the suitability of the surrogate matrix used as calibrators in the newly developed LC-MS/MS biomarker assays.

Unfortunately, no such certified controls typically exist for new biomarker candidates. This is especially true when it comes to complex biomarker panels; they are hardly obtainable. Consequently, there is a need for a specific validation experiment to evaluate the suitability of a surrogate matrix for its use as a basis for creating calibrators.

To make the authentic and surrogate matrices comparable, i.e. analyte-free, a SIL compound, not naturally occurring anywhere, was employed as the analyte, and the nonlabelled, endogenous compound was used as the internal standard. Since the roles of the analyte and its SIL analogue were inverted, we refer to the new validation experiment as the *Isotope Inversion Experiment*.

This *Isotope Inversion Experiment* consists of two parts: in the first part, the SIL compound is spiked into both the authentic and surrogate matrices to create *inverse calibrators*. Then, the resulting *inverse calibration curves* in the authentic and surrogate matrices were compared with respect to their linear regression.

In the second part of the experiment, *Inverse Quality Controls* were created in the authentic matrix and quantified by the *inverse calibration curve* established in the surrogate matrix. The calculated accuracy of these controls served as the decisive figure of merit for the whole *Isotope Inversion Experiment*, since this result reflects the circumstances of the *Standard Assay*, i.e. quantification of the unknown samples in the authentic matrix by a surrogate matrix calibration.

As a proof-of-concept application, the *Isotope Inversion Experiment* was successfully integrated into the validation process of a LC-MS/MS assay for a corticosteroid biomarker panel (for details, see section 2.1).

Thus, this newly developed validation experiment will be a valuable tool in the validation of LC-MS/MS methods for addressing new biomarkers (or panels) and has

the potential for facilitating the development of a specific validation protocol for LC-MS/MS biomarker assays.

1.3. Evaluation of *ferromagnetic particles based deproteination* as suitable sample preparation technique for demanding endogenous biomarker panels

Ferromagnetic particles are a very promising tool in analytics, since they can be handled as a suspension by pipetting but can also be manipulated by magnetic force so that they temporarily become a solid phase. Depending on their surface coating, these particles can be used for i) the extraction of analytes, ii) the removal of disturbing substances from a sample or iii) to deliver a reagent. These particles are widely used in immunoanalysers and play an important role in (automatic) nucleic acid extraction [21]. Since the handling of these particles requires no centrifugation, vacuum or pressure, they could also be a valuable tool for the convenient automation of sample preparation prior to LC-MS/MS analysis [22].

The second possible area of application for ferromagnetic particles mentioned above, i.e. the removal of interfering matrix components from human samples, involves a rather generic approach that is independent of the analyte's chemistry. Thus, the employment of ferromagnetic particles for sample preparation of heterogeneous biomarker panels prior to LC-MS/MS analysis seems very promising. Eicosanoids represent one group of demanding biomarkers. The analysis of these arachidonic acid metabolites is quite challenging due to their low levels in human body fluids, their instability and the possibility of their artificial formation during/after blood sampling. Furthermore, this group of potential biomarkers is characterised by broad physicochemical variety and many isobaric compounds [23]. In general, LC-MS/MS biomarker assays that address eicosanoids usually employ offline SPE for sample preparation. On the one hand, this technique offers a selection of analytes and the opportunity to concentrate them, but it also involves multiple time-consuming steps and is difficult to automate. To overcome these obstacles, we decided to evaluate the applicability of *ferromagnetic particle enhanced deproteination* in combination with online SPE as a semi-automated sample preparation technique for a biomarker assay addressing this group of demanding analytes. The prerequisite for this evaluation was the establishment of a respective analytical method. For this purpose, an LC-MS/MS assay for seven exemplary eicosanoids was successfully developed

and a thorough validation involving precision, accuracy, matrix effect, ruggedness and stability experiments was performed. The validation results were very satisfactory overall (compare section 2.2).

The results clearly demonstrate that *ferromagnetic particle enhanced deproteination* is a robust and automatable tool (in combination with online SPE), that is not only suitable for the LC-MS/MS analysis of xenobiotica but also for demanding biomarker panels.

1.4. Summaries

Summary in English

Due to its selectivity and versatility, liquid chromatography tandem mass spectrometry (LC-MS/MS) is a key technology in the analysis of complex biomarker panels. However, several issues hamper the significant advance of LC-MS/MS in routine laboratory medicine, e.g. limited automation possibilities and the lack of validation regulations and certified external controls.

Solutions were developed and investigated to address some of these issues within the scope of this research project: In the first project, a novel validation experiment to address the suitability of a surrogate matrix for calibrators and controls was developed. The feasibility of this experiment could be successfully demonstrated in the validation of a previously established biomarker marker assay for six corticosteroids. Based on these results, a substantial gap could be filled in the validation process of LC-MS/MS assays for endogenous compounds.

In the second project, *ferromagnetic particle enhanced deproteination* was evaluated as a potential automatable sample preparation technique for biomarker panels with a broad variety of physicochemical properties. As a demonstration application, an eicosanoid panel including arachidonic acid and six of its metabolites was selected. The validation results confirm that *ferromagnetic particle enhanced deproteination* in combination with online solid phase extraction is a suitable sample preparation technique for demanding biomarker panels. This generic and automatable sample preparation technique could represent an attractive and novel solution for LC-MS/MS assays for biomarker panels.

In summary, the two subprojects of this doctoral thesis deliver valuable solutions to advance the use of biomarker profiling via LC-MS/MS in clinical diagnostics.

Zusammenfassung - Summary in German

Aufgrund ihrer hohen Selektivität und großen Vielseitigkeit nehmen Flüssigkeitschromatographie-Tandemmassenspektrometrie(LC-MS/MS)-Methoden eine Schlüsselrolle beim Biomarker-Profiling ein. Nichtsdestotrotz gestaltet sich die Verwendung dieser Technik in der labormedizinischen Routineanalytik bis heute kompliziert. Dies liegt unter anderem an dem noch recht geringen Automatisierungsgrad der LC-MS/MS, am Fehlen von offiziellen

Validierungsrichtlinien sowie der Schwierigkeit, zertifizierte externe Kontrollen für neue Biomarkerpanele zu bekommen.

Im Rahmen dieser Doktorarbeit wurden Lösungen für einige der zuvor erwähnten Punkte erarbeitet: Das erste Projekt beinhaltete die Entwicklung eines neuen Validierungsexperiments um die tatsächliche Verwendbarkeit einer Surrogatmatrix-Kalibrierung für die Quantifizierung von nativen Realmatrix-Proben mit unbekanntem Gehalt zu testen. Dieses neue Experiment konnte erfolgreich in die Validierung eines ebenfalls neu entwickelten Biomarkerassays für sechs Corticosteroide eingebunden werden. Es ist hervorzuheben, dass dieses Experiment eine nennenswerte Lücke in der Validierung von LC-MS/MS Assays für endogene Substanzen schließt.

Ziel des zweiten Projekts war es herauszufinden, ob Magnetpartikel basierte Proteindepletion als Probenvorbereitung für anspruchsvolle Biomarkerpanele, welche Analyten mit einer großen Bandbreite physiko-chemischer Eigenschaften umfassen, geeignet ist. Als exemplarische Anwendung diente ein selbst entwickelter LC-MS/MS Assay für sieben Eicosanoide. Im Rahmen einer umfassenden Validierung konnte gezeigt werden, dass Magnetpartikel – in Kombination mit einer Online-Festphasenextraktion – für die Probenvorbereitung solcher komplexen Biomarkerpanele sehr gut geeignet sind. Somit stellt diese Art der Probenvorbereitung eine breit anwendbare und zudem automatisierbare Option für LC-MS/MS Assays dar.

Abschließend ist festzustellen, dass im Rahmen dieser Doktorarbeit wertvolle Lösungen entwickelt und erprobt wurden, um das LC-MS/MS basierte Biomarker-Profiling in der klinischen Diagnostik voran zu bringen.

2. ORIGINAL ARTICLES

2.1. Suhr, A. C., Vogeser M., Grimm, S. H. *Isotope Inversion Experiment evaluating the suitability of calibration in surrogate matrix for quantification via LC-MS/MS – Exemplary application for a steroid multi-method*. J Pharm Biomed Anal 2016; 124:309-18

2.2. Suhr A. C., Bruegel, M., Maier, B., Holdt, L. M., Kleinhempel, A., Teupser, D., Grimm, S. H., Vogeser, M. *Ferromagnetic particles as a rapid and robust sample preparation for the absolute quantification of seven eicosanoids in human plasma by UHPLC-MS/MS*. J Chromatogr B Analyt Technol Biomed Life Sci 2016, 1022:173-182

3. APPENDIX

3.1. List of abbreviations

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
NMR	nuclear magnetic resonance
GC	gas chromatography
MS	mass spectrometry
LC	liquid chromatography
SIL	stable isotope labelled
SID	stable isotope dilution
AD	Alzheimer’s disease
EMA	European Medicines Agency
FDA	Food and Drug Administration
CLSI	Clinical & Laboratory Standards Institute
UHPLC	ultra-high performance liquid chromatography
SPE	solid phase extraction

3.2. References

[1] K. Strimbu, J.A. Tavel, What are biomarkers?, Curr Opin HIV AIDS, 5 (2010) 463-466.
[2] D.I. Ellis, W.B. Dunn, J.L. Griffin, J.W. Allwood, R. Goodacre, Metabolic fingerprinting as a diagnostic tool, Pharmacogenomics, 8 (2007) 1243-1266.
[3] H.G. Gika, G.A. Theodoridis, R.S. Plumb, I.D. Wilson, Current practice of liquid chromatography-mass spectrometry in metabolomics and metabonomics, J Pharm Biomed Anal, 87 (2014) 12-25.
[4] E. Ciccimaro, I.A. Blair, Stable-isotope dilution LC-MS for quantitative biomarker analysis, Bioanalysis, 2 (2010) 311-341.
[5] S. Becker, L. Kortz, C. Helmschrodt, J. Thiery, U. Ceglarek, LC-MS-based metabolomics in the clinical laboratory, J Chromatogr B Analyt Technol Biomed Life Sci, 883-884 (2012) 68-75.
[6] A. Roux, D. Lison, C. Junot, J.F. Heilier, Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: A review, Clin Biochem, 44 (2011) 119-135.
[7] W. Arlt, M. Biehl, A.E. Taylor, S. Hahner, R. Libe, B.A. Hughes, P. Schneider, D.J. Smith, H. Stiekema, N. Krone, E. Porfiri, G. Opocher, J. Bertherat, F. Mantero, B. Allolio, M. Terzolo, P. Nightingale, C.H. Shackleton, X. Bertagna, M. Fassnacht, P.M. Stewart, Urine steroid metabolomics as a biomarker tool for detecting malignancy in adrenal tumors, J Clin Endocrinol Metab, 96 (2011) 3775-3784.
[8] M. Mapstone, A.K. Cheema, M.S. Fiandaca, X. Zhong, T.R. Mhyre, L.H. MacArthur, W.J. Hall, S.G. Fisher, D.R. Peterson, J.M. Haley, M.D. Nazar, S.A. Rich, D.J. Berlau, C.B. Peltz, M.T. Tan, C.H. Kawas, H.J. Federoff, Plasma phospholipids identify antecedent memory impairment in older adults, Nat Med, 20 (2014) 415-418.
[9] H. Luan, L.F. Liu, Z. Tang, M. Zhang, K.K. Chua, J.X. Song, V.C. Mok, M. Li, Z. Cai, Comprehensive urinary metabolomic profiling and identification of potential noninvasive marker for idiopathic Parkinson's disease, Sci Rep, 5 (2015) 13888.
[10] M. Vogeser, K.G. Parhofer, Liquid chromatography tandem-mass spectrometry (LC-MS/MS)--technique and applications in endocrinology, Exp Clin Endocrinol Diabetes, 115 (2007) 559-570.
[11] B. Shushan, A review of clinical diagnostic applications of liquid chromatography-tandem mass spectrometry, Mass Spectrom Rev, 29 (2010) 930-944.
[12] R.P. Grant, High throughput automated LC-MS/MS analysis of endogenous small molecule biomarkers, Clin Lab Med, 31 (2011) 429-441.
[13] N.C. van de Merbel, Quantitative determination of endogenous compounds in biological samples using chromatographic techniques, TrAC Trends in Analytical Chemistry, 27 (2008) 924-933.
[14] R. Houghton, C. Horro Pita, I. Ward, R. Macarthur, Generic approach to validation of small-molecule LC-MS/MS biomarker assays, Bioanalysis, 1 (2009) 1365-1374.
[15] EMA, Guideline on Bioanalytical Method Validation, 2011, European Medicines Agency, http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf, accessed February 2014.
[16] CLSI, *Liquid Chromatography-Mass-Spectrometry Methods; Approved Guideline,* CLSI document C62-A, Wayne, PA: Clinical and Laboratory Standards Institute, 2014.

[17] FDA, Guidance for Industry Bioanalytical Method Validation, 2001, U.S. Department of Health and Human Services, Food and Drug Administration, http://www.fda.gov/downloads/Drugs/Guidance/ucm070107.pdf, accessed February 2014.
[18] J.W. Lee, V. Devanarayan, Y.C. Barrett, R. Weiner, J. Allinson, S. Fountain, S. Keller, I. Weinryb, M. Green, L. Duan, J.A. Rogers, R. Millham, P.J. O'Brien, J. Sailstad, M. Khan, C. Ray, J.A. Wagner, Fit-for-purpose method development and validation for successful biomarker measurement, Pharm Res, 23 (2006) 312-328.
[19] D. Tsikas, A proposal for comparing methods of quantitative analysis of endogenous compounds in biological systems by using the relative lower limit of quantification (rLLOQ), J Chromatogr B Analyt Technol Biomed Life Sci, 877 (2009) 2244-2251.
[20] M. Vogeser, F. Kirchhoff, Progress in automation of LC-MS in laboratory medicine, Clin Biochem, 44 (2011) 4-13.
[21] N. Pamme, On-chip bioanalysis with magnetic particles, Curr Opin Chem Biol, 16 (2012) 436-443.
[22] K. Konig, S.F. Goethel, V.M. Rusu, M. Vogeser, Deproteination of serum samples for LC-MS/MS analyses by applying magnetic micro-particles, Clin Biochem, 46 (2013) 652-655.
[23] G. Astarita, A.C. Kendall, E.A. Dennis, A. Nicolaou, Targeted lipidomic strategies for oxygenated metabolites of polyunsaturated fatty acids, Biochim Biophys Acta, 1851 (2015) 456-468.

3.3. Publications

Articles

- J. Zander, B. Maier, A. Suhr, M. Zoller, L. Frey, D. Teupser, M. Vogeser, Quantification of piperacillin, tazobactam, cefepime, meropenem, ciprofloxacin and linezolid in serum using an isotope dilution UHPLC-MS/MS method with semi-automated sample preparation, Clin Chem Lab Med, 53 (2015) 781-791.
- A.C. Suhr, M. Vogeser, S.H. Grimm, Isotope Inversion Experiment evaluating the suitability of calibration in surrogate matrix for quantification via LC-MS/MS-Exemplary application for a steroid multi-method, J Pharm Biomed Anal, 124 (2016) 309-318.
- A.C. Suhr, M. Bruegel, B. Maier, L.M. Holdt, A. Kleinhempel, D. Teupser, S.H. Grimm, M. Vogeser, Ferromagnetic particles as a rapid and robust sample preparation for the absolute quantification of seven eicosanoids in human plasma by UHPLC-MS/MS, J Chromatogr B Analyt Technol Biomed Life Sci, 1022 (2016) 173-182.

Talks (with published abstracts)

- A.C. Suhr, B. Maier, M. Bruegel, A. Kleinhempel, D. Teupser, M. Vogeser. Ferromagnetic particles as a rapid and robust sample preparation for the absolute quantification of eicosanoids. MSACL 2015 EU, Salzburg, Austria, Sep 8th -11th, 2015. Abstract book page 54
- A.C. Suhr, M. Vogeser, S.H. Grimm. Isotope Inversion Experiment evaluating the suitability of calibration in surrogate matrix for quantification via LC-MS/MS – Exemplary application for a steroid multi-method. German Conference of Laboratory Medicine (DKLM), Mannheim, Sep 28th – 30th, 2016. Talk FV06

Posters (with published abstracts)

- A.C. Suhr, J. Briegel, D. Teupser, M. Vogeser. Sensitive 2D-UHPLC-MS/MS method for simultaneous quantification of seven corticosteroids to investigate adreno-cortical dysfunction in critical ill patients. MSACL 2014 EU, Salzburg, Austria, Sep 2nd -5th, 2014. Poster #40, Abstract book page 86
- A.C. Suhr, J. Briegel, D. Teupser, M. Vogeser. Adreno-cortical dysfunction in critically ill patients – Development of a sensitive 2D-UHPLC-MS/MS method for the simultaneous quantification of seven corticosteroids. German Conference of Laboratory Medicine (DKLM), Mannheim, Sep 24th – 27th, 2014. Clin Chem Lab Med: P099 (2014)
- A.C. Suhr, B. Maier, M. Bruegel, A. Kleinhempel, D. Teupser, M. Vogeser. Ferromagnetic particles as a rapid and robust sample preparation for the absolute quantification of eicosanoids. German Conference of Laboratory Medicine (DKLM), Leipzig, Oct 14th – 17th, 2015. Clin Chem Lab Med: P040 (2015)

3.4. Acknowledgement

First, I would like to express my gratitude to Michael Vogeser for his kind and competent supervision during my thesis, for his trust in my research and for giving me the ability to work on interesting projects.

I am also very thankful for the expert advice and moral support of Barbara Maier and Stefanie Grimm.

Further, I thank Daniel Teupser for the ability to do my PhD studies at the Institute of Laboratory Medicine and I would like to extend my thanks to all cooperation partners of my research projects.

I am very grateful for the financial support of my PhD studies by the Hans Fischer Society Munich.

Moreover, I love to say thank you to all colleagues at Laboratory Medicine for their various ways of support as well as for entertaining lunch times. In particular, I thank Bernd Northoff and Alexander Tolios for their scientific spirit.

My thanks also include Shafie Rooshani and his whole team at Herz-Apotheke Poing for keeping the pharmacist in me alive.

My very special thanks are dedicated to my friends and my family for cheering me up and supporting me whenever needed. I owe my deepest gratitude to my parents for constantly believing in me.

Last but not least I thank my beloved Florian who is always there for me – including inspiring discussions about mass spectrometry.