iC42

integrated solutions in systems biology
clinical research & development

Summary of Services

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Introduction

iC42 is a unique clinical research and development facility that combines quantitative mass spectrometry (drugs, drug metabolites, other small molecules and large molecules, endogenous compounds), metabolic and protein profiling technologies in a regulatory compliant environment under one roof. We are designed and uniquely qualified to carry out the bioanalytics for complex clinical trials involving drug quantification and molecular marker strategies. In addition, iC42 carries out research and development projects including: molecular marker discovery and qualification, translational research, strategy development and the identification of molecular mechanisms. Projects range from the development and validation of assays and strategies with only a few study samples and clinical therapeutic drug monitoring to serving as the central laboratory for phase III clinical trials with more than 50 clinical centers and more than ten thousand samples- from bioanalysis to strategic research partnerships. In addition, to research and bioanalytical services, activities include consulting and interactions with regulatory agencies.

iC42 is committed to advancing individualized medicine by examining the unique biology of an individual to assess truly personalized treatments. Other major *foci* are the evaluation of pediatric pharmacokinetics, drug metabolism and the development of clinical management tools for pediatric patients. In addition, we promote and enhance research in transplantation through the Colorado Center for Transplantation Care, Research and Education.

We are the source for all of your research and development needs and we look forward to collaborating with you,

Sincerely,
Uwe Christians MD, PhD, MRQA
iC42 Laboratory Director

About iC42

Clinical Research & Development provides integrated solutions to systems biology. iC42 is mainly a cutting-edge mass spectrometry laboratory. Our staff is a unique and complementary blend of highly successful academic researchers and bioanalytical specialists with decades of industry experience. We serve as a regulatory compliant laboratory under the umbrella of one of the leading Medical Schools in the United States, the University of Colorado Denver. This collaboration gives us full and instant access to its resources and expertise. Our scientists, who are highly qualified with strong academic track records and extensive industry experience, facilitate innovative projects and strategize developments that are far beyond the capabilities of most established Commercial Research Organizations. Thus, it is not surprising that iC42 Clinical Research & Development has developed into a global resource for "high-end' bioanalytics that currently collaborates with more than 40 entities including academic groups, clinical networks, biotech and pharmaceutical companies all over the world. Our unique facility is located at the Fitzsimons Bioscience Park and our services include:

- Highly Sensitive LC-MS/MS Bioanalytics
- Quantification of endogenous compounds and multiplexing technologies (metabolomics, proteomics)
- Molecular Marker Discovery, Qualification & Development
- Complex Assay Development & Validation
- GLP Compliant, CAP Accredited, CLIA Certified
- Quantification of Drugs & Metabolites
- Isolation of Drug Metabolites & Structural ID
- Clinical Therapeutic Drug Monitoring
- Complete PK/PD Package Managed Under One Roof
- Consulting & Strategic Research Partnership

Our State of the Art Facility is Located in Colorado

In September 2008, iC42 moved into a new facility in the Fitzsimons Bioscience East less than 20 min from Denver International Airport and occupies Suite 100. This suite comprises 7500 sqft of state-of-the-art space specifically designed for a biotech company that carries out pre-clinical drug development and clinical bioanalytics. Special features include FDA-compliant server and archive rooms (fire proof shell, independent security and access, halon extinguisher system, temperature and humidity control, motion sensors), magnet card access control and a dedicated server tracking movements, security system with motion sensors that alarms the police, monitoring of freezers, and a dedicated room for handling and storage of reference materials. Our facility has two main laboratories. One laboratory is used for quantitative mass spectrometry in a regulatory compliant environment, the other is dedicated to discovery and research including drug metabolism. The laboratories are clearly separated from the office space. All offices have ethernet access to Clinical Research & Development servers and the University of Colorado ethernet with all its resources including library access. The front door, like all other access doors are always kept locked, and access can only be granted by the receptionist. All visitors and contractors have to sign in, are issued visitor badges and cannot move in the facility without being accompanied by an authorized iC42 employee.

Our Dedicated Research and Service Team

To meet our team, please visit our website at www.bioanalytics.us.
We are also on Facebook: http://www.facebook.com/pages/IC42-Integrated-Solutions-in-Systems-Biology/192314414156199?sk=wall

1. Bioanalytical Services

1.1 Quantitative Bioanalytical Methods

Quantitative Bioanalytical Methods Compounds

Compound	Matrix	Lower Limit of Quantitation	Instrument
ADMA, Homocysteine, Arg	Plasma	0.3 µM	API 4000
Alfentanil	Plasma	0.1 ng/mL	API 5000
Aprotinin	Tissue (Rat Kidney)	80 ng/mL	ELISA
Biolimus	Blood, Tissue, Stents	0.005 ng/mL	API 5000
Cyclosporine / Metabolites	Blood	0.1-1.0 ng/mL	API 5000
DMXB	Plasma/Brains	0.1 ng/mL	API 5000
Duet DNA	Monocytes	Ratio	GC-MS
Everolimus	Blood	0.1 ng/mL	API 5000
Felbamate	CSF, Brain, Serum	0.1 ng/mL	API 5000
Fentanyl	Plasma/DBS	0.1 ng/mL	API 4000
Free-Fatty Acids	Plasma, Tissue, Blood	100 µM	GC-MS
GSH	Plasma	10 µM	API 4000
Glucocorticiods	Plasma	0.1 ng/mL	API 5000
[^{13}C] Glucose	Plasma	10 µM	GC-MS
[^{13}C] Glycerol	Plasma	10 µM	GC-MS
High Energy Phosphates	Tissues	0.25 µM	API 4000
Isoprostanes	Plasma, Urine	0.01 ng/mL	API 5000
Ketamine	Blood	1.0 ng/mL	UPLC-MS/MS
Ketarolac	Plasma	1.0 ng/mL	API 4000
Lamotrigine	Plasma	1.0 ng/mL	API 4000
Leflunomide	Blood	0.1 ng/mL	API 5000
Lidocaine	Plasma	0.5 ng/mL	API 4000
Lovastatin	Plasma	0.1 ng/mL	API 5000
Metabolic Profiling	Plasma, Urine, Tissue	_____	Exactive
Morphine / Metabolites	Plasma/DBS	1 - 2.5 ng/mL	API 5000
MPA	Plasma	1.0 ng/mL	API 4000
Naltrexone	Plasma	0.1 ng/mL	API 5000
Nicotine	Hair	0.1 ng/mL	API 5000

Quantitative Bioanalytical Methods

Compounds

Compound	Matrix	Lower Limit of Quantitation	Instrument
Pravastatin	Plasma	0.5 ng/mL	API 4000
Phenytoin	Plasma	0.1 ng/mL	API 5000
Phenytoin	Plasma	0.1 ng/mL	API 5000
PhIP	Plasma / Microsomes	0.1 ng/mL	API 4000
Propofol	Plasma	0.5 ng/mL	API 4000
Sirolimus	Tissues / Blood	0.01 ng/mL	API 4000
Steroid Hormones	Plasma	0.1 ng/mL	API 5000
Tacrolimus / Metabolites	Blood	0.1 ng/mL	API 4000
Temsirolimus / Metabolites	Blood	0.1 ng/mL	Exactive
Valproic Acid	Serum	1.0 ng/mL	GC/MS
Vitamin D and Metabolites	Plasma	0.1 ng/mL	API 5000
Paclitaxel	Blood	0.25ng/mL	API 5000
Methadone	Plasma/DBS	0.25ng/mL	API 4000

Quantitative Bioanalytical Methods
Amino Acids

Amino Acids	Matrix	Lower Limit of Quantitation	Instrument
Ala-Gin	Plasma	20nM/mL	API 5000
Alanine	Plasma	20nM/mL	API 5000
Arginine	Plasma	20nM/mL	API 5000
Asparagine	Plasma	20nM/mL	API 5000
Aspartic Acid	Plasma	20nM/mL	API 5000
(Cysteine) 2	Plasma	20nM/mL	API 5000
Citruline	Plasma	20nM/mL	API 5000
Glutamic Acid	Plasma	20nM/mL	API 5000
Glutamine	Plasma	20nM/mL	API 5000
Glycine	Plasma	20nM/mL	API 5000
Gly-Gin	Plasma	20nM/mL	API 5000
Histidine	Plasma	20nM/mL	API 5000
Hydroxyproline	Plasma	20nM/mL	API 5000
Isoleucine	Plasma	20nM/mL	API 5000
Lysine	Plasma	20nM/mL	API 5000
Mehtionine	Plasma	20nM/mL	API 5000
Mehtionine–d3*	Plasma	20nM/mL	API 5000
Ornithine	Plasma	20nM/mL	API 5000
Phenylalanine	Plasma	20nM/mL	API 5000
Proline	Plasma	20nM/mL	API 5000
Threonine	Plasma	20nM/mL	API 5000
Tryptophan	Plasma	20nM/mL	API 5000
Tryptohan-d5*	Plasma	20nM/mL	API 5000
Tyrosine	Plasma	_20nM/mL	API 5000

1.2 LC-MS/MS Quantification of Endogenous Compounds

Free Isoprostanes
Method : LC/LC-MS/MS
Status : fully validated
Matrices : human plasma, human urine, rat plasma and urine, tissue (partially validated)
Reference : Haschke M, Zhang YL, Kahle C, Klawitter J, Korecka M, Shaw LM, Christians U. Quantification of 15-F2t-isoprostane in human urine and plasma using high-performance liquid chromatography - atmospheric pressure chemical ionization-tandem mass spectrometry. Clin Chem 2007; 53:489-497.

Total Isoprostanes
Method : LC/LC-MS/MS
Status : partially validated
Matrices : human plasma, human urine, rat plasma and urine
Reference : N/A. Modification of the free isoprostane assay including release of bound isoprostanes using KOH human plasma, human urine, rat plasma and urine and extraction with affinity columns.

Vitamin D Profiling
VitD2, VitD3, 25(OH)VitD2, 25(OH)VitD3, 1(OH)VitD3, 1,25(OH)2VitD2 and 1, 25(OH)2VitD3
Method : LC/LC-MS/MS
Status : partially validated
Matrices : human plasma
Reference : ASMS presentation, publication in preparation

Steroid Hormones
Testosterone (total and free), estrogen (total)
Method : LC/LC-MS/MS
Status : partially validated
Matrices : human plasma
Reference : publication in preparation

In Preparation:
Endothelin, angiotensin, aldosterone

Endothelial Dysfunction Panel (LC-MS/MS)

- ADMA
- Arginine
- Homocysteine
- Cysteine
- Glutathione
- Isoprostanes
- Amino acid profiles in plasma
- Fatty acid profiles in blood cells
- HODE/ HETE (bioactive lipids)

The Identification of Unknown Molecular Mechanisms *In Vitro*, *In Vivo* and The Identification of Clinically Relevant Pharmacodynamic and Toxicodynamic Mechanisms in Trials

Modern technologies such as genomics/transcriptomics, proteomics, and biochemical profiling (metabolomics), allow for the detection and monitoring of early changes of cell signal transduction regulation and biochemistry with high sensitivity and specificity. Thus, they are able to detect disease processes and drug effects before histopathological and pathophysiological changes occur. Two major strategies can be differentiated, *i.e.*, non-targeted and targeted approaches. Non-targeted approaches try to non-selectively capture as much data as possible, thus reducing the risk of falsely negative, but increasing the risk of falsely positive results. Since non-targeted approaches are typically hypothesis generating, they are discovery tools. Development of molecular markers into a clinical tool or a tool for drug development typically follows several stages, namely, discovery using non-targeted and targeted approaches, identification of a lead, mechanistic verification of the molecular marker, validation of appropriate targeted assays, confirmation of appropriate sensitivity and specificity in clinical trials (qualification), and, if necessary, regulatory approval. An attractive strategy is the development of combinatorial molecular markers. These are molecular marker patterns that typically consist of five or more individual parameters, each of which usually must be changed in a specific direction to yield a significant result. In comparison to a single marker, it is reasonable to expect that the measurement of combinatorial molecular markers will result in better specificity and sensitivity. Metabolomic combinatorial markers become a special practical interest considering that the measurement of cell metabolites, in most cases, are validated and quantitative assays are already available.

It is reasonable to expect that the availability of specific and sensitive molecular markers will impact drug development as follows: *i*) faster and more efficient pre-clinical and early clinical development; *ii*) selection of lead drug candidates with a better therapeutic index; *iii*) earlier and better detection of toxic effects; *iv*) monitoring of pharmacodynamics and

toxicodynamics during preclinical and clinical development; *v*) development of more efficient and predictive animal models; *vi*) identification of mechanisms of actions and better understanding of cause-effect relationships; *vii*) access to indications that cannot be assessed by conventional strategies due to time limitations, such as chronic disease processes or disease prevention; *viii*) guidance of dose finding studies; *ix*) better long-term safety and efficacy; *x*) stratification of patient populations during clinical trials; *xi*) identification of "enriched" populations with better chance of therapeutic efficacy and tolerability; *xii*) diagnostic tools for clinical management of drugs in clinical practice.

For more details please see:

1. Christians U, Schmitz V, Klawitter J, Klawitter J. Proteo-metabolomic strategies in the future of drug development. In: The Applicability of Analytical Techniques to Clinical Studies. Caroli S, Záray G (eds.) John Wiley and Sons, Inc. New York (in press)
2. Christians U, Albuisson J, Klawitter J, Klawitter J. The role of metabolomics in the study of kidney diseases and in the development of diagnostic tools. In: Biomarkers of Kidney Disease. Edelstein C (ed.) Elsevier, San Diego (2010), pp. 39-100.
3. Christians U, McCrery S, Klawitter J, Klawitter J. The role of proteomics in the study of kidney diseases and in the development of diagnostic tools. In: Biomarkers of Kidney Disease. Edelstein C (ed.) Elsevier, San Diego (2010), pp. 101-176.
4. Christians U, Klawitter J, Klawitter J, Brunner N, Schmitz V. Biomarkers of immunosuppressant organ toxicity after transplantation- status, concepts and misconceptions. Expert Opin Drug Metabol Toxicol 2011; 7:175-200
5. Christians U, Klawitter J, Hornberger A, Klawitter J. How unbiased is non-targeted metabolomics and is targeted pathway screening the solution? Curr Pharm Biotechnol 2011 (in press)

Biochemical Profiling (Metabolomics)

The metabolome is a collection of all small molecule metabolites or chemicals (< 1500 da) that can be found in a cell, organ, or organism. Biochemical profiling or metabolomics is closely related to genomics and proteomics, but focuses on the small molecule, catabolic and metabolic products that arise from the interactions of the large macromolecules (DNA, RNA, proteins) and measures the downstream products of multiple protein, gene, and environmental interactions. The measurement of small molecules to assess disease processes and drug responses is a well established concept and has been an integral part of clinical chemistry, clinical practice, and drug development for more than a century. Current clinical chemistry practice is to quantify one or sometimes a few small molecules in one assay. In comparison, metabolomics can be viewed as ultra-high throughput clinical chemistry, assessing hundreds and sometimes thousands of metabolites in a single analytical run.

Technological advances in NMR and MS have opened a new chapter in biochemistry by introducing metabolomics as an approach to study metabolism and its regulation in relation to disease, genetic, and environmental factors. Since the metabolome is the most predictive of phenotype, metabolomics holds the promise to extensively contribute to the understanding of phenotypic changes as an organism's answer to disease, genetic changes, and nutritional, toxicological, environmental, and pharmacological influences. While it usually takes hours, days, and sometimes weeks for protein and mRNA expression to change in response to a challenge, metabolic responses can often be measured within seconds or minutes.

It has been argued that while transcriptomics and proteomics are important research tools, metabolic profiling will offer the greatest impact on the field of personalized health and as an outcomes parameter. One reason is that metabolomics reflects best the interaction between phenotype and environment. Other than genes and protein, metabolites are often tissue and species-independent (14). Metabolite markers are therefore attractive in terms of translating results during pre-clinical and from pre-clinical into clinical development.

Metabolomics strategies can be non-targeted or targeted. Non-targeted metabolomics describes the "unbiased" analysis of the metabolome by examination of metabolite patterns in different experimental groups with the subsequent classification of these patterns into a "fingerprint". "Unbiased" means that preferably the complete metabolite information is captured by using non-selective assay strategies. Samples can be classified if the metabolite fingerprints differ between groups allowing for sample clustering. In most cases, ^{1}H-NMR and mass spectrometry-based assays are used for metabolic

fingerprinting. In ^{1}H-NMR-based assays, the chemical shift and area-under-the-peak in mass-spectrometry-based assays, the mass-to-charge ratios (m/z) and the signal intensities are used to describe a specific fingerprint. If separation steps such as gas chromatography or high-performance liquid chromatography are used to separate compounds before detection; retention times provide additional information for indexing metabolites.

Important factors potentially introducing bias into a metabolomic analysis may include, but may not be limited to timing of sample collection, the sample collection procedure, sample processing, stabilization, stability and storage, extraction procedures, dilution of sample, type and number of analytical methods used, preferences of analytical assays for metabolites with certain physico-chemical properties, ion suppression (LC-MS), derivatization (GC-MS), sensitivity of the assay, range of reliable response and the ability to allow at least for semi-quantitative comparison.

iC42 has extensive experience with sample collection and handling for metabolomics studies and we have all major metabolomic profiling technologies available, including non-targeted NMR, GC-MS and LC-MS metabolmics assays as well as targeted and validated multiplexing assays, e.g. for lipids, fatty acids, bioactive lipids, leukotrienes, and prostaglandins. Based on our experience, a combination of targeted and non-targeted assays is the most potent approach for discovery studies.

A unique, robust and reliable approach established at iC42 is to screen for changes in known metabolic pathways using a set of validated, quantitative multiplexing LC-MS assays (targeted pathway screening, TAPAS). Instead of non-selectively screening for changes in metabolite patterns, TAPAS screens for changes in metabolic pathways. Since such assays are designed for specific groups of metabolites, TAPAS can cover a larger number of metabolic pathways including metabolites of a wide variety of physicochemical properties and concentration ranges and thus, although base on targeted assays, TAPAS may ultimately be less biased than current non-targeted metabolomics strategies.

Representative recent examples of metabolomics studies conducted at iC42:

1. Klawitter J, Gottschalk S, Hainz C, Leibfritz D, Christians U, Serkova NJ. Immunosuppressant neurotoxicity in rat brain models: oxidative stress and cellular metabolism. Chem Res Toxicol 2010; 23: 608-619.
2. Klawitter J, Haschke M, Kahle C, Dingmann C, Klawitter J, Leibfritz D, Christians U. Toxicodynamic effects of ciclosporin are reflected by metabolite profiles in the urine of healthy individuals after a single dose. Br J Clin Pharmacol 2010; 70: 241-251.
3. Gottschalk S, Cummins CL, Leibfritz D, Christians U, Benet LZ, Serkova NJ. Age and sex differences in the effects of the immunosuppressants cyclosporine, sirolimus and everolimus on rat brain metabolism. Neurotoxicol 2011; 32:50-57

1.3 Amino Acids

Method : LC-MALDI-TOF/MS
Status : fully validated
Matrices : human plasma
Reference : Armstrong M, Jonscher K, Reisdorph NA. Analysis of 25 underivatized amino acids in human plasma using ion-pairing reversed-phase liquid chromatography/time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 2007;21(16):2717-26.

Analytes:

Compound	Molecular Formula	Exact mass [M+H]	Extracted ion window	Low cal std (nM/mL)	High cal std (nM/mL)	S/N ratio (pM injected)	IS used for Quantitation
Taurine	C2H7N03S	126.0224	126.00-126.04	1.56	400	851 (125)	Glutamine-d5
Aspartic acid	C4H7N04	134.0453	134.01-134.05	1.56	400	52.5 (125)	Glutamine acid-d3
Hydroxyproline	C5H9N03	132.0660	132.03-132.07	1.56	400	1750 (125)	Glutamine-d5
Serine	C3H7NO3	106.0504	106.02-106.06	1.56	400	267 (125)	Glutamine acid-d3
Glycine	C2H5NO2	76.0398	76.01-76.05	25	3200	22.3 (3125)	Glutamine acid-d3
Glutamine-d*	C5H5D5N203	152.1000	152.05-152.15	NA	NA	637 (1000)	NA
Glutamine	C5H10N2O3	146.0769	147.04-147.08	25	3200	1450 (3125)	Glutamine-d5
Asparagine	C4H8N2O3	133.0613	133.03-133.07	1.56	400	94.9 (125)	Glutamine-d5
Threonine	C4H9NO3	120.0660	120.03-120.07	1.56	400	315 (125)	Glutamine acid-d3
Glutamic acid-d*	C5H6D3NO4	151.1000	151.05-151.15	NA	NA	15.2 (1000)	NA
Glutamic acid	C5H9NO4	148.0609	148.03-148.07	12.5	1600	714 (1562)	Glutamine acid-d3
Alanine	C3H7N02	90.0555	90.02-90.06	12.5	1600	345 (125)	Leucine-d10

Compound	Molecular Formula	Exact mass [M+H]	Extracted ion window	Low cal std (nM/mL)	High cal std (nM/mL)	S/N ratio (pM injected)	IS used for Quantitation
(Cysteine)2	C6H12N2O4S2	241.0316	241.01-241.05	1.56	400	1160 (125)	Methionine-d3
Citrulline	C6H13N3O3	176.1035	176.01-176.05	1.56	400	383 (125)	Glutamine-d5
Proline	C5H9NO2	116.0711	116.04-116.08	1.56	400	355 (125)	Glutamine-d5
Gly-Gln*	C7H13N3O4	204.2000	204.10-204.30	NA	NA	2560 (1000)	NA
Ala-Gln	C8H15N3O4	218.1140	218.08-218.12	1.56	400	508 (125)	Gly-Gln
Valine	C5H11NO2	118.0868	118.05-118.09	1.56	400	163 (125)	Leucine-d0
Methionine-d3*	C5H8DNO2S	153.1000	153.05-153.15	NA	NA	1810 (1000)	NA
Methionine	C5H11NO2S	150.0588	150.03-150.07	1.56	400	737 (125)	Methionine-d3
Tyrosine	C9H11NO3	182.0817	182.05-182.09	1.56	400	722 (125)	Leucine-d10
Isoleucine	C6H13NO2	132.1024	132.08-132.12	1.56	400	340 (125)	Leucine-d10
Leucine-d10*	C6H3D10NO2	142.2000	142.00-142.30	NA	NA	1450 (1000)	NA
Leucine	C6H13NO2	132.1024	132.08-132.12	1.56	400	228 (125)	Leucine-d10
Phenylalanine	C9H11NO2	166.0868	166.06-166.10	1.56	400	1010 (125)	Leucine-d10
Histidine	C6H9N3O2	156.0773	156.05-156.08	1.56	400	1090 (125)	Tryptophan-d5
Tryptophan	C11H12N2O2	205.0977	205.08-205.12	1.56	400	383 (125)	Tryptophan-d5
Tryptophan-d5*	C11H7D5N2O2	210.1000	210.00-210.30	NA	NA	1110 (1000)	NA
Arginine	C6H14N4O2	175.1195	175.09-175.13	1.56	400	1700 (125)	Tryptophan-d5
Ornithine	C6H12N2O2	133.0977	133.08-133.12	1.56	400	544 (125)	Tryptophan-d5
Lysine	C6H14N2O2	147.1133	147.09-147.13	1.56	400	448 (125)	Tryptophan-d5

* Internal standard

1.4 High Energy Phosphates (LC-MS)

Method : LC-MS
Status : fully validated
Matrices : Tissues
Reference : Klawitter J, Schmitz V, Klawitter J, Leibfritz D, Christians U. Development and validation of an assay for the quantification of 11 nucleotides using LC/LC-electrospray ionization-MS. Analytes Biochem. 2007 Jun15;365(2):230-9.

Analytes:

AMP (m/z= 346)

ADP (m/z= 426)

ATP (m/z= 506)

GDP (m/z= 442)

GTP (m/z= 522)

UDP (m/z= 403)

UTP (m/z= 483)

CDP (m/z= 402)

CTP (m/z= 482)

NAD (m/z= 662)

FAD (m/z= 784)

Internal Standard 6-aminohexyl-ADP (m/z= 525

1.5 Metabolic Profiling (GC-MS and LC-MS)

Method : GC-MS
Status : partially validated
Matrices : human and rat plasma and tissues
Reference : N/A : N/A

Analytes:

3OH-butyrate
Acetic acid
Alanine
Aminomalonate
Creatinine
Galactonic acid
Glucitol
Glucoronic acid
Glutamine
Glycine
Hippurate
Histidine
Indolacetate
Isocitrate
Isovalerate
Lactic acid
Oxalate
Proline
Pseudouridine
Pyruvic acid
Ribose
Serine
Succinate
Threonine
Tyrosine
Uric acid
Valine
Xylitol

Please note that only the major metabolites are listed. Typically, more than 100 metabolites can be detected within one GC-MS run, allowing either for quantitation or semi-quantitative comparison.

In addition, LC-TOF and LC-MS-TOF assays are set up and available. Metabolites can be identified using the METLIN / HMBD databases. However, it must be noted that due to the inherent problem of ion suppression in the electrospray source use of this data for semi-quantitative comparison may be limited.

1.6 Metabolic Profiling (^{1}H NMR)

Method : proton nuclear magnetic resonance spectroscopy (500, 600 and 900 MHz)
Status : partially validated
Matrices : human and rat plasma, urine and tissues
Reference : N/A

Analytes:

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
1	Leucine	0.94	22	●	●
2	Leucine	0.96	23	●	●
3	Valine	0.97	18	●	
4	Isoleucine	1.00	16	●	●
5	Valine	1.03	19	●	
6	(isobutyrate / 3oxo-isovalerate)	1.13	23.1		●
7	3-hydroxybutyrate	1.19	23	●	
8	Threonine	1.29	20.5	●	
9	Lactate	1.32	21.3	●	●
10	Lysine	1.43 + 1.47	22.8	●	
11	Alanine	1.46	17.5	●	●
12	Arginine	1.67	25.3	●	
13	Leucine	1.68	25.0	●	●
14	Leucine	1.69	41.0	●	
15	Leucine	1.69	40.2	●	
16	Lysine	1.70	27.6	●	
17	2-hydroxyglutarate	1.83	31.9		●
18	Lysine	1.87	31.5	●	

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
19	Arginine	1.87	29.3	●	
20	2-Hydroxyglutarate	1.98	31.5		
21	Acetate	2.02	23.4	●	●
22	Glutamate	2.08	28.3	●	
23	Glutamine	2.12	28	●	
24	Hydroxyglutarate	2.26	34.3		●
25	Glutamate	2.32	34.7	●	
26	Succinate	2.43	34		●
27	Glutamine	2.44	32.1	●	
28	2-Oxoglutarate	2.45	31.5		●
29	Glutathione (Glu) both froms	2.49	32.7	●	
30	Citrate	2.56	46.2		●
31	Dimethylamine	2.70	35.7		●
32	Citrate	2.71	46.2		●
33	Trimethylamine	2.87	45.7		●
34	GSSG (Cys) oxidized	2.95	39.9	●	
35	2-Oxoglutarate	2.97	36.8		●
36	Lysine	3.01	40.3	●	
37	Creatine	3.02	38.3	●	●
38	Creatinine	3.04	31.4	●	●
39	R-N+-$(CH_3)_3$ several signals	3.18 -3.27	53.1-55.5	●	●
40	Taurine	3.20	49.9	●	●
41	β-Glucose C2	3.23	75.3	●	●
42	Arginine	3.23	41.7	●	
43	Trimethylamine N-oxide	3.25	60.8	●	● 3.3 ppm

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
44	Phenylalanine	3.28	36.9		●
45	GSSG (Cys) oxidized	3.30	39.8	●	
46	Taurine	3.32	36.8	●	●
47	α, β-Glucose C4	3.40	70.8	●	●
48	β-Glucose C3/C5	3.48	77.1	●	●
49	α-Glucose C2	3.52	72.6	●	●
50	Glycine	3.54	42.8	●	●
51	Glutathione+Gln+Glu	3.69	55.7	●	
52	α-Glucose C3	3.69	73.9	●	●
53	α, β-Gglucose C6	3.72	61.9	●	●
54	Glutathione (Gly) both forms	3.75	44.7	●	
55	Alanine	3.75	51.7	●	●
56	α-Glucose C5	3.82	72.6	●	●
57	α, β-Glucose C6	3.87	61.9	●	●
58	Creatine	3.91	55.0	●	●
59	Hippurate	3.94	45.1		●
60	Creatinine	4.09	56.9		●
61	Lactate	4.09	69.7	●	●
62	β-Glucose C1	4.64	97.1	●	●
63	α-Glucose C1	5.21	93.3	●	●
64	Urea	5.80	------		●
65	Phenylalanine	7.31	130.5		●
66	Phenylalanine	7.34	128.0		●
67	Phenylalanine	7.41	129.9		●
68	Hippurate	7.50	129.9		●

1.6 Metabolic Profiling (^{1}H NMR)

No.	Metabolite	1H [ppm]	13C [ppm]	blood	urine
69	Hippurate	7.59	133.2		●
70	Hippurate	7.79	128.2		●
71	Fumarate	8.46	147.9		

1.7 High Energy Phosphates (^{31}P NMR)

Method : phosphorous nuclear magnetic resonance spectroscopy
Status : partially validated
Matrices : Tissues
Reference : N/A

Analytes:

- phosphomonoesthers (PME)
- phosphodiesters (PDE) (both precursors for membrane phospholipid metabolism)
- sugar phosphates (UDPG) phosphocreatine (PCr)
- NAD,
- NMP
- NDP
- NTP
- Anorganic phosphate (can be used for monitoring in vivo pH changes in perfused organs and cells)
- NMP, NDP and NTP: nucleotide mono, di and tri phosphates such as AMP, ADP, ATP etc.

Lipidomics can be defined as the comprehensive identification and quantification of all lipid molecular species in a biological system. Lipids are loosely defined as biological compounds that are generally hydrophobic in nature and soluble in organic solvents. Lipids serve as membrane components, as mediators in cell signaling and as fuel and energy storage and are of interest in areas such as cardiovascular disease, cancer, inflammation, and nutrition. Their distinct solubility properties often dictate their separate analysis in metabolomics experiments. iC42 assays for lipid profiling are based on ^{1}H-NMR, GC-MS and LC-MS/MS and, as aforementioned, include non-targeted screening as well as targeted, validated LC-MS/MS assays for bioactive lipids, isoprostanes, prostaglandins and leukotrienes.

1.8 Fatty Acid Profiling (GC-MS)

Method : GC-MS
Status : partially validated
Matrices : human and rat plasma and tissues
Reference : N/A

Analytes:

C12:0 dodecanoic acid (lauric acid)

C14:0 tetradecanoic acid (myristic acid)

C14:1 tetradecenoic acid (myristoleic acid)

C16:0 hexadecanoic acid (palmitic acid)

C16:1 hexadecenoic acid (palmitoleic acid)

C17:0 heptadecanoic acid (margaric acid) as internal standard

C18:0 octadecanoic acid (stearic acid)

C18:1 octadecenoic acid (oleic acid)

C18:2 octadecadienoic acid (linoleic acid)

C18:3 octadecatrienoic acid (linolenic acid)

C20:4 eicosatetraenoic acid (arachidonic acid)

C22:0 docosanoic acid (behenic acid)

C24:0 tetracosanoic acid (lignoceric acid)

1.9 Lipid Patterns (^{1}H NMR)

Method : proton nuclear magnetic resonance spectroscopy
Status : human and rat blood, plasma and tissues
Matrices : human and rat blood, plasma and tissues
Reference : N/A

Analytes:

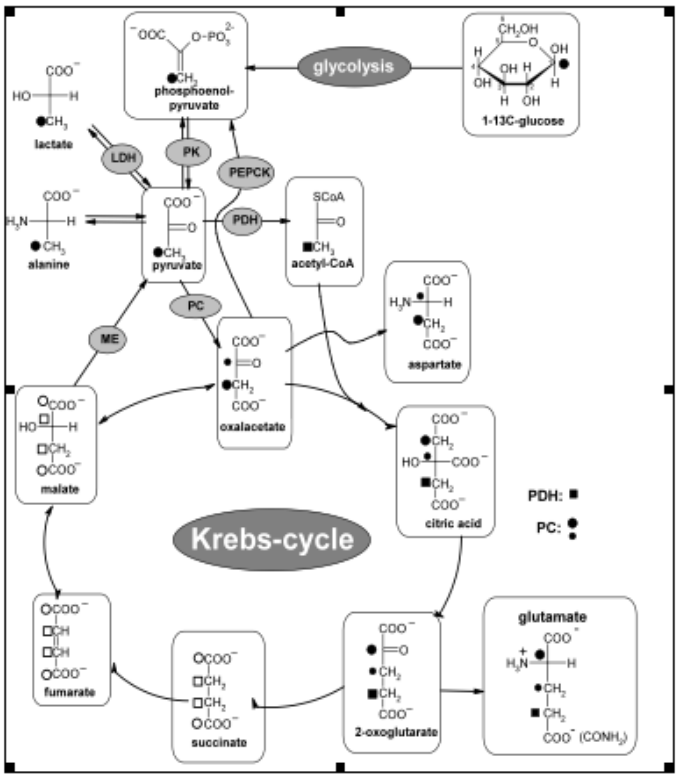
- poly- and mono-unsaturated fatty acids (PUFA and MUFA)
- total fatty acids
- triacylglycerols
- glycerophosphates
- phosphatidylcholine
- phosphatidylethanolamine
- cholesterol

1.10 ^{13}C-labeled tracers (^{13}C NMR)

A powerful non-biased, non-targeted screening metabolomics tool to identify unknown molecular mechanisms, is the assessment of fluxes in the metabolic network of a cell, organ, or organism: **Fluxomics**. By capturing the metabolome in its functional interactions with the environment and the genome, it provides a true dynamic picture of the phenotype. The most reliable strategy to do this is *via* ^{13}C-labeled substrates: the ^{13}C atoms are incorporated into the newly formed downstream metabolites in distinct numbers and specific positions allowing identification of distinct isotopomers and the evaluation of metabolite fluxes through specific pathways and the changes hereof as caused by drugs and disease processes.

Method	^{13}C nuclear magnetic resonance spectroscopy
Status	partially validated
Matrix	tissues, *ex vivo* perfused organs, perfused and extracted cell cultures
Reference	N/A

Analytes: Depending on ^{13}C labeled tracer used. For example in the case of 1-^{13}C-glucose:



Metabolic fate of ^{13}C-label from [1–^{13}C]glucose. Label distribution in glycolytic and tricarboxylic acid (TCA) cycle intermediates during metabolism of [1–^{13}C]glucose

1.11 Isotope Ratio Mass Spectrometry and Measurement of Isotope Enrichment Using GC-MS

Carbon Dioxide Enrichment

Method : Automated carousel and reference gas system in combination with a double-focusing sector field mass spectrometer
Status : partially validated
Matrices : breath and blood
Reference : N/A

Singly Labeled Water for Determination of Total Body Water and Doubly Labeled Water Enrichment for the Measurement of Total Energy Expenditure

Method : double-focusing sector field mass spectrometer with autosampler, an HD collector, dual inlet system, and an H/device for reduction of H_2O to H_2
Status : partially validated
Matrices : human and rat plasma
Reference : N/A

Labeled Glucose and Glycerol in Plasma

Method : GC-MS
Status : fully validated
Matrices : human and rat plasma
Reference : N/A

The assay was developed and validated using 6,6-d2-glucose and d5 glycerol. This method can be easily adapted to glucose and glycerol with other ^{13}C and deuterium labeled analogues.

Labeled Essential and Non-Essential Amino Acids

Method : GC-MS
Status : fully validated
Matrices : human and rat plasma
Reference : N/A

1-^{13}C-labeled Fatty Acids

Method : GC-MS
Status : fully validated
Matrices : human and rat plasma
Reference : N/A

Protein profiling (proteomics)

To date > 228,000 proteins and more than 300 different post-translational modifications have been described; however, the number of different components of the human proteome that has been estimated to add up to approximately 1 million, vastly exceeds the number of different genes in the human genome (20-40,000). The reasons include single nucleotide polymorphisms and post translational modification. The proteome is also constantly in flux. Post-translational modifications such as phosphorylation can be temporary and diseases can influence proteins.

Until the 1990's, enzymatic or chemical evaluation, such as Edman degradation of highly purified proteins, constituted the mainstream methods for the determination of amino acid sequences of polypeptides and proteins. Nowadays, MS in combination with library searches has evolved as the backbone of proteomics and allow for the simultaneous structural identification of multiple proteins in complex mixtures). MS is typically combined with a preceding separation or protein selection technology. This may include extraction of proteins by activated surfaces, removal of high abundance proteins, antibody and protein interactions, enrichment of the target cells, organelles or cell fractions, separation of 2D-gels and/or separation by 2D-HPLC. The removal of high-abundance proteins, e.g., from a plasma sample before further proteomic analysis is a common approach. But even if immunoaffinity columns are used, it is important to keep in mind that this also may remove proteins and peptides bound to high abundance proteins. Derivatization of proteins with isotope-coded affinity tags allows for direct semi-quantitative comparison of several proteomes and even for protein quantification. Proteomic approaches can broadly be separated into techniques that are based on separation and detection of the intact proteins (top-down proteomics) and techniques that involve digestion of the proteins into peptides, separation, MS detection of the peptides, and identification of the protein based on specific peptides (bottom-up proteomics).

iC42 has extensive experience in all relevant mass spectrometry-based proteomics technologies including labeling and targeted quantification of proteins.

Recent examples:

1. Klawitter J, Klawitter J, Kushner E, Jonscher KR, Bendrick-Peart J, Leibfritz D, Christians U, Schmitz V. Association of immunosuppressant-induced protein changes in the rat kidney with changes in urine metabolite patterns: A proteo-metabonomic study. J Proteome Res 2010; 9: 865-75

2. Klawitter J, Shokati T, Moll V, Christians U, Klawitter J. Effects of lovastatin on breast cancer cells: a proteo-metabonomic study. Breast Cancer Res 2010; 12(2):R16.
3. Klawitter J, Klawitter J, Gurshtein J, Corby K, Fong S, Tagliaferri M, Quattrochi M, Cohen I, Shtivelman E, Christians U. Bezielle (BZL101)-induced oxidative stress damage followed by redistribution of metabolic fluxes in breast cancer cells: a combined proteomic and metabolomic study. Int J Cancer 2011 (in press)

1.12 Non-Targeted Proteomics

Extraction, Separation and Analytical Technologies:

- 1D and 2D-gel electrophoresis
- Spot cutting, trypsination
- Immunoprecipitation
- Fractionation by semi-preparative HPLC
- 1D- and 2D HPLC, nano-LC, chromatography chip
- MALDI
- Protein identification: iontrap-MS spectrometry, quadrupole-time-of-flight mass spectrometry, triple stage quadrupole mass spectrometry, quadrupole- linear ion trap mass spectrometry in combination with nano-LC and database searches.

Labeling Technologies:

- SILAC (in combination with cell culture facility)
- iTRAQ

Qualification of Database Hits:

- Western blot
- PCR
- gene knock down (in combination with cell culture facility)

Proteomics Services

From sample preparation to generating high quality data for publication, in the following areas:

- Proprietary sample preparation technology
- 2D DIGE (2-Dimensional Differential In-Gel Electrophoresis) for detection of differential protein expression
- 2D Phosphoprotein and Glycoprotein profiling
- Protein identification by Mass Spectrometry
- 2D membrane service and fluorescent 2-D Western Blot
- Serum Proteomics

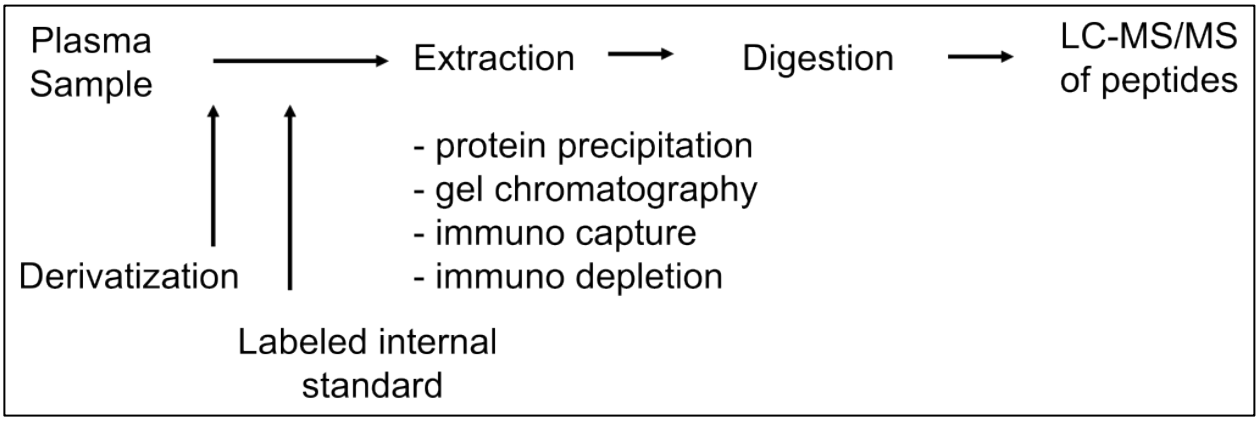
Our dedicated service team and cutting-edge technology platforms offer the following:

- Customized study design and sample preparation

- High sensitivity: 0.2ng / spot
- High accuracy: in-gel analysis of up to 3 samples & cross-gel analysis of > 3 samples
- Fast turnaround time: one week
- Cost-effectiveness: price covers experimental design & sample preparation
- High-quality data: ready for publication and presentation

1.13 Targeted Quantification of Peptides and Proteins

General strategic approach:



All assays were developed as part of contract research and further details cannot be revealed.

1.14. Drug Metabolism Studies

iC42 offers a range of drug metabolism services (please see below). These studies are carried out in compliance with applicable FDA guidances following the "fit for purpose" principle. These include:

- In vitro drug metabolism studies
- Identification of metabolite structures
- Generation and isolation of pure metabolites (please also see 1.15.)
- Quantification of metabolites drug metabolites in animal toxicokinetic studies
- Quantification of drug metabolites in clinical trials and pharmacokinetics
- Clinical drug-drug interaction studies
- Pediatric drug metabolism

In vitro drug metabolism services:

(I) Interspecies Comparison of In Vitro Metabolism: Metabolic profiling of different species using liver slices, hepatocytes and microsomes, cytochrome P450 phenotyping, cytochrome P450 inhibition, and cytochrome P450 induction, and if necessary, metabolism by hepatic cytosol, hepatic mitochondrial fractions and evaluation of phase II metabolism.

(II) Metabolic Pathway Identification (Reaction Phenotyping): Identification of cytochrome P450 and other enzymes involved in metabolism of new medications using expressed isolated cytochrome P450 and/or other drug metabolizing enzymes, specific inhibitory antibody and specific chemical inhibitors.

(III) Cytochrome P450 Inhibition: Estimation of apparent inhibition constants (Ki) and half-maximal inhibition constants (IC_{50}) values.

(IV) Induction of cytochrome P450 enzymes: Evaluation of the potential induction of cytochromes by a new compound.

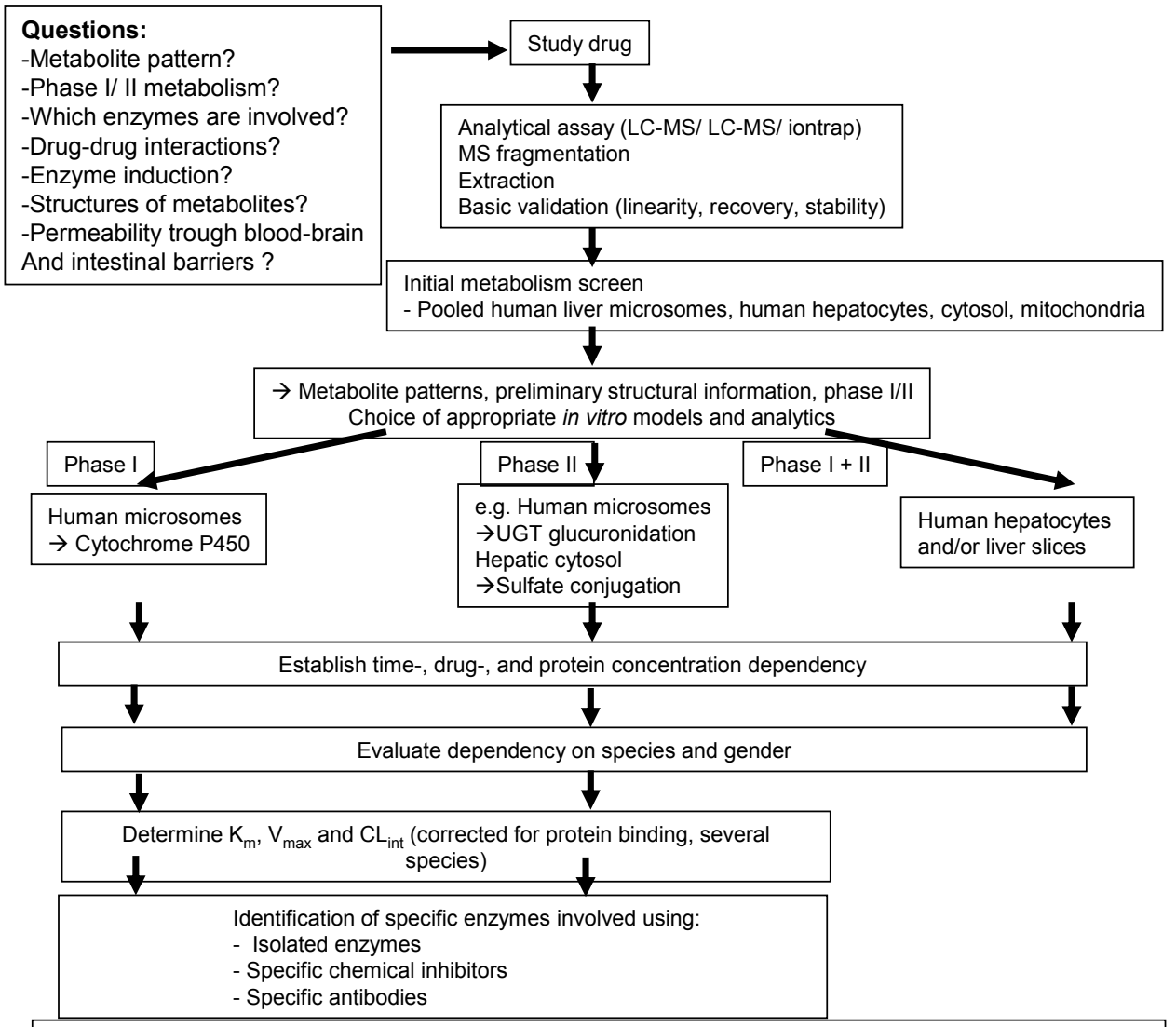
(V) Metabolite Identification: Isolation of drug metabolites, identification of metabolite structures using mass spectrometry and NMR spectroscopy, development and validation of LC/MS, LC/MS/MS or GC/MS assays for quantification of parent drugs and metabolites in blood and tissues, analysis of study samples from pharmacokinetic studies.

(VI) Metabolite stability: Metabolism of a new medication using hepatic microsomes or hepatocytes from a variety of species for metabolite stability and estimate of intrinsic clearance (Cl_{int}).

(VII) In vitro permeability studies: Determine the intestinal permeability and/or blood-brain barrier permeability of new compounds.

The investigators have extensive experience and expertise in the field of drug metabolism, analytics, pharmacokinetics, toxicology, and FDA drug approval procedures. Our laboratory is CAP accredited, and cGLP compliant and has a full DEA license as well as an accredited animal facility.

Overview Typical Strategic Approach for Drug Metabolism Studies for a New Drug Candidate. Please note that this approach may be changed by the study director/ principal investigator based on the nature of the test article and based on scientific reasoning.



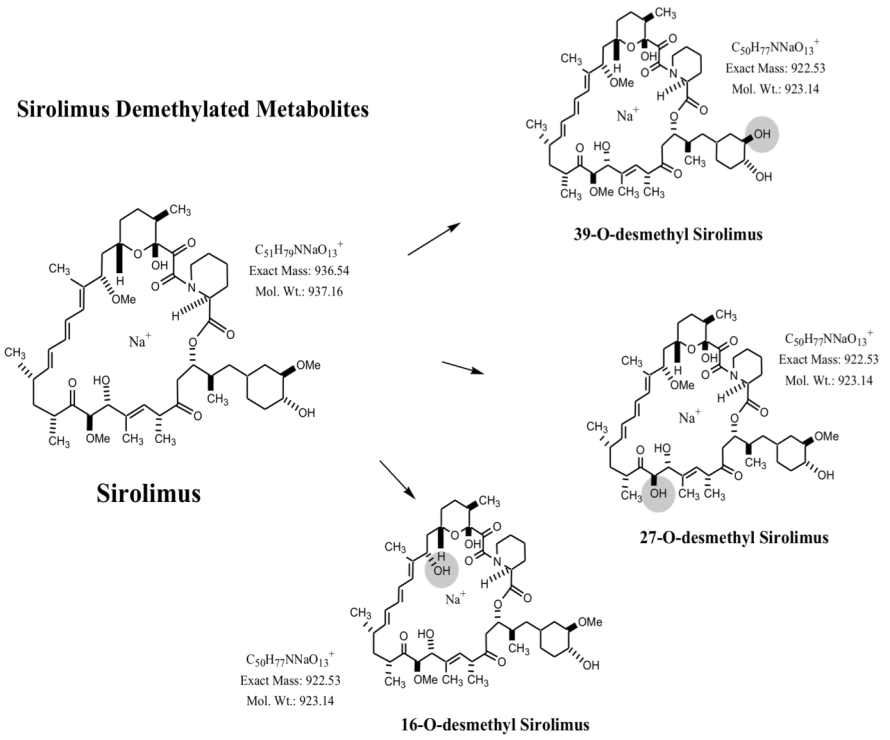
Drug-drug interactions (inhibition, inhibitors will be selected on the basis of results above):
-Effect of study drugs on metabolism of other drugs
-Effect of other drugs on metabolism of study drug
-Mechanism-based inhibition by study drug

Drug-drug interactions (induction):
→ Western blotting, metabolism of specific CYP probes, gene expression → gene arrays (if necessary)
- Human hepatocytes
- After treatment of rats and isolation of liver microsomes

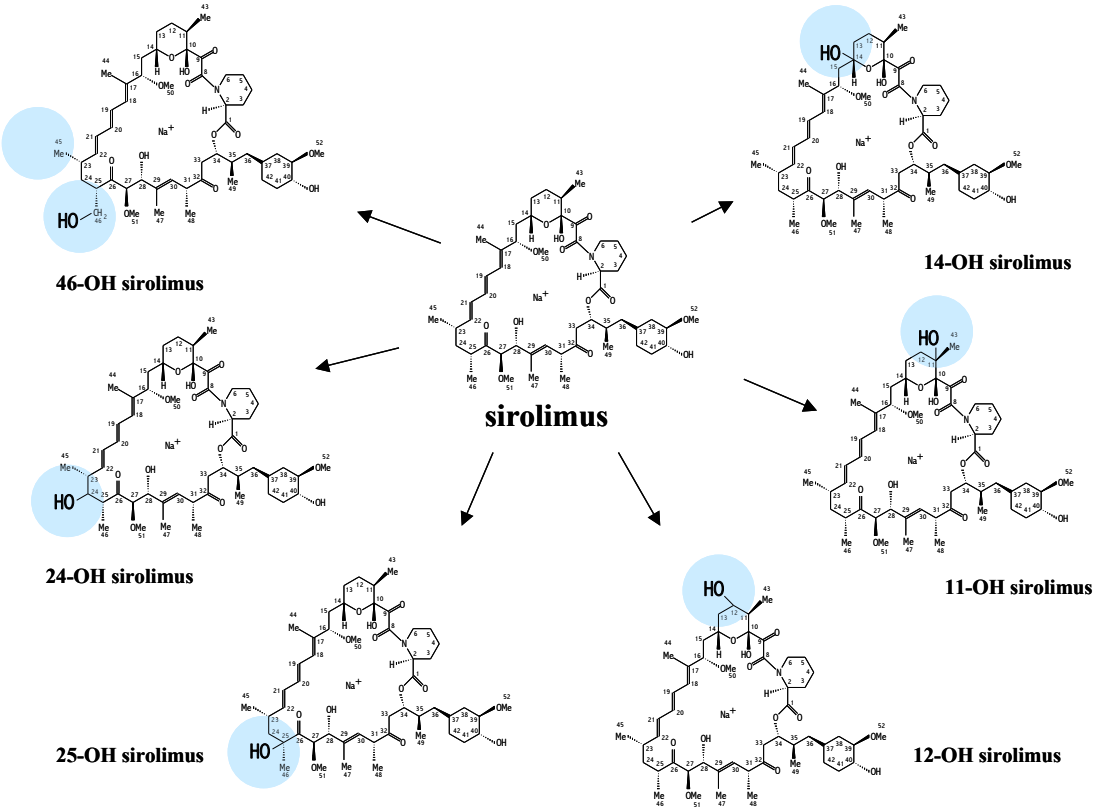
Structural identification of metabolites:
-If necessary: upscale production of metabolites (microsomes, animals, bacterial culture and preparative HPLC)
-Establish quantity and purity of metabolites (mass spectrometry, HPLC/UV)
-Mass spectrometry (MS/MS, MS/ion trap, MS/time-of-flight, validate fragment structures→ HR/MS, H/D exchange)
-Mono- and heteronuclear, one- and two-dimensional high-resolution NMR, LC/NMR

Develop and validate LC/MS/MS or GC/MS assays of parent and metabolites
-High-throughput assays for animal studies and samples from other experimental sources
(required for enzyme kinetics, drug-drug interaction and permeability studies)

1.15 Purified and Certified Immunosuppressant Metabolites



Sirolimus hydroxylated metabolites



Sirolimus

Atom numbering follows the IUPAC nomenclature

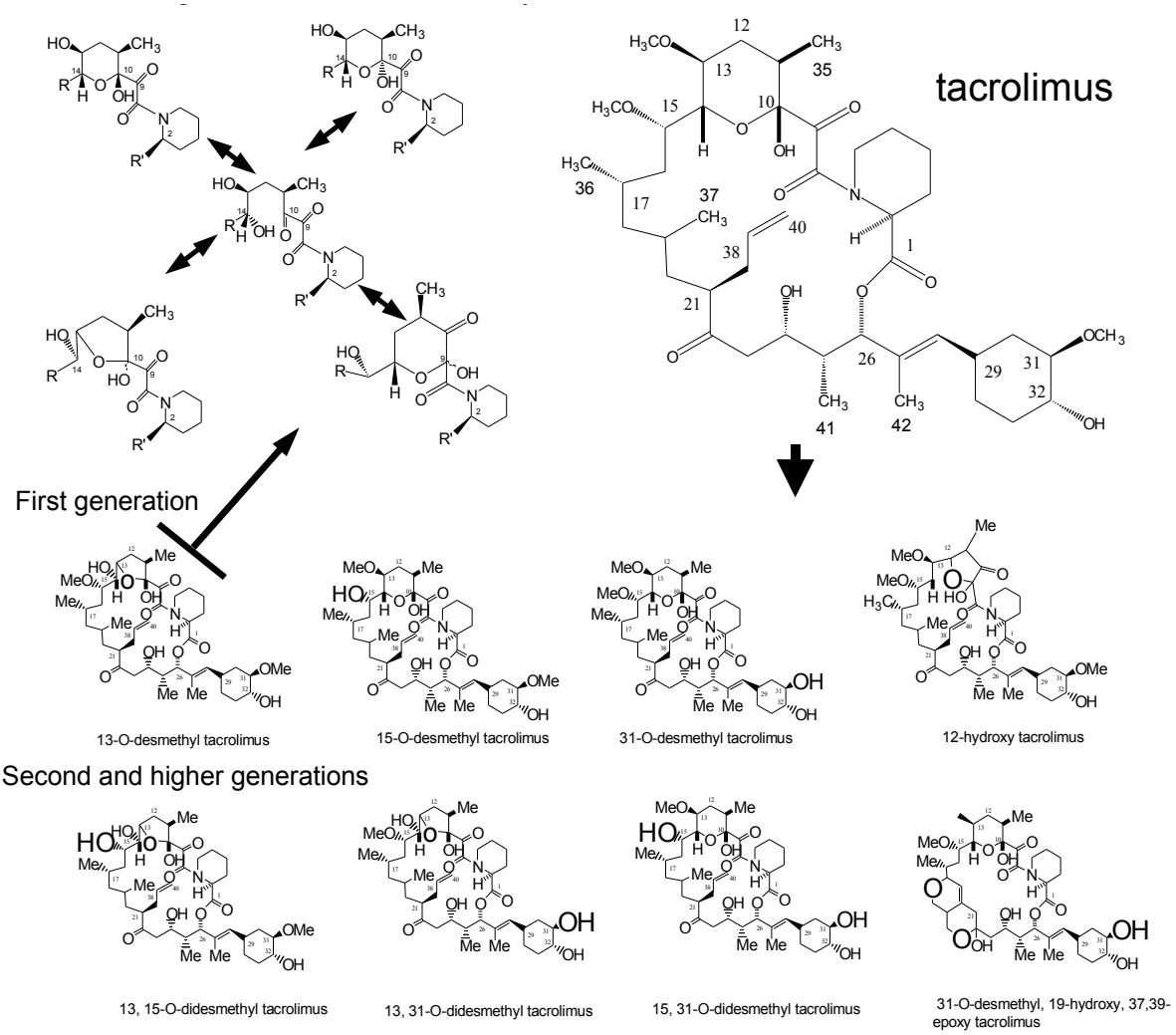
All 10-50 µg/ batch

- 12-hydroxy sirolimus
- 24-hydroxy sirolimus
- 25-hydroxy sirolimus
- 46-hydroxy sirolimus
- Piperidine hydroxy sirolimus
- 49-hydroxy sirolimus
- 16-O-desmethyl sirolimus
- 27-O-desmethyl sirolimus
- 39-O-desmethyl sirolimus
- Selected didesmethyl sirolimus Metabolites

Publications:

1. Christians U, Sattler M, Schiebel HM, Kruse C, Radeke HH, Linck A, Sewing KF. Isolation of two immunosuppressive metabolites after in vitro metabolism of rapamycin. Drug Metab Dispos 1992, 20: 186-191
2. Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF, Cytochrome P4503A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. Drug Metab Dispos 1992; 20: 753-761
3. Streit F, Christians U, Schiebel HM, Napoli KL, Ernst L, Linck A, Kahan BD, Sewing KF. Sensitive and specific quantification of sirolimus (rapamycin) and its metabolites in blood of kidney graft recipients by HPLC/electrospray-mass spectrometry. Clin Chem 1996; 42: 1417-1425
4. Streit F, Christians U, Schiebel HM, Meyer A, Sewing KF. Structural identification of four metabolites of the macrolide immunosuppressant sirolimus after in vitro metabolism by electrospray-MS/MS. Drug Metab Dispos 1996; 24: 1272-1278
5. Lampen A, Zhang Y, Hackbarth I, Benet LZ, Sewing KF, Christians U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. J Exp Pharmacol Ther 1998; 285: 1104-1112.
6. Jacobsen W, Serkova N, Hausen B, Morris RE, Benet LZ, Christians U. Comparison of the in vitro metabolism of the immunosuppressants sirolimus and RAD. Transplant Proc 2001; 33: 514-515
7. Filler G, Bendrick-Peart J, Strom T, Zhang YL, Johnson G, Christians U. Characterization of sirolimus metabolites in pediatric solid organ transplant recipients. Pediatr Transplant. 2009, 13; 44-53

1.15. Purified and Certified Immunosuppressant Metabolites (continued)



Structure of tacrolimus (A), major metabolic pathways (B), and structural isomers of 13-O-demethyl tacrolimus (C).

Tacrolimus

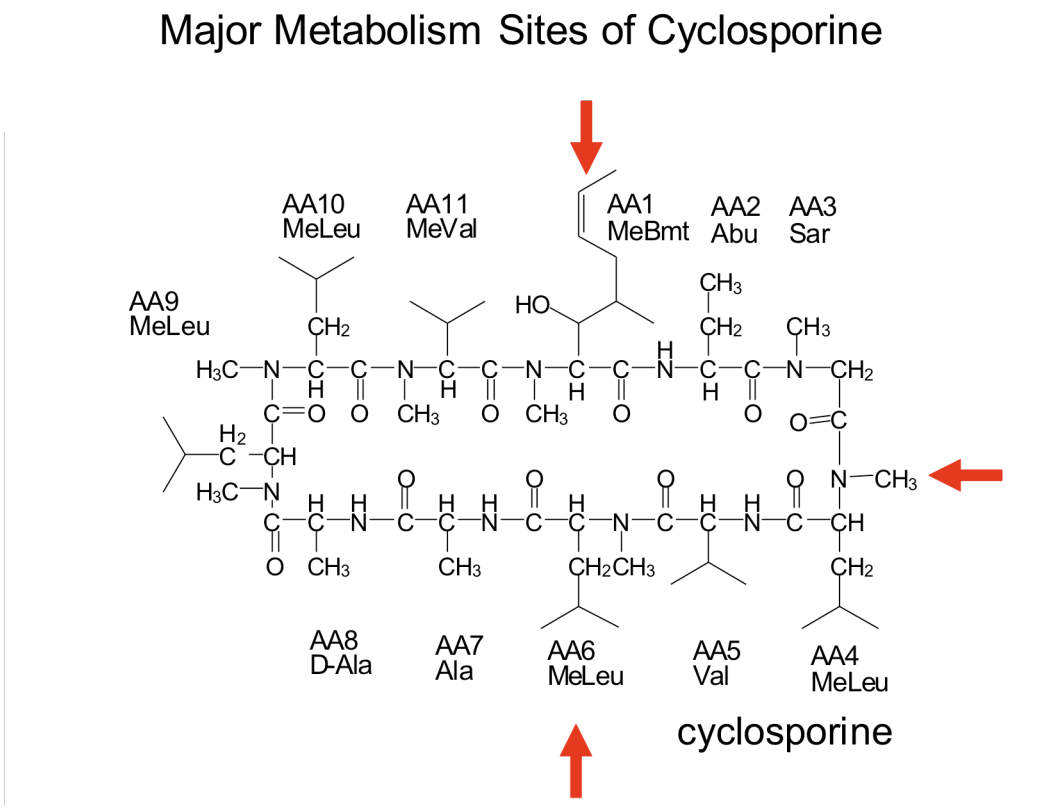
Atom numbering follows the IUPAC nomenclature

- 13-O-desmethyl tacrolimus: 250-500 µg
- 15-O-desmethyl tacrolimus: 50-100 µg
- 31-O-desmethy tacrolimus: 50-100 µg
- 12-hydroxy tacrolimus: 10-25 µg
- 13,31-di-O-desmethyl tacrolimus: 10-25 µg
- 15,31-di-O-desmethyl tacrolimus: 10-25 µg
- 13,15-di-O-desmethyl tacrolimus: 10-25 µg
- 31-O-desmethyl and ring formation between C19, C36 and C37: 10-25 µg

Publications:

1. Christians U, Radeke HH, Kownatzki R, Schottmann R, Sewing KF. Isolation of an immunosuppressive metabolite of FK506. Clin Biochem 1991; 24: 271-275
2. Christians U, Braun F, Schmidt M, Kosian N, Schiebel HM, Ernst L, Winkler M, Kruse C, Linck A, Sewing KF. Specific and sensitive measurement of FK506 and its metabolites in blood and urine of liver graft recipients. Clin Chem 1992; 38: 2025-2032
3. Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF, Cytochrome P4503A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. Drug Metab Dispos 1992; 20: 753-761
4. Schüler W, Christians U, Schmieder P, Schiebel HM, Holze I, Sewing KF, Kessler H. Structural identification of 13-demethyl-FK506 and its isomers generated by in-vitro metabolism of FK506 using human liver microsomes. Helv Chim Acta 1993; 76: 2288-2302
5. Lampen A, Christians U, Guengerich FP, Watkins P, Kolars JC, Bader A, Dralle H, Hackbarth I, Sewing KF. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions and interindividual variability. Drug Metab Dispos 1995; 23: 1315-1324

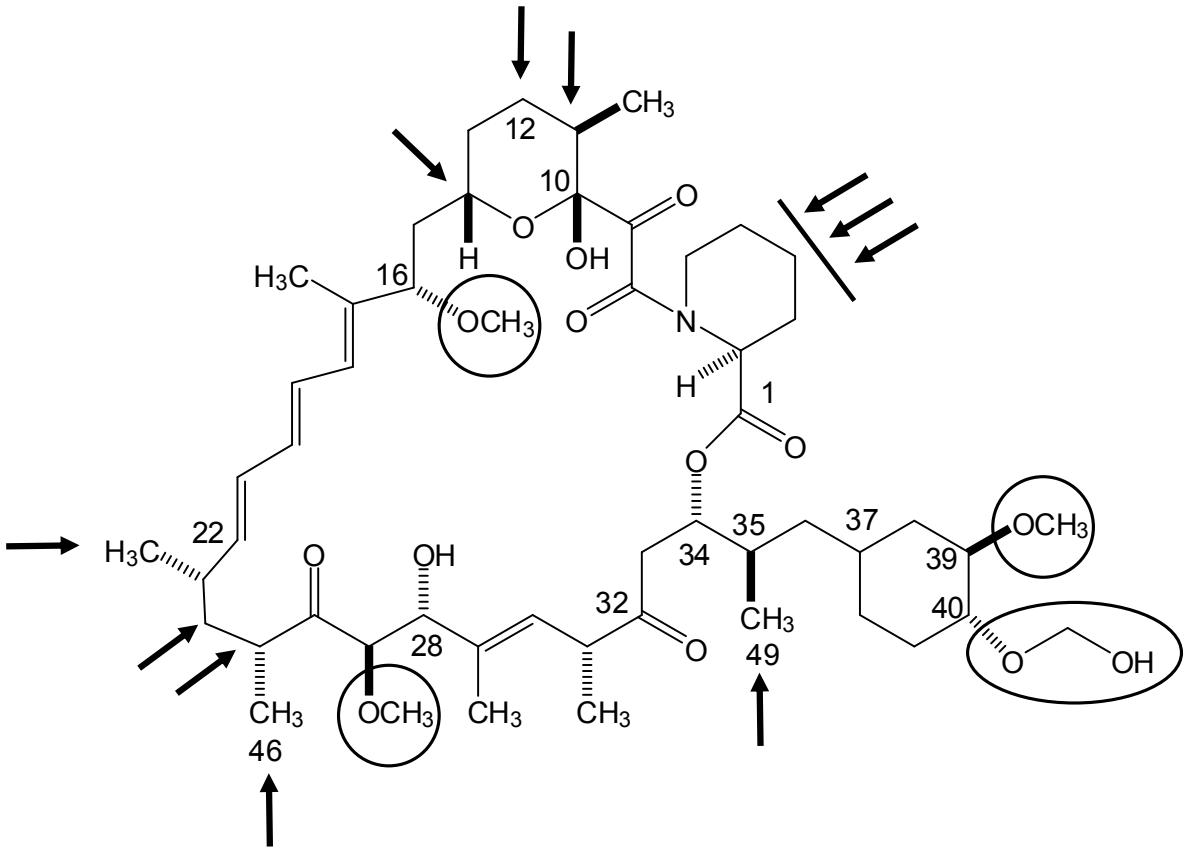
1.15. Purified and Certified Immunosuppressant Metabolites (continued)



Publications:

1. Christians U, Strohmeyer S, Kownatzki R, Schiebel HM, Bleck JS, Greipel J, Kohlhaw K, Schottmann R, Sewing KF. Investigations on the metabolic pathways of cyclosporine: I. Excretion of cyclosporine and its metabolites in human bile- Isolation of 12 new cyclosporine metabolites. Xenobiotica, 21 (1991) 1185-1198
2. Christians U, Strohmeyer S, Kownatzki R, Schiebel HM, Bleck J, Kohlhaw K, Schottmann R, Sewing KF. Investigations on the metabolic pathways of cyclosporine: Elucidation of the metabolic pathway in vitro by human liver microsomes. Xenobiotica, 21 (1991) 1199-1210
3. Christians U, Sewing KF. Ciclosporin metabolism in transplant patients. Pharmacol Ther, 57 (1993) 291-345
4. Christians U, Sewing KF. Alternative cyclosporine metabolic pathways and toxicity. Clin Biochem, 28 (1995) 547-559
5. Lampen A, Christians U, Bader A, Hackbarth I, Sewing KF. Drug interactions and interindividual variability of ciclosporin metabolism in the small intestine. Pharmacology 52 (1996) 159-168

1.15. Purified and Certified Immunosuppressant Metabolites (continued)



Everolimus

Numbering follows the IUPAC nomenclature, the arrows indicate hydroxylation positions, circles O-dealkylation.

All 10-50 µg/ batch

- 12-hydroxy everolimus
- 24-hydroxy everolimus
- 25-hydroxy everolimus
- 46-hydroxy everolimus
- Piperidine hydroxy everolimus
- 49-hydroxy everolimus
- 16-O-desmethyl everolimus
- 27-O-desmethyl everolimus
- 39-O-desmethyl everolimus
- Selected didesmethyl everolimus metabolites
- Selected dihydroxy everolimus metabolites
- seco everolimus and other selected degradation products

Publications

1. Jacobsen W, Serkova N, Hausen B, Morris RE, Benet LZ, Christians U. Comparison of the in vitro metabolism of the immunosuppressants sirolimus and RAD. Transplant Proc 2001; 33: 514-515
2. Strom T, Haschke M, Boyd J, Roberts M, Arabshahi L, Marbach P, Christians U. Cross reactivity of the major everolimus metabolites with the Innofluor Certican immunoassay for therapeutic drug monitoring of everolimus. Ther Drug Monit 2007 29:743-749.
3. Strom T, Haschke M, Bendrick-Peart J, Boyd J, Roberts M, Arabshahi L, Marbach P, Christians U. Everolimus metabolite patterns in the blood of kidney transplant patients. Ther Drug Monit 2007; 29: 592-599

1.16. Drug Eluting Stents

Angioplasty is the mechanical widening of a narrowed or totally-obstructed blood vessel. These obstructions are often caused by atherosclerosis. At first, angioplastic balloons were used to widen coronary arteries. Unfortunately, a small percentage of arteries collapsed immediately after the balloon was deflated. Secondly, a substantial portion of arteries began to close up again, a process called restenosis. Restenosis proved to be the body's response to the 'controlled injury' of angioplasty, similar to a scar forming over an injury, rather than a recurrence of coronary artery disease.

In the 1980's and 90s, metal devices called 'stents' were developed to assist cardiologists to overcome the balloon angioplasty related issues. These metal mesh-like tubes eliminated many of the complications of abrupt artery collapse, but restenosis persisted. Although the restenosis occurrence improved to about 25% of the cases, bare metal stents still experienced reblocking, necessitating a new surgical procedure typically at six-months.

In order to further reduce the process of restenosis after angioplasty, the next generation stents consist of a regular metal stent that is coated with a drug that is known to interrupt the biological processes that cause restenosis. In the clinical data gathered so far, these so called drug-eluting stents appear to reduce restenosis to the single digits. There are three major components to a drug-eluting stent:

- Type of stent that carries the drug coating
- Method by which the drug is delivered/eluted by the coating to the arterial wall
- The drug itself – how does it act in the human body to prevent restenosis?

iC42 has more than 15 years of experience in the development of drug eluting stents and provides a complete range of innovative capabilities to support their development:

- Quantification of drugs, their metabolites and degradation products in blood, tissues and on stents
- Drug stability testing in forced degradation studies and on stents (production process and aging) following ICH guidelines
- Structural identification of drug degradation products
- Matrix degradation profiling
- Elution testing from stents
- Lot release testing

- Drug development for new drugs including:
  a. Drug metabolism studies including structural identification and activity testing of drug metabolites
  b. Drug distribution studies
  c. Drug-drug interaction studies
  d. Animal toxicology
  e. Phase I clinical trials (single ascending and multiple ascending dose studies)
  f. Bioanalysis and pharmacokinetic analysis for clinical trials after stent implantation

- Consulting, interactions with regulatory agencies and strategy development

iC42 has extensive experience in the development and validation of LC/LC-MS/MS assays with ultra-high sensitivity for the quantification of drugs eluted from stents in a cGLP/ CAP-accredited environment.

Depending on the drug, assays with lower limits of quantitation of less than 10 pg/mL in whole blood and lower limits of detection of less than 1 pg/mL have been developed and successfully been utilized for clinical trials.

Representative Publications:

2. Ikonen TS, Gummert JF, Serkova N, Hayase M, Honda Y, Kobayase Y, Hausen B, Yock PG, Christians U, Morris RE. Efficacies of sirolimus (rapamycin) and cyclosporine in allograft vascular disease in non-human primates: trough levels of sirolimus correlate with inhibition of progression of arterial intimal thickening. Transpl Int 2000; 13 Suppl 1: S314-20.
3. Zhang YL, Bendrick-Peart J, Strom T, Haschke M, Christians U. Development and validation of a high-throughput assay for quantification of the proliferation inhibitor ABT-578 using LC/LC-MS/MS in blood and tissue samples. Ther Drug Monit 2005; 27: 770-778.
4. Ostojic M, Sagic D, Jung R, Zhang YL, Nedeljkovic M, Mangovski L, Stojkovic S, Debeljacki D, Colic M, Beleslin B, Milosavljevic B, Orlic D, Topic D, Karanovic N, Paunovic D, Christians U; NOBORI PK Investigators. The pharmacokinetics of Biolimus A9 after elution from the Nobori stent in patients with coronary artery disease: the NOBORI PK study. Catheter Cardiovasc Interv. 2008; 72: 901-908.
5. Clavijo C, Storm T , Moll V , Betts R, Zhang YL, Christians U, Bendrick-Peart J. Development and validation of a semi-automated assay for the highly sensitive quantification of Biolimus A9 in human whole blood using high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877: 3506-3514.
6. Steudel W, Dingmann C, Zhang YL, Bendrick-Peart J, Clavijo C, Shulze J, Betts R, Christians, U. A randomized, double-blind, placebo-controlled, single intravenous dose escalation study to evaluate the safety, tolerability and pharmacokinetics of the novel coronary smooth muscle cell proliferation inhibitor Biolimus A9 in healthy individuals. J Clin Pharmacol 2011; 51: 29-39.
7. Tada N, Virmani R, Grant G, Bartlett L, Black A, Clavijo C, Christians U, Betts R, Savage D, Su S, Shulze J, Kar S. Polymer-free biolimus A9 coated stent demonstrates more sustained intimal inhibition, improved healing and reduced inflammation in comparison to a polymer-coated sirolimus eluting Cypher stent in a porcine model. Circulation Cardiovasc Interv 2010; 3:174-183.
8. Ostojic MC, Perisic Z, Sagic D, Jung R, Zhang YL, Bendrick-Peart J, Betts R, Christians U. The pharmacokinetics of Biolimus A9 after elution from the BioMatrix II stent in patients with coronary artery disease: The Stealth PK study. Eur J Clin Pharmacol 2011; 67: 389-398.

2. Instruments and Software for Molecular Marker Discovery, Qualification and Quantification

2.1 Instruments

iC42 owns or has access to the following instruments

GC/MS
Agilent GC-MSD

Single Stage Quadrupole Instruments
Agilent Mass Selective Detector

Triple Stage Quadrupole Instruments
MDS Sciex API 4000 (multiple)
MDS Sciex API 5000 (multiple)
MDS Sciex API 5500

Ion Traps
Thermo Scientific Orbitrap with UPLC & Turbulent Flow Inline Extraction System
MDS Sciex API 4000 Qtrap
Agilent LC/MSD Trap XCT Ultra (several)

Time-of-Flight Mass Spectrometers
Quadrupole TOF

Isotope ratio Mass Spectrometer
Thermo Electron DELTA V advantage

NMR Spectroscopy Instruments
Varian INOVA NMR 600MHz spectrometer

HPLC Systems
Agilent 1100 Series HPLC
HTC PAL Leap Autosamplers

2.2 Software Packages for the Identification of Molecular Markers after Metabolite, Peptide and Protein Profiling Equipment

Metabolic Profiling

LightSight	Complete environment for finding metabolites in complex mixtures
ACD/MS Manager	For metabolite identification in combination with Light Sight
AMIX	Conversion of nuclear magnetic resonance and mass spectra into bucket tables and statistical analysis
METLIN	Database for metabolite identification after LC-MS analysis
Wiley.7N, Mass Spectral Libraries	Database with over 800.000 entries to identify metabolites after GC-MS analysis

Peptide/ Protein Profiling

MASCOT	Search engines for the identification of peptides/ proteins after mass spectrometry analysis
Spectrum Mill	Spectrometry analysis
Protein Pilot	Software for streamlining peptide and protein identification and quantification . Contains workflows for SILAC, ICAT and iTRAQ labeling technologies and the MIDAS workflow for setting up MS/MS experiments for quantification of peptides/ proteins.

Software for the Identification of Molecular Markers

GeneSpringMS	Comprehensive software package for the statistical analysis of spectral data to identify molecular makers
MarkerView	Metabolomics and protein/peptide profiling workflows