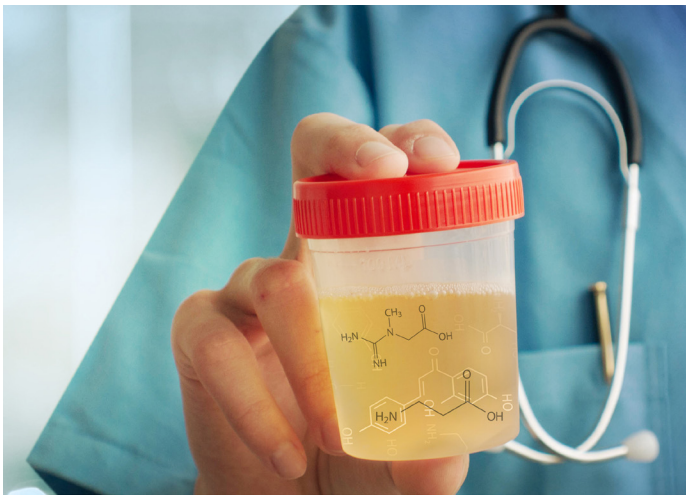
B.I.QUANT-UR™ (for research use only)



Because of the easy access to urine and the fact that there is mostly no interference with metabolite binding proteins, urine is an important and often used bodyfluid. Furthermore, urine is an extremely information-rich but very complex bodyfluid that contains metabolic breakdown products from a wide range of nutrients, drugs, environmental contaminants, endogenous metabolites and bacterial by-products.

Due to the appearance of large cohorts in clinical studies, the demand of technologies able to analyze a large number of measurements in an automated and robust way is increasing. NMR is an established metabolomics tool for obtaining metabolic phenotypes.

With the IVDr platform, a solution optimized for clinical/translational research, and using the standardized SOPs in B.I.Methods™ it is already possible to generate automatically highest quality spectra under full reproducibility and transferability from urine measurements ensuring the highest precision and sensitivity of analytical results. We have already introduced the lipoprotein subclass analysis for plasma/serum in 2016 (B.I.LISA™) and expand our analysis support in 2017 with another powerful tool for the IVDr platform providing automatic quantification of a variety of metabolites in urine directly after the measurement. The automated quantification is based on in house developed algorithms involving fitting predefined ^{1}H signals.

The methods and solutions described here are for research use only and not for use in clinical diagnostic procedures.

Benefits of B.I.QUANT-UR

- Completely automated quantification tool of up to 150 metabolites
- Validation of all LODs has been done following ISO 17025 guidelines for wet spiking
- Validation of all LODs by a Bruker in house technology called numerical spiking, allowing to test a large set of samples with different overlap situations
- Broad spectrum of analytes which can be quantified in a single run, at the same time allowing un-targeted analysis as used in the neonatal version
- Absolute and relative concentration for each of the up to 150 metabolites due to the calibration with **one** Quantification Reference sample (B.I.Methods™)
- One preparation, measurement and analysis can replace multiple conventional analysis methods such as GC-MS, LC-MS and Ion Exchange Chromatography
- On a daily basis 80-100 samples can be measured using the SOPs in B.I.Methods.

Requirements for B.I.QUANT-UR

- IVDr platform or compatible AVANCE III or AVANCE III HD 600 systems
- Use of B.I. Methods and SOPs for urine
- Absolute temperature, solvent suppression and quantification reference sample must be checked regularly (preferably daily)
- Access to Bruker Data Analysis server for fully automated remote analysis (transfer of spectra after measurement to Bruker server via private ftp, back-transfer of result PDF report or XML file)

The urine quantification B.I.QUANT-UR offers 3 versions

- B.I.QUANT-UR b: basic version, 50 compounds with concentration ranges, occurring in most human urines
- B.I.QUANT-UR e: extended version, 150 compounds with concentration ranges, age 6 month and up, also including IEM and other disease markers
- B.I.QUANT-UR ne: neonates extended version , 150 compounds with concentration ranges, also including disease markers and non-targeted classification against healthy newborn model

B.I.QUANT-UR b is used in the following cases

- Epidemiology
- Frequent diseases like kidney damage, diabetes, metabolic syndrome, obesity and cancer
- Ability to monitor and optimize treatment
- Microbiom related health problems
- Food and environmental influence to health
- Monitor compound concentrations in personalized urinary metabolic profile

The extended versions are used in the same cases as B.I.QUANT-UR b *plus*:

- Pediatrics
- Drug efficiency and treatment monitoring for IEM patients
- Functional food efficiency and dosage/composition
- Selective screening
- Neonatal health

Why are 2 age ranges offered individually?

Metabolite quantification in human urine has proven a challenging task due to complex compositional changes from test person to test person and even from the same person at different time points. Typical DIN Spiking wet methods to detect LOD are not delivering sufficient information for safe quantification. With innovative Bruker validation tools it is possible to break this barrier. The new routines introduced for urine quantification also take into account substantial matrix changes going from neonates to children/adults, which all affect metabolite chemical shifts and lead to varying overlap situations due to ionic matrix changes in the 2 age classes.

The changing overlap situation is visualized for 3 different neonatal urine spectra in Figure 1

Figure 1

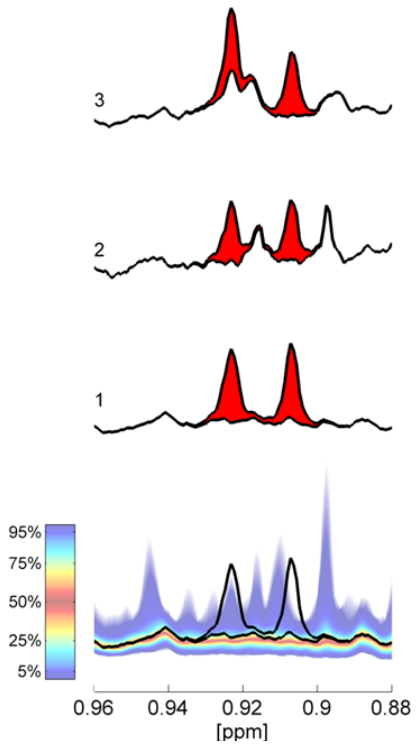


Figure 1 Example of numerical spiking for 2-oxoisocaproic acid

In Figure 1 numerical spiking has been done for 2-oxoisocaproic acid (signals in red) into 3 different neonatal urine spectra (25mmol/mol Creatinine). The bottom spectrum shows the spiking (black) on top of a quantile plot of all neonatal spectra used for validation.

Figure 2

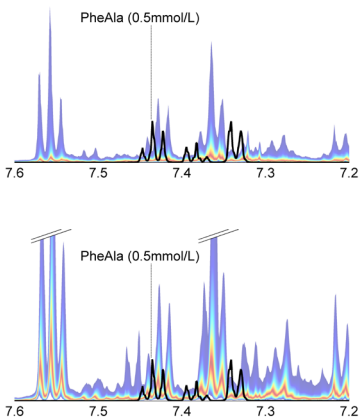


Figure 2 Example of the change of composition for Phenylalanine for the 2 age ranges

In Figure 2 the change in composition of a urine spectra going from neonates (top) to adults (bottom) is visualized. From the 2 spectra the complexity of overlap can clearly be seen. The result leads to the fact, that LODs have to be determined in both age groups separately to obtain correct quantification information, such increasing the accuracy of both versions substantially.

More than 600 urine spectra have been used to estimate LODs for each age class. Such massive coverage is impossible to achieve with wet spiking only.

Figure 3 shows an example of B.I.QUANT-UR ne at work. In black color, the actual urine spectrum is shown, the filled

Figure 3

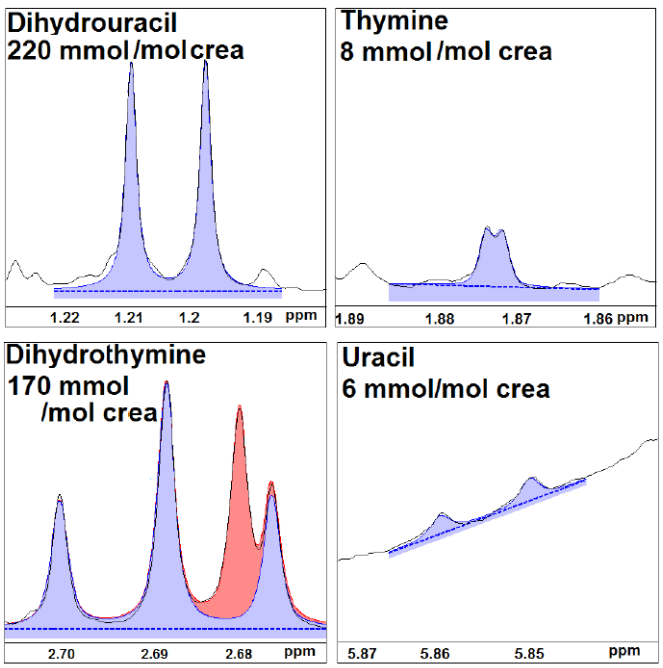


Figure 3 Visualization of quantification results

blue sections describe how the quantification of 4 relevant metabolites (Dihydrouracil, Thymine, Dihydrothymine and Uracil) in defects in purine metabolism was performed. For Dihydrothymine the handling of the overlapping signals of another metabolite is visualized. For Uracil, it is obvious, how the broad underlying signal of urea is subtracted.

In table 1 an extract of the report automatically generated by B.I.QUANT-UR ne is shown. Metabolites are sorted into chemical classes. Quantification values are given absolute and relative to Creatinine, LODs are listed as well as a 95% concentration range derived from the validation spectra set. The actual sample is shown as a black bar in the concentration range, if the corresponding metabolite is detected.

The chemical classes and additional categories used for each metabolite are

1. Creatinine
2. Alcohols and derivatives
3. Amines and derivatives
4. Amino acids and derivatives
5. Benzene and substituted derivatives
6. Carboxylic acids
7. Cosmetics, vitamines, drugs and drug metabolites
8. Fatty acids and derivatives
9. Hydroxy acids and derivatives
10. Keto acids and derivatives
11. Purine,Pyridine and Pyrimidine derivatives
12. Sugars and derivatives

Table 1

1.9 Hydroxy acids and derivatives

Compound	Conc. mmol/L	Conc. mmol/mol Crea	LOD mmol/mol Crea	95% Range mmol/mol Crea	Graphics (*)
3-Hydroxyglutaric acid	< 0.07	< 41	41	≤ 44	
3-Hydroxypropionic acid	< 0.17	< 93	93	≤ 93	
D-Galactonic acid	< 0.23	< 130	130	≤ 130	
D-Gluconic acid	< 0.34	< 190	190	≤ 550	
Glycolic acid	2.7	1500	190	≤ 480	
Malic acid	< 0.14	< 81	81	≤ 250	

(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

1.10 Keto acids and derivatives

Compound	Conc. mmol/L	Conc. mmol/mol Crea	LOD mmol/mol Crea	95% Range mmol/mol Crea	Graphics (*)
2-Ketobutyric acid	< 0.10	< 54	54	≤ 54	
2-Oxoglutaric acid	0.55	300	160	≤ 590	
2-Oxoisocaproic acid	< 0.01	< 5	5	≤ 10	
2-Oxoisovaleric acid	< 0.01	< 4	4	≤ 4	
3-Hydroxybutyric acid	< 0.17	< 97	97	≤ 100	
3-Methyl-2-oxovaleric acid	< 0.03	< 19	19	≤ 19	
4-Hydroxyphenylpyruvic acid	< 0.08	< 45	45	≤ 45	
Acetoacetic acid	0.09	49	5	≤ 28	
Acetoine	< 0.02	< 9	9	≤ 9	
Acetone	0.17	97	14	≤ 110	
DL-Kynurenin	< 1.4	< 790	790	≤ 790	
Oxaloacetic acid	1.9	1100	44	≤ 210	
Pyruvic acid	0.06	31	13	≤ 41	
Succinylacetone	< 0.73	< 410	410	≤ 410	

(*) Gray horizontal boxes represent 95% concentration range, black vertical lines represent sample value.

Table 1 Extract of B.I.QUANT-UR ne

The results are provided twice, in PDF format as well as in XML format, which allows easier transfer into other programs for statistical analysis or EXCEL sheets. For members of the IVDr Forum the XML file can be uploaded into a Bruker dedicated version of the Metagene Knowledge Base on Inborn Errors of Metabolism to see potential relationships of quantified metabolites with rare diseases.

Further information

For detailed metabolite lists and more technical info, please contact Manfred.spraul@bruker.com and for sales information your local sales representative.

B.I.QUANT-UR modules are offered as flat rate for 1 year, 3 years with remote result generation and as pay per use transferring datasets to be analyzed by email or other means.

Disclaimer

The B.I.QUANT modules are for research use only and are not released for clinical diagnostics.

For further information, visit the following websites:

IVDr platform
https://www.bruker.com/products/mr/nmr/avance-ivdr/overview.html

B.I.Methods
https://www.bruker.com/products/mr/nmr-preclinical-screening/bi-methods.html

B.I.Quant-UR
https://www.bruker.com/products/mr/nmr-preclinical-screening/bi-quant-ur.html

B.I.LISA
https://www.bruker.com/products/mr/nmr-preclinical-screening/lipoprotein-subclass-analysis.html

IVDr Forum
https://www.bruker.com/products/mr/nmr/ivdr-forum.html

Bruker BioSpin
Providing NMR Solutions for Metabolomics

NMR is an advantageous technique for metabolomic research, providing high reproducibility, simple sample preparation and the ability to measure different small molecule metabolites simultaneously. Today, new advances in software and hardware platforms have made NMR more effective, easier to use and more cost efficient. Discover for yourself how NMR can help illuminate metabolic networks.

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