

Metabolome and Lipidome Analysis of Human Urine Samples Using the MxP® Quant 500 Kit

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1 Introduction

Human urine is a commonly used matrix for biomarker discovery research. Sample collection is non-invasive, and urine is suited for both proteomics- and metabolomics-based research. In addition, sample handling before analysis is relatively simple compared to other biofluids, and normalization of metabolite concentrations to creatinine levels is a useful approach to minimize the effects of fluid intake. There are several recommendations, how to collect, process, and store urine samples¹⁻³. In general, fresh samples should be kept on ice or 4 °C for max. 8 hours before centrifugation. The supernatant should then be stored at -80 °C.

This application note describes the expected metabolite and lipid panel in human urine samples based on measurements using the MxP® Quant 500 kit.

2 Methods

Only 10 µL of urine per sample are required to run the MxP® Quant 500 kit for urine analysis. The data presented here is based on the analysis of 20 individual human urine samples in three technical replicates using Waters' Xevo® TQ-S system. Some modifications and additional kit components are required. For details, please refer to the technical note 35041 "Analysis of

Urine Using the MxP® Quant 500 Kit on SCIEX 5500+ and 6500+”.

In summary:

- Urine-specific data acquisition and quantitation methods
- Internal standard with d3-creatinine
- Alternative sample derivatization protocol
- Creatinine calibrators
- Urine zero samples

3 Results and Conclusion

The MxP® Quant 500 kit enabled the detection and quantification of 72 analytes above the limit of detection (LOD) in more than 90% of the samples. Thereof, 31 analytes can only be analyzed using the MxP® Quant 500 kit, not with the Absolute/DQ® p180 kit. The concentration ranges after normalization to creatinine concentration and the comparison to the metabolite and lipid coverage of the Absolute/DQ® p180 kit are shown in Table 1. Table 2 lists an additional 39 metabolites and lipids that were detected in 50-90% of the tested samples. Thereof, 23 analytes can only be analyzed using the MxP® Quant 500 kit, not with the Absolute/DQ® p180 kit.

Taken together, the MxP® Quant 500 kit enables the detection and quantification of 111 analytes >LOD in a typical human urine sample. Thereof, 54 metabolites and lipids, mainly bile acids, carboxylic acids, fatty acids, and triglycerides,

can only be analyzed using the MxP® Quant 500 kit, not with the Absolute/IDQ® p180 kit.

In conclusion, the data described here shows the utility of the MxP® Quant 500 kit in identifying dozens of analytes and determining their concentration ranges in human urine samples.

Table 1: Concentrations of 72 analytes detected >LOD using the MxP® Quant 500 kit in more than 90% of the human urine samples.

Detected in more than 90% of the tested samples (n = 20)				
Analyte Class	Analyte	Short Name	Concentration Range [µM/mmol Crea]	Absolute/IDQ® p180
Alkaloids	Trigonelline	Trigonelline	0.157-4.13	
Amine oxides	Trimethylamine N-oxide	TMAO	7.14-212.3	
Amino acids	Alanine	Ala	10.9-65.4	x
Amino acids	Arginine	Arg	0.807-6.88	x
Amino acids	Asparagine	Asn	2.97-13.3	x
Amino acids	Cysteine	Cys	1.91-12.1	
Amino acids	Glutamate	Glu	1.16-5.13	x
Amino acids	Glutamine	Gln	21.1-88.6	x
Amino acids	Glycine	Gly	23.2-254.3	x
Amino acids	Histidine	His	18.1-152.7	x
Amino acids	Isoleucine	Ile	0.45-1.69	x
Amino acids	Leucine	Leu	0.751-3.74	x
Amino acids	Lysine	Lys	2.27-95.9	x
Amino acids	Phenylalanine	Phe	1.67-6.86	x
Amino acids	Serine	Ser	14.3-41.3	x
Amino acids	Threonine	Thr	2.75-24.4	x
Amino acids	Tryptophan	Trp	2.37-7.71	x
Amino acids	Tyrosine	Tyr	2.37-17.8	x
Amino acids	Valine	Val	1.28-4.3	x
Amino acid related	α-Amino adipic acid	alpha-AAA	1.24-6.48	x
Amino acid related	Acetylornithine	Ac-Orn	0.014-0.071	x
Amino acid related	Asymmetric dimethylarginine	ADMA	2.09-5.18	x
Amino acid related	Anserine	Anserine	0.003-2	
Amino acid related	5-Aminovaleric acid	5-AVA	0.026-0.364	
Amino acid related	β-Aminobutyric acid	BABA	0.059-0.579	
Amino acid related	Carnosine	Carnosine	0.25-13	x
Amino acid related	Citrulline	Cit	0.137-1.09	x
Amino acid related	Creatinine	Creatinine	1000-1000	x
Amino acid related	Cystine	Cystine	8.19-167	
Amino acid related	Homoarginine	HArg	0.013-0.431	
Amino acid related	Homocysteine	HCys	0.501-4.52	
Amino acid related	trans-4-Hydroxyproline	t4-OH-Pro	0.014-0.89	x
Amino acid related	Methionine sulfoxide	Met-SO	0.089-0.912	x

Detected in more than 90% of the tested samples (n = 20)				
Analyte Class	Analyte	Short Name	Concentration Range [µM/mmol Crea]	Absolute/DQ [®] p180
Amino acid related	1-Methylhistidine	1-Met-His	5.41-13.9	
Amino acid related	3-Methylhistidine	3-Met-His	0.199-8.96	
Amino acid related	Phenylacetylglycine	PAG	0.008-0.291	
Amino acid related	Proline betaine	ProBetaine	0.109-13.7	
Amino acid related	Sarcosine	Sarcosine	0.026-0.446	x
Amino acid related	Symmetric dimethylarginine	SDMA	3.14-6.78	x
Amino acid related	Taurine	Taurine	1.35-130.1	x
Amino acid related	Tryptophan betaine	TrpBetaine	0.007-0.289	
Bile acids	Glycolithocholic acid sulfate	GLCAS	0.006-0.095	
Biogenic amines	β-Alanine	beta-Ala	0.066-1.86	
Biogenic amines	γ-Aminobutyric acid	GABA	0.054-0.289	
Biogenic amines	Phenylethylamine	PEA	0-0.035	x
Biogenic amines	Putrescine	Putrescine	0.021-0.276	x
Carboxylic acids	Aconitic acid	AconAcid	2.77-100.7	
Carboxylic acids	Hippuric acid	HipAcid	5.97-60.9	
Carboxylic acids	3-Hydroxyglutaric acid	OH-GlutAcid	0.715-8.82	
Carboxylic acids	Lactic acid	Lac	0-125.8	
Cresols	p-Cresol sulfate	p-Cresol-SO4	2.5-19.4	
Fatty acids	Arachidonic acid	AA	0-0.182	
Hormones and related	Abscisic acid	AbsAcid	0-7.16	
Hormones and related	Cortisol	Cortisol	0.001-0.034	
Hormones and related	Dehydroepiandrosterone sulfate	DHEAS	0.017-1.23	
Indoles and derivatives	3-Indoleacetic acid	3-IAA	0.045-5.54	
Indoles and derivatives	Indoxyl sulfate	Ind-SO4	13.4-119.2	
Nucleobases and related	Hypoxanthine	Hypoxanthine	1.51-16.6	
Nucleobases and related	Xanthine	Xanthine	5.03-39.9	x
Vitamins and cofactors	Choline	Choline	0.481-3.09	
Acylcarnitines	Carnitine	C0	1.92-46.6	x
Acylcarnitines	Acetylcarnitine	C2	0.211-19.9	x
Acylcarnitines	Propionylcarnitine	C3	0.018-0.401	x
Acylcarnitines	Malonylcarnitine (Hydroxybutyrylcarnitine)	C3-DC (C4-OH)	0.015-0.087	x
Acylcarnitines	Butyrylcarnitine	C4	0.333-2.18	x
Acylcarnitines	Butenylcarnitine	C4:1	0.005-0.033	x
Acylcarnitines	Valerylcarnitine	C5	0.121-0.623	x
Acylcarnitines	Glutarylcarnitine (Hydroxyhexanoylcarnitine)	C5-DC (C6-OH)	0.076-0.172	x
Acylcarnitines	Methylglutarylcarnitine	C5-M-DC	0.03-0.116	x
Acylcarnitines	Hydroxyvalerylcarnitine (Methylmalonylcarnitine)	C5-OH (C3-DC-M)	0.084-0.198	x
Acylcarnitines	Tiglylcarnitine	C5:1	0.051-0.136	x
Cholesteryl esters	Cholesteryl ester (22:2)	CE (22:2)	0-0.412	

Table 2: Concentrations of 39 analytes detected >LOD using the MxP® Quant 500 kit in 50-90% of the human urine samples.

Detected in 50-90% of the tested samples (n = 20)				
Analyte Class	Analyte	Short Name	Concentration Range [µM/mmol Crea]	Absolute/IDQ® p180
Amino acids	Methionine	Met	0-2.12	x
Amino acids	Proline	Pro	0-1.15	x
Amino acid related	Betaine	Betaine	0-61.8	
Amino acid related	Kynurenine	Kynurenine	0.074-0.599	x
Amino acid related	Nitrotyrosine	Nitro-Tyr	0.022-0.129	x
Amino acid related	Ornithine	Orn	0.398-4.99	x
Amino acid related	Phenylalanine betaine	PheAlaBetaine	0.001-0.017	
Bile acids	Chenodeoxycholic acid	CDCA	0.019-0.11	
Bile acids	Glycoursodeoxycholic acid	GDCA	0-0.018	
Biogenic amines	Dopamine	Dopamine	0.047-0.303	x
Biogenic amines	Serotonin	Serotonin	0.021-0.086	x
Carboxylic acids	Tetradecanedioic acid	DiCA(14:0)	0-0.046	
Carboxylic acids	Succinic acid	Suc	2.19-58.3	
Fatty acids	Eicosapentaenoic acid	EPA	0-0.519	
Hormones and related	Cortisone	Cortisone	0.004-0.057	
Indoles and derivatives	3-Indolepropionic acid	3-IPA	0-0.016	
Indoles and derivatives	Indole	Indole	24.1-142.3	
Acylcarnitines	Hexanoylcarnitine (Fumaryl carnitine)	C6 (C4:1-DC)	0.029-0.092	x
Acylcarnitines	Pimeloylcarnitine	C7-DC	0.036-0.118	x
Acylcarnitines	Octanoylcarnitine	C8	0.087-0.253	x
Acylcarnitines	Nonanoylcarnitine	C9	0.296-2.78	x
Acylcarnitines	Decanoylcarnitine	C10	0.03-0.093	x
Acylcarnitines	Dodecanoylcarnitine	C12	0.017-0.087	x
Acylcarnitines	Hydroxytetradecadienoylcarnitine	C14:2-OH	0.001-0.008	x
Acylcarnitines	Hexadecanoylcarnitine	C16	0.003-0.017	x
Acylcarnitines	Hydroxyhexadecanoylcarnitine	C16-OH	0.001-0.005	x
Diglycerides	Diglyceride (14:0_14:0)	DG(14:0_14:0)	0-0.022	
Diglycerides	Diglyceride (14:0_18:1)	DG(14:0_18:1)	0.001-0.007	
Diglycerides	Diglyceride (14:0_18:2)	DG(14:0_18:2)	0-0.106	
Diglycerides	Diglyceride-O (14:0_18:2)	DG-O(14:0_18:2)	0.002-0.028	
Triglycerides	Triglyceride (18:0_38:6)	TG(18:0_38:6)	0.009-0.11	
Triglycerides	Triglyceride (20:2_34:4)	TG(20:2_34:4)	0.001-0.024	
Triglycerides	Triglyceride (20:2_36:5)	TG(20:2_36:5)	0-0.016	
Triglycerides	Triglyceride (20:3_34:0)	TG(20:3_34:0)	0-0.05	
Triglycerides	Triglyceride (20:4_33:2)	TG(20:4_33:2)	0-0.044	
Triglycerides	Triglyceride (20:4_34:3)	TG(20:4_34:3)	0-0.032	
Triglycerides	Triglyceride (20:4_36:4)	TG(20:4_36:4)	0.003-0.084	
Triglycerides	Triglyceride (22:2_32:4)	TG(22:2_32:4)	0.002-0.461	
Triglycerides	Triglyceride (22:4_32:2)	TG(22:4_32:2)	0-0.018	

References

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3. Rotter M, Brandmaier S, Prehn C, et al. Stability of targeted metabolite profiles of urine samples under different storage conditions. *Metabolomics* 2017;13:4.