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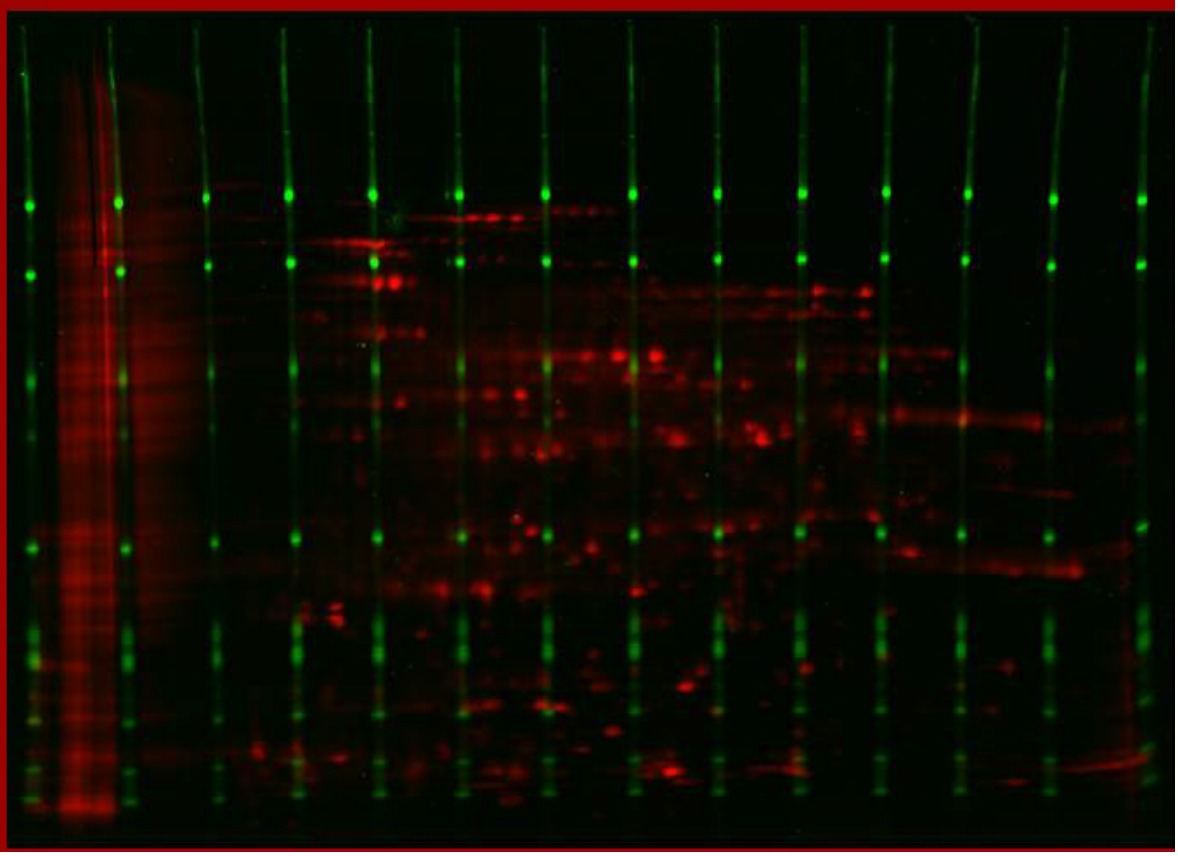

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https://campus.uni-muenster.de/cu-proteomics
Edited by Simone König
CUP/IZKF, Röntgenstr. 21, 48149 Münster
koenigs@uni-muenster.de
Ph. +49-251-8357164

Cover image
Mercator gel (run by D. Ackermann at CUP) representing the award-winning CoFGE technology for standardized gel electrophoresis

Short Protocol

Extraction of δ-aminolevulinic acid-induced protoporphyrin IX from blood and serum and measurement using analytical chromatography coupled to mass spectrometry

*Insa Peters and Simone König**

[1] Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster, Münster, Germany

*Corresponding author: koenigs@uni-muenster.de

Abstract

In continuation of earlier work on the detection of δ-aminolevulinic acid (ALA)-induced protoporphyrin IX (PPIX) in blood and serum both the extraction method and the analysis were improved considerably. Liquid chromatography (LC) analysis was transferred from capillary equipment to an analytical workflow reducing the experiment time from several hours to 10 min. These promising results enable LC coupling to modern mass spectrometers which boosts the sensitivity about 100-fold.

Experimental

This work [1] continues earlier developments [2,3].

Extraction

PPIX extraction from both blood and serum was simplified by using protein precipitation with optimized solvents (Figure 1). Four parts of water were added to blood (50 µl for recovery tests, 200 µl for real analyses), the solution was shaken for 30 min for hemolysis. Ten parts of acetonitrile (ACN) were added with shaking for 1 h. Subsequently, the sample was centrifuged (30 min, 14000 rcf); the supernatant was dried and redissolved in 200 µl DMSO for LC-MS (2-5 µl injection). In order to determine the recovery of PPIX and the internal standard mesoporphyrin (MPIX), samples were spiked at different points in the procedure (A-D; 50 µl biofluid + 6.66 µl of 10 pmol/µl). Porphyrines could be recovered at better than 90% from both blood and serum. For the detection of endogenous PPIX, the required blood volume was thus decreased to 200 µl (from 1 ml [2]) and 500 µl for serum (from 4 ml [2]).

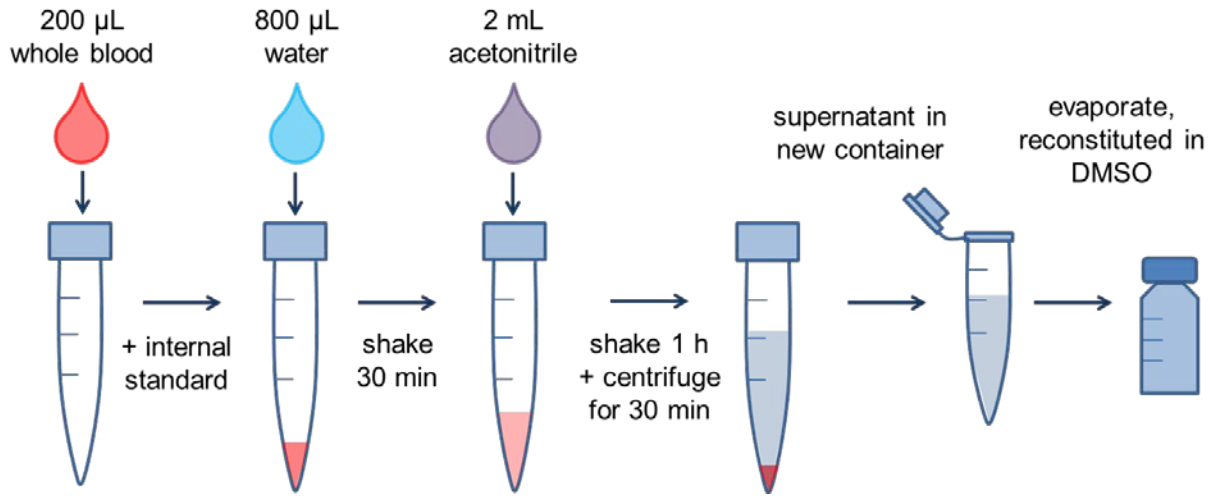

Figure 1: Schematic for the extraction workflow [1]. Four parts of water and 10 parts of acetonitrile were added to 1 part blood followed by vortexing and centrifuging. The supernatant was dried and redissolved in DMSO for LC-MS.

Chromatography

The HP1100 LC system was changed from capillary to analytical flow (300 µl/min) using a semi-porous column (Poroshell 120 EC-C18, endcapped, 2.1 x 100 mm, 2.7 µm particle; guard column: UHPLC Guard 3PK Poroshell120 EC-C18, 2.1 x 5 mm, 2.7 µm; Agilent). The method is shown in Table 1. On the coupled mass spectrometer (Esquire3000, Bruker), the source parameter were adjusted to the higher flow (Table 2). Results are shown in Figure 2. The new LC setup considerably improved the separation, eliminated peak tailing and shortened the run time to 10 min.

Table 1: HPLC parameter

Parameter	Value	
Injection volume	5 µL	
Flow rate	0,3 mL/min	
Eluent B	H_2O/ACN/formic acid (4,9%:95%:0,1%)	
Gradient	**Time [min]**	**Eluent B [%]**
	0	70
	1	100
	5	100
	5,5	70
	10	70

Table 2: MS parameter

Parameter	Value
Source temperatur [°C]	320
Cone voltage [V]	4500
Desolvation gas [psi]	20
Dry gas [L/min]	6

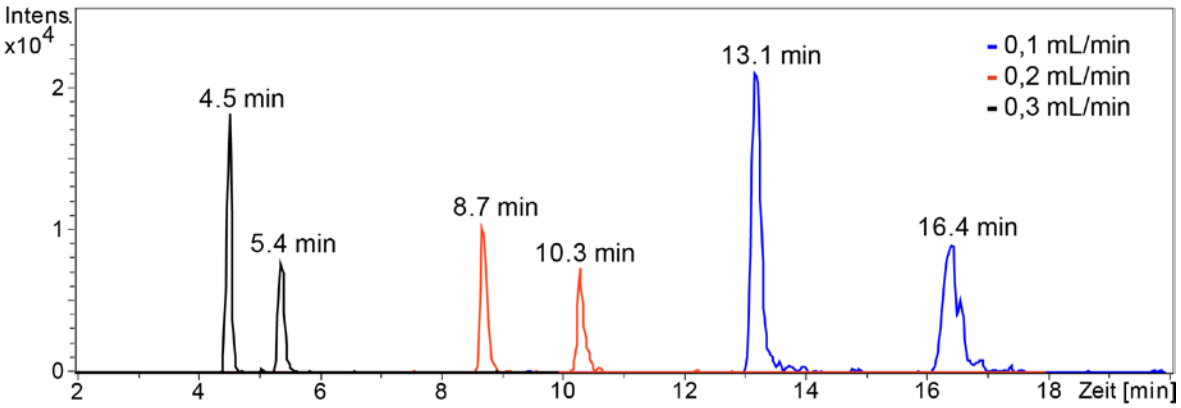

Figure 2: Three chromatograms overlayed to illustrate the influence of different flow rates. Extracted ion chromatograms for fragment ions at *m/z* 445.3 and 449.3. MPIX elutes before PPIX.

Conclusion

The new protocol improves several aspects of the workflow with respect to experimental time and handling effort. Further sample preparation is needed, because high-throughput is still hampered by easy column clogging. The LC-method is however ready for transfer to a modern mass spectrometer, which will boost sensitivity by two orders of magnitude.

Acknowledgements

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References

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[3] Walke A, Suero Molina E, Müther M, Stummer W, König S (2019) Determination of δ-aminolevulinic acid-induced protoporphyrin IX in blood. Mercator J Biomol Mass Spectrom 3: 1-13. urn:nbn:de:hbz:6-84199486146.