Project Report: 58p2

Two-dimensional liquid chromatography coupled with tandem mass spectrometry for cell-membrane phospholipid bioanalysis

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As mentioned in the application and reviewed in the literature [1], the chromatographic separation of whole phospholipids is one of well-known challenges in lipidomics due to their intrinsic complexity and broad distribution of physical and chemical properties and molecular species.

Research visit activities: Under the frame of this project, Prof. Dr. Pavel Jandera has visited the Laboratory in Graz twice for total 10 days of duration and co-supervised the chromatographic optimization, especially the choice of mobile phases for elution. Dr. Jiri Urban visited Graz once for 6 days to have helped with the optimization of elution conditions. Dr. Xinghua Guo and his student Mr Mathias Eisenhut have visited the Laboratory in Pardubice for 5 days respectively.

Detailed research visits supported by this project:

Pardubice to Graz:

Prof. Dr. Pavel Jandera	11-17.10.2010	7 days
	30.08.01.09.2011	3 days
Dr. Jiri Urban	26.06-01.07.2011	6 days

Graz to Pardubice:

Dr. Xinghua Guo	05-08.12.2010	5 days
Mr. B.Sc. Mathias Eisenhut	23-27.05.2011	5 days

Realized work: Our project started in September 2010 with the preparation of dedicated monolithic columns at the University of Pardubice (Pardubice, Czech). Using thermal polymerization [2], 5 revered-phase capillary columns and 5 hydrophilic interaction chromatographic (HILIC) capillary columns were first prepared in two batches. After an initial characterization, they were subjected to intensive tests for the separation of phospholipids at the Graz University of Technology (Graz, Austria). As model polar compounds for optimization, some small sugar carboxylic acids were also used to guide the choice of mobile phases and their gradients.

The tests at the Graz University of Technology focused on developing chromatographic elution methods for LC-MS separation, which aimed at as one of the two chromatographic dimensions in the future for 2D-HPLC. Depending on the polarities of the selected

compounds, both positive and negative electrospray ionization were applied under the multiple reaction monitoring (MRM) mode. In the first batch, the standard monolithic capillary columns with the ID 75 µm did not work properly with the applied Agilent 1100 HPLC system due to the incompatibility of the low flow rate required and high pressures generated. To prepare monolithic columns with bigger diameter (about ID 0,5 mm) has been a known challenge due to the possible poor homogeneity of polymerization (thus, the sorbent material) and the loose interaction between the capillary inner wall and the sorbent material. Technically this problem was partially overcome and some columns with ID of 0,53 mm were prepared and characterized initially. The test indicated that the required optimized flow rate could be supported then with our HPLC systems. Several gradient methods have been tested to separate small polar carboxylic acids. However, further long-lasting tests indicated that the capillary columns (with ID 0,53 mm) were actually not so durable as those with smaller diameters. One of the major problems came from the collapse of monolithic materials at a certain pressure. This could be observed by the movement of the sorbent slowly in the flow direction to have resulted some hollow capillary gaps along the columns. The applied pressure was not extremely high in this case (about 280-300 Bar), and this was likely resulted from the relatively weak interaction between the capillary inner wall and the sorbent materials. Another problem was the fragileness of the capillary column (ID 0,53 mm) under its working pressure, although a special care has been taken during its handling. As expected, among the tested HILIC and reversed-phase columns, the HILIC ones have always shown better retention capability than the reversed phase for he selected testing analytes of small carboxylic acids, while the reversed phase resulted only retentions for less polar analytes and all polar small acids eluted immediately with the mobile phase after injection. The best retention as long as 11-14 min was achieved with the HILIC column (ID 0,53 mm, 15 cm long) for the major carboxylic acids (Figure 1). However, the attempt to separate them more and to increase the resolution with the fine tuning of the elution gradient and the mobile phases was only with limited success. The low resolution and broad peaks may partially result from the low flow rate (50 µL/min) compared to the column dead volume. Further increase of the flow rate would cause rapid increase of the back column pressure on this HPLC system.

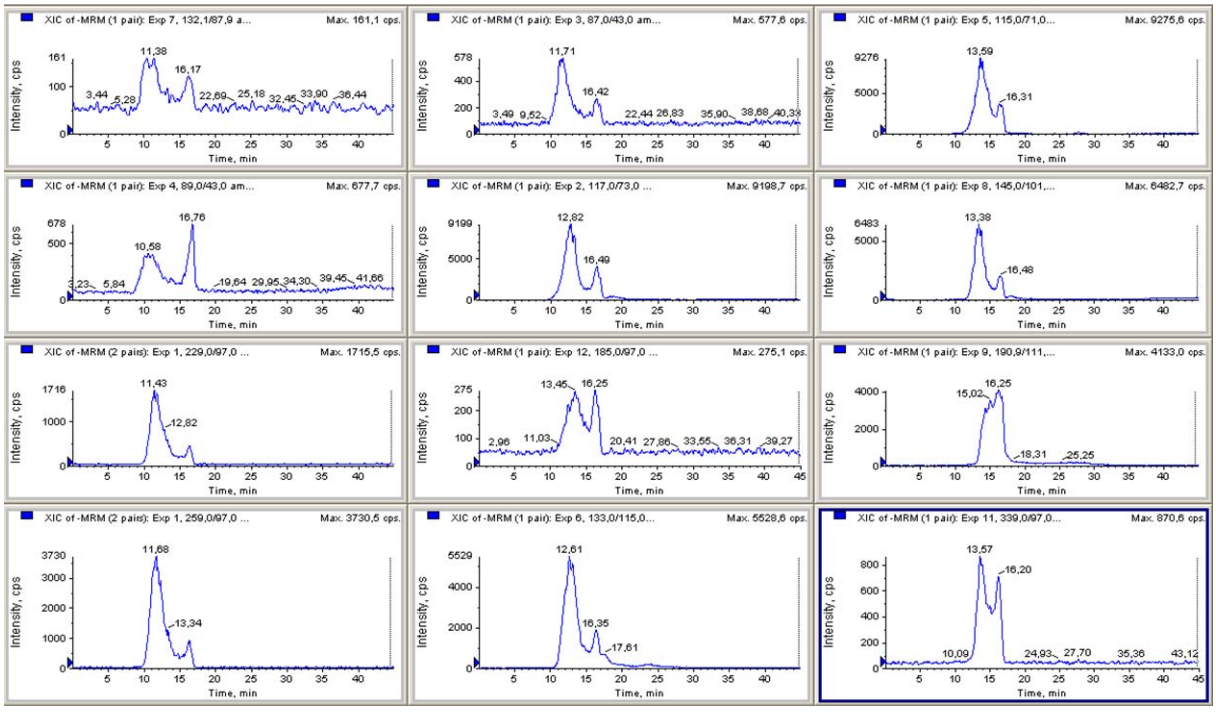

Figure 1 MRM LC-MS chromatogram obtained with the HILIC capillary column (ID 0,53 mm, 15 cm) for the selected testing analytes (MRM pair, RT): L-Aspartic acid (132/88, 11.4 min.), L-Lactic acid (89/43, 10.6 min.), Ribose-5-phosphate (229/97, 11.4 min.), Glucose-6-phosphate (259/97, 11.7 min.), Pyruvic acid (87/43, 11.7 min), Succinic acid (117/73, 12.8 min.), 3-Phosphoglycerate (185/97, 13.5 min.), L-Malic acid (133/115, 12.6 min.), Fumaric acid (115/71, 13.6 min.), alpha-Ketoglutaric acid (145/101, 13.4 min.), Citric acid (191/111, 15.0 min.) and Fructose-1,6-biphosphate (339/97, 13.6 min.), respectively.

Future work: Due to the complexity and difficulties of this work, it turns out that more time is needed to finish the whole research proposed in the project application. Although a method of comprehensive two-dimensional LC×LC-mass spectrometric method for simultaneously unbiased separation and characterization of phospholipid molecular species of complex mixtures has not been fully realized during the period of this project, we have collected some very useful experience and data for the future development. Under mutual agreement, the research formulated in this project will be continued as our collaborative topics in the coming years. If further significant progresses are achieved, another joint project based on this could be possibly developed.

Finally we would like to thank the colleagues at the organization offices in Prague and the ÖAD office in Graz for their kind support during the project, with that the administration of this one-year project has been run smoothly. Although still many works to be carried out on this topic, the both research institutions consider this bilateral project as an effective way to stimulate some international collaborations.

References

1. Hu C, van der Heijden R et al.: Analytical strategies in lipidomics and applications in disease biomarker discovery. ***J. Chrom. B.*** 877, 2836-2846 (2009).
2. Jandera Pavel; Urban J et al.: Polymethacrylate monolithic and hybrid particle-monolithic columns for reversed-phase and hydrophilic interaction capillary liquid chromatography. ***J. Chrom. A*** 1217(1), 22-33 (2010).

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