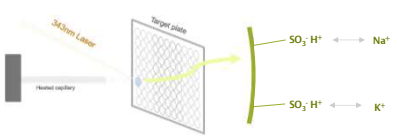
[1]Department of Chemistry, University or Reading, Reading, UK
[2]Waters Corporation, Wilmslow, UK

Quick and easy on-target sample cleanup for Liquid Atmospheric Pressure (LAP)-MALDI

Agata Kowalczyk[1] | Jeff Brown[2] | Rainer Cramer[1]

Introduction

Sample desalting in proteomic workflows is essential to reduce ion suppression and improve mass spectrometric sensitivity. For off-line MS sample preparation as it is the case for MALDI MS, conventional methods, such as spin columns or ZipTips®, can be costly or difficult to integrate into high-throughput applications. To address this, a rapid and cost-effective on-target desalting approach using strong cation exchange (SCX) beads was assessed. This method offers a simple, inexpensive and scalable alternative, improving analyte enrichment directly on the target plate. In particular where the sample is a liquid droplet, SCX beads can be effectively employed as it is the case with LAP-MALDI MS.



Methods

SCX beads (DOWEX® 50WX8) were purified with 1:1 (v:v) methanol/water washing followed by regeneration with 5% formic acid or 2% hydrochloric acid and a final LC-MS -grade water washing step until reaching a neutral pH. Beads were used as a slurry suspended in water or pH-equilibrated with 10mM ammonium salt buffers. LAP-MALDI samples were prepared to 1-1.75µL volumes by on-target mixing of 0.5µL analyte, 0.25µL equimolar aqueous NaCl + KCl (or water), 0.05-0.5µL of the bead slurry, and 0.5µL of the liquid MALDI matrix (10mg/ml CHCA in 1:1 water/acetonitrile + 60% propylene glycol) – in this order. Standards were tested with residual and known added amounts of abundant salts (NaCl/KCl). To compare efficiency of bead preparations, the total bead surface area was approximated using image analysis. They were also tested on a milk extract and during a pH-controlled enzymatic assay using a SYNAPT™* G2-Si mass spectrometer with a custom-built LAP-MALDI source.

Ion intensities were extracted from mass spectra using a custom-built Python script. Bead shapes and radii were extracted with a semi-automated script implementing the Hough Circle Transform (HCT) algorithm.

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Results

Improvements in mass spectral quality

Adduct peaks are minimised, proving that Na and K ions are captured by the SCX beads. Absolute protonated analyte ion intensities were improved for proteins but decreased for a single peptide while milk lipid ion signal also benefitted (see Figure 1).

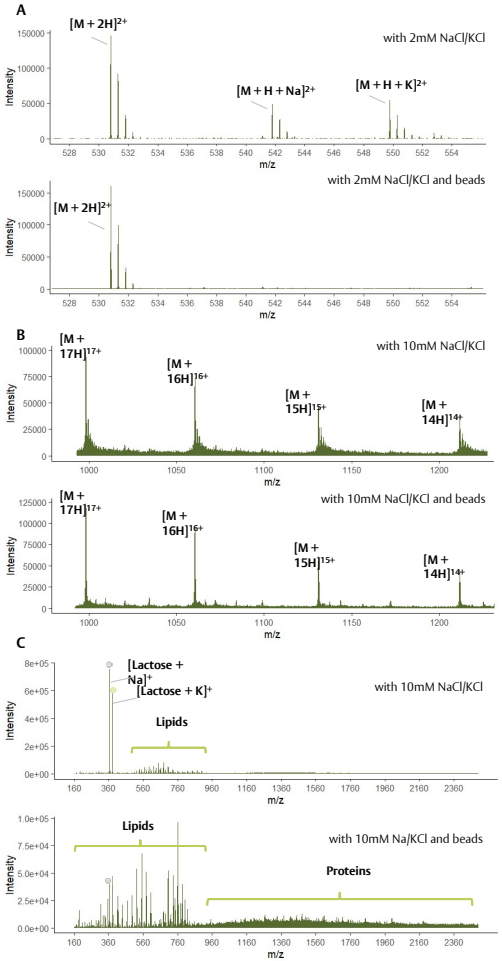


Figure 1. LAP-MALDI mass spectra presenting the effects of on-plate desalting using on-target: **A)** 5pmol bradykinin, **B)** 20pmol horse heart myoglobin, **C)** cow milk extract

Adduct removal in presence of known amounts of salts

What is the extent of Na/K adduct removal and is it scalable?

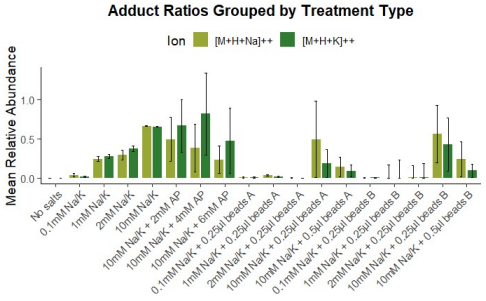
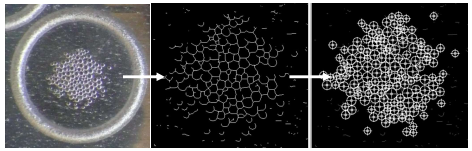


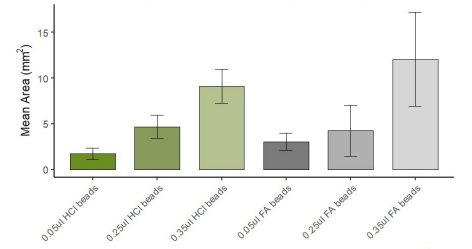
Figure 2. Comparison of Na/K adduct removal on doubly charged bradykinin ions in the presence of different salt levels. The data is based on 5 replicates including 10 scans each (1 scan/s). Mean relative abundances are presented as intensity ratios of the adduct ion signal relative to the protonated ion signal. Concentrations are given as approximate on-target values (1 mM = 1 nmol on target). Data for the addition of ammonium phosphate (AP) is also provided for a comparison to the addition of SCX beads.

Comparison of bead treatments

Does treating beads with a weak vs strong acid make a difference to the removal efficiency?

A Determination of SCX bead size distribution via image analysis





B

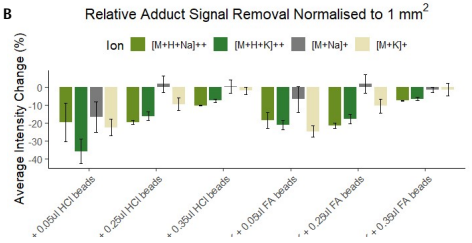


Figure 3. A) Bead area estimation and **B)** investigation of treatment effects on adduct ion signal intensities.

SCX bead application in enzyme assays

The addition of SCX beads was successfully employed during on-target enzymatic reaction monitoring, where Na/K ions are often abundant .

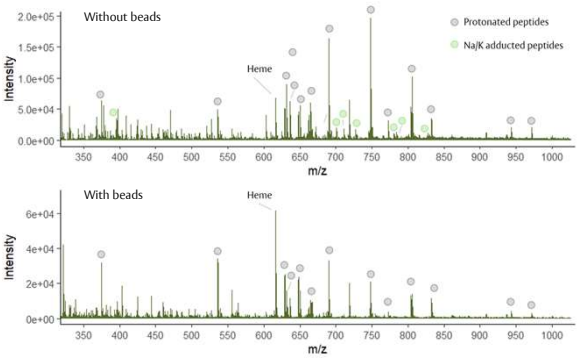


Figure 4. Spectral complexity decreases after adding SCX beads to an on-target myoglobin digestion with trypsin in 10mM ammonium bicarbonate (AmBic) and a pH-8 liquid matrix (CHCA/3-AQ in 10mM AmBic + 60% PG). Peptides and adduct ions are identified tentatively.

Conclusions

- On-target desalting using SCX beads decreases adduct ion peak intensities in a LAP-MALDI sample and can contribute to higher S/N of protonated larger molecules (myoglobin, milk proteins).
- Only the smallest working amount of beads should be used for clean-up as too many beads can have a detrimental effect on overall signal intensity, in particular for small peptides.
- There is no effect on the adduct ion removal efficiency with respect to the acids used for the SCX bead preparation.
- Small peptide binding effects to SCX beads need to be investigated further.

References

1. Ryumin, P. and Cramer, R. (2015). Efficient Production of Multiply Charged MALDI Ions. Springer eBooks, pp.37–47. doi:https://doi.org/10.1007/978-3-319-04819-2_2.
2. Pistori, H. and Costa, E.R. (2004). Hough Circle Transform Plugin. University of California, Berkeley Vision Sciences. Available at: https://github.com/llamero/Local_Hough_Circle

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Contact information

- Email: a.kowalczyk@reading.ac.uk