

High Performance TLC-MALDI

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Introduction

- HPTLC is routinely used as efficient separation method for difficult analytes such as lipids
- Automated devices provide robust high performance TLC separations
- Hyphenation with MALDI-TOF analysis permits high resolution molecular readout directly from the TLC/HPTLC-plates [1,2]
- MALDI-MS/MS provides structural information where required

Methods

- Lipid standards (Avanti Polar Lipids)
- HPTLC silica gel 60 F₂₅₄ aluminum backed sheets, 50×75 mm, 200 µm layer thickness (Merck, # 1.05556.0001)
- ATS4 (CAMAG) for automated sample application
- DHB applied using the ImagePrep matrix coating device (Bruker)
- TLC-adapted target for MALDI (Bruker)
- TLC-MALDI software (Bruker) settings: 100 µm raster width
- UltrafleXtreme MALDI-TOF (Bruker) acquisition time ~ 5 min/lane

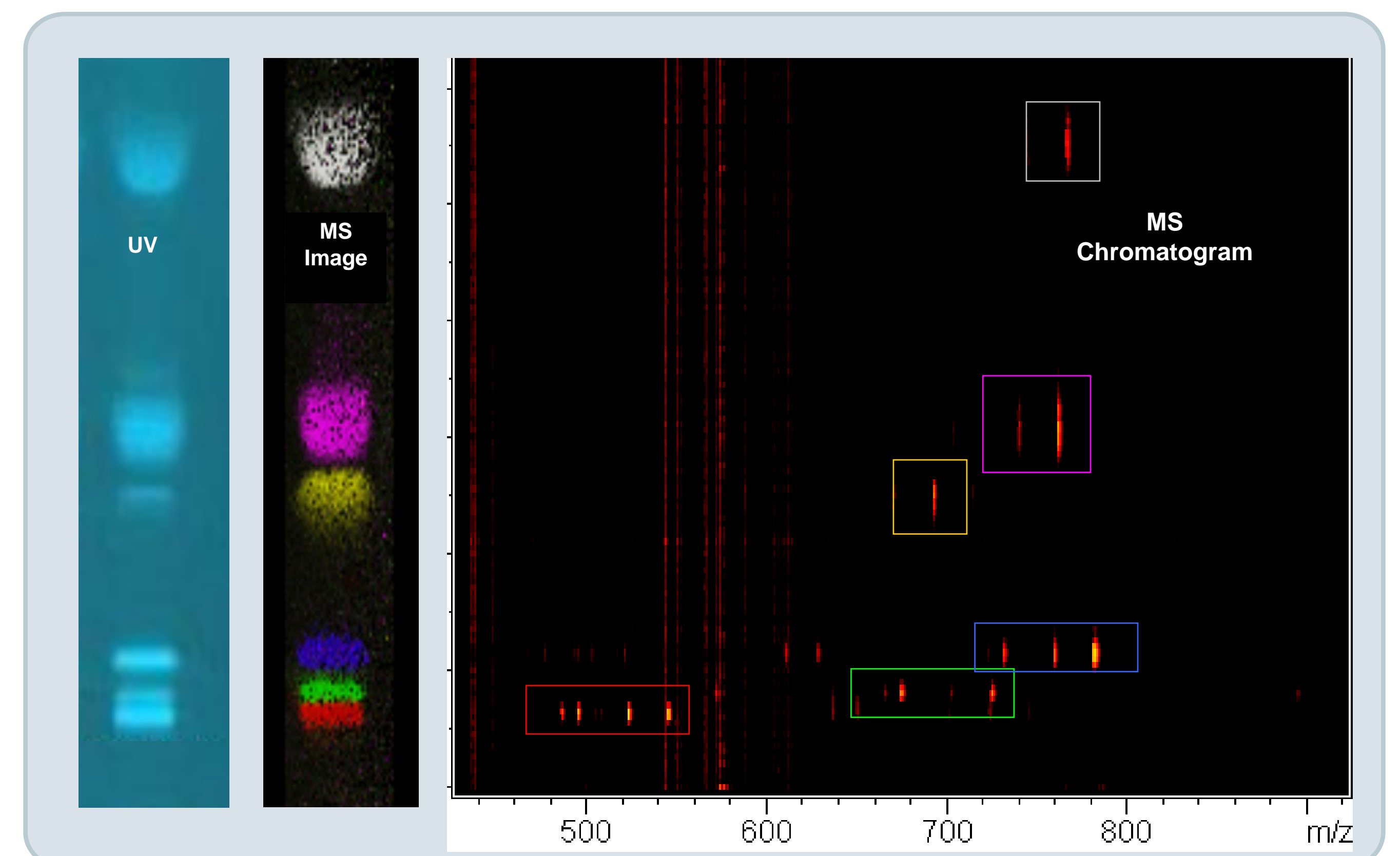


Fig. 1: HPTLC analysis of 532 ng of a lipid standard mixture: (UV) primuline, fluorescence at 366 nm; (MS Image) MALDI-TOF image analysis, false color representation of ion distribution; and (MS Chromatogram) heat map mass chromatogram with lipid signals (R_f vs. m/z) boxed corresponding to the MS image. The chromatographic representation provides a 2D high resolution access to TLC-MALDI data. DHB was used as MALDI matrix.

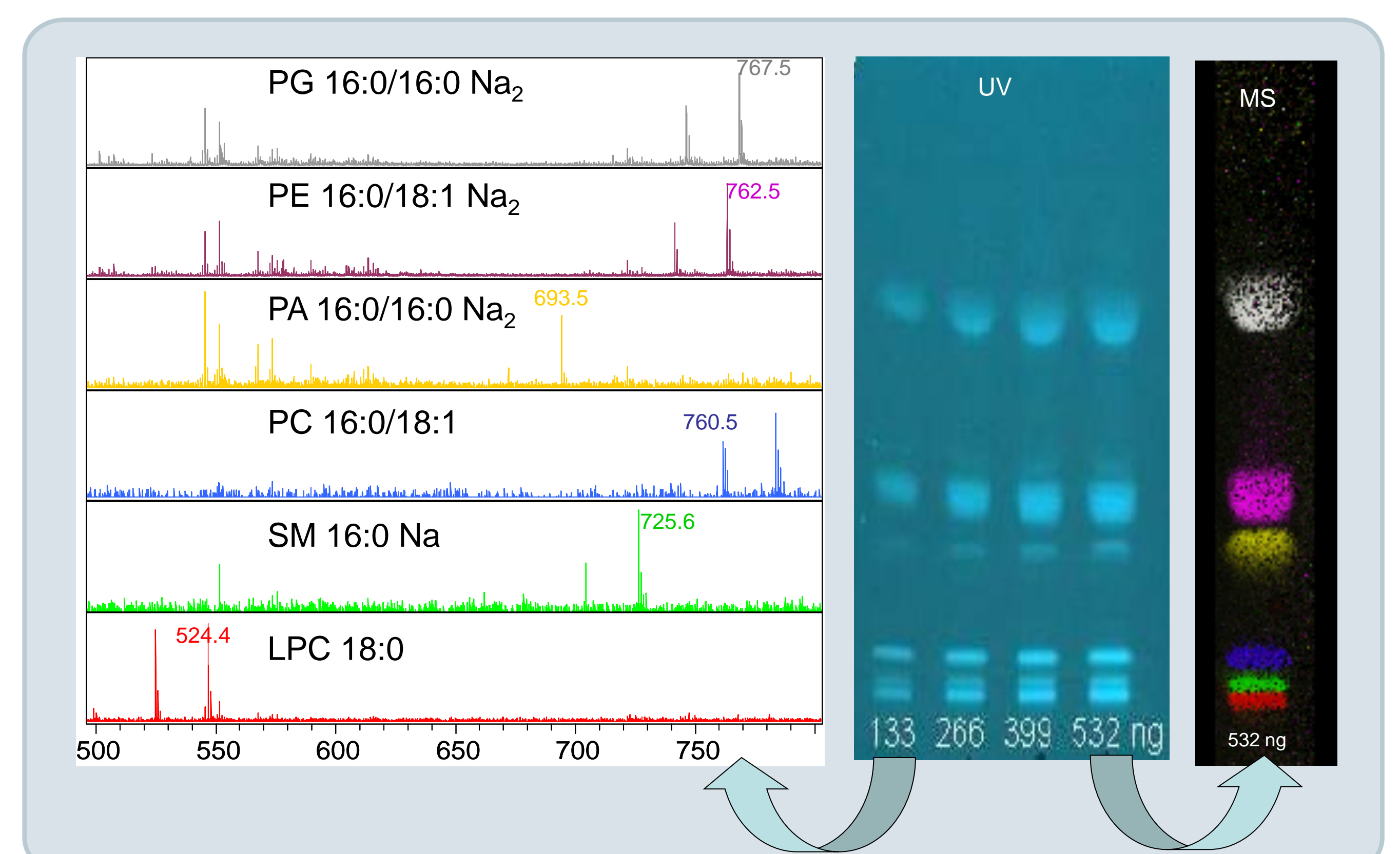


Fig. 3: Detectability of HPTLC-MALDI: A dilution series of lipid standard containing 133-532 ng of each lipid was analyzed. Even at the 133 ng level intense MALDI spectra were obtained that can be well interpreted. Parent ion masses refer to MH⁺ or sodiated peaks.

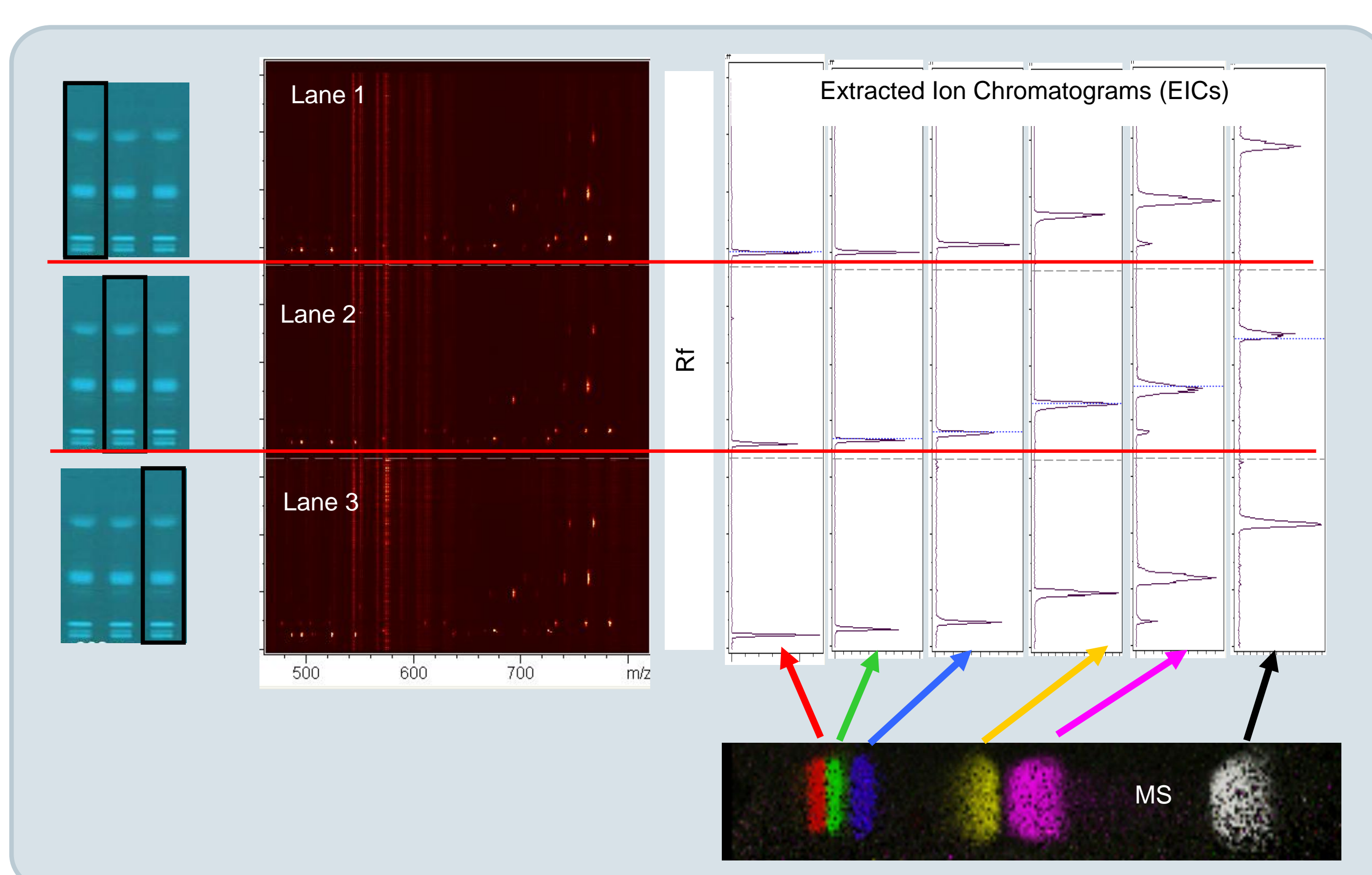


Fig. 2: Repeatability of HPTLC-MALDI. 3 repetitive analyses with 330 ng lipid standard on 2 TLC plates were developed by primuline (left) and MALDI (center). Repeatability of the intensities of extracted parent ion chromatograms (right) is **better than 10 %**.

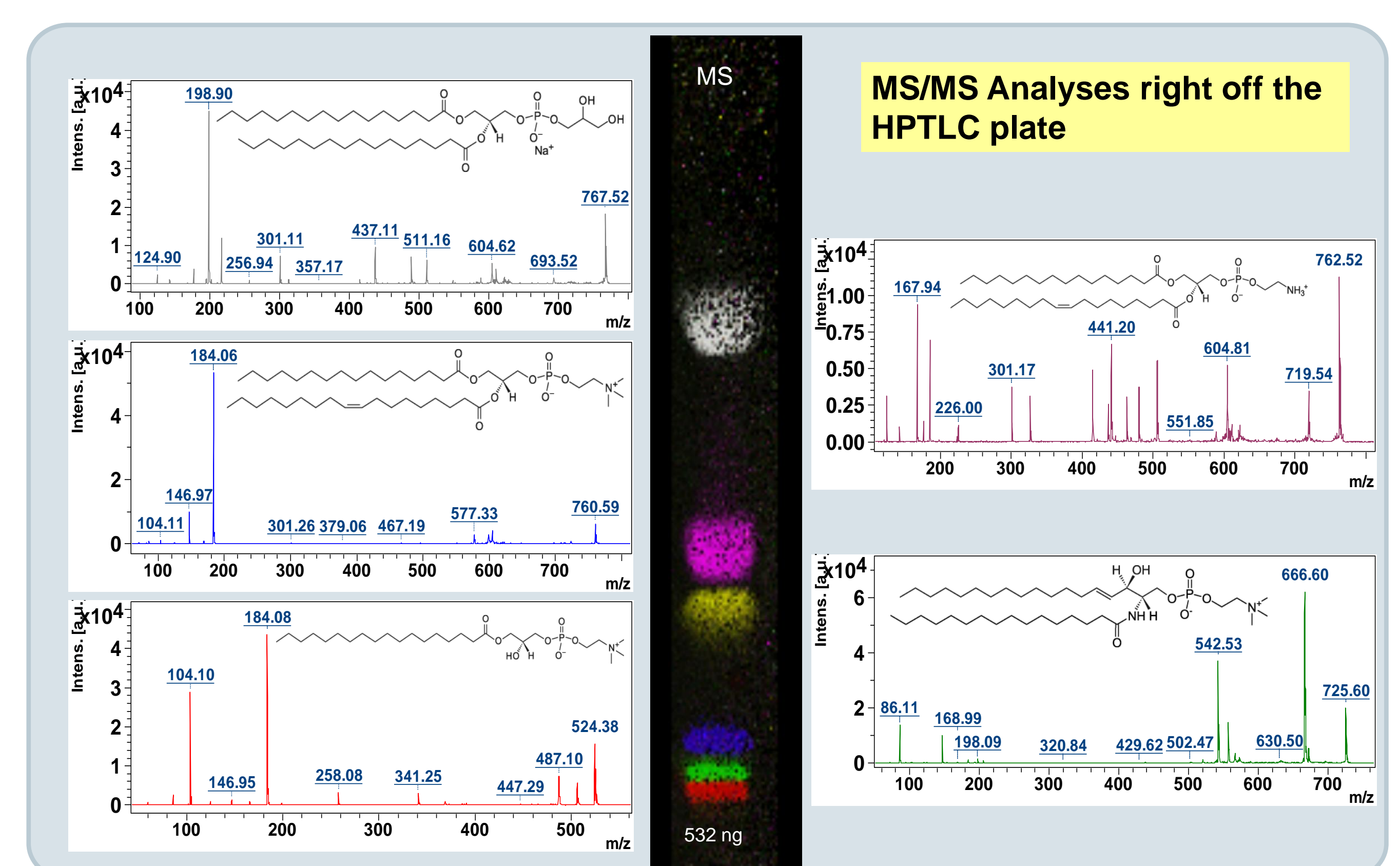


Fig. 4: Structural elucidation Software supported MALDI-TOF/TOF MS/MS spectra acquisition right from the HPTLC plate after MS acquisition. Fragment ions correlate with the respective lipid structure and permit its unequivocal identification and head group assignment.

References

- B Fuchs et al. *A direct and simple method of coupling matrix-assisted laser desorption and ionization time-of-flight mass spectrometry (MALDI-TOF MS) to thin-layer chromatography (TLC) for the analysis of phospholipids from egg yolk*, ABC 2007, **389**: 827-834
- B Fuchs et al. *Analysis of stem cell lipids by offline HPTLC-MALDI-TOF MS*, ABC 2008, **392**: 849-860

Conclusions

HPTLC-MALDI is a largely automated, software-supported new method:

- Chromatographic resolution of HPTLC is maintained throughout the MALDI analysis **Fig. 1**
- Reproducibility of HPTLC-MALDI is in the 10 % range **Fig. 2**
- Sensitivity of HPTLC-MALDI for lipids is in the 100 ng range **Fig. 3**
- Structural analysis can directly follow up using HPTLC-MALDI-MS/MS **Fig. 4**
- **suitable for lipid analysis for clinical, nutritional, pharmaceutical and cosmetic analysis**

HPTLC-MALDI