

Matrix-free TLC-MALDI Analysis of Porphyrin Mixtures

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Introduction

Thin-layer chromatography (TLC) remains important in many laboratories with several applications. Lately, TLC-MALDI has been developed to increase the scope of qualitative analysis and compound identification from complex mixtures. Typically, a matrix is applied to the developed TLC plate to detect analyte species.

In this study, mixtures of porphyrin and related compounds were prepared and analyzed by TLC-MALDI without purification and without added matrix. LD-MS analysis of porphyrinoids without added matrix on a conventional target is known, but the methodology had not been previously extended to MALDI-TLC.

Porphyrins are of biological relevance as exemplified by heme and chlorophyll. Due to their rich coordination, electrochemical, and photochemical properties, porphyrins also find application in several domains such as in medical imaging and therapy, as well as in light harvesting and energy/electron transfer processes.

The synthesis of porphyrins and related porphyrinoids can afford complex mixtures of porphyrins/porphyrinoids and other oligomers. Matrix-free, MALDI-TLC has the potential to provide a simple and rapid analytical method for identifying a target compound from other byproducts, and thereby confirming the formation and guiding the chromatographic purification of the desired porphyrin/porphyrinoid, even among compounds of with similar polarity and R_f values

Methods

A Bruker Autoflex maX MALDI-TOF mass spectrometer, equipped with a 2000 Hz SmartBeam laser was used in positive reflector mode with 20 kV acceleration voltages for the analysis of TLC separated sample spots.

The porphyrin reaction mixtures were applied on to a silica TLC plate and developed in CH₂Cl₂/hexanes (1:1).

Results

The potential for matrix-free, MALDI-TLC analysis is shown by the detection of four porphyrinoids from a mixture spotted on a silica TLC plate (Figure 1).

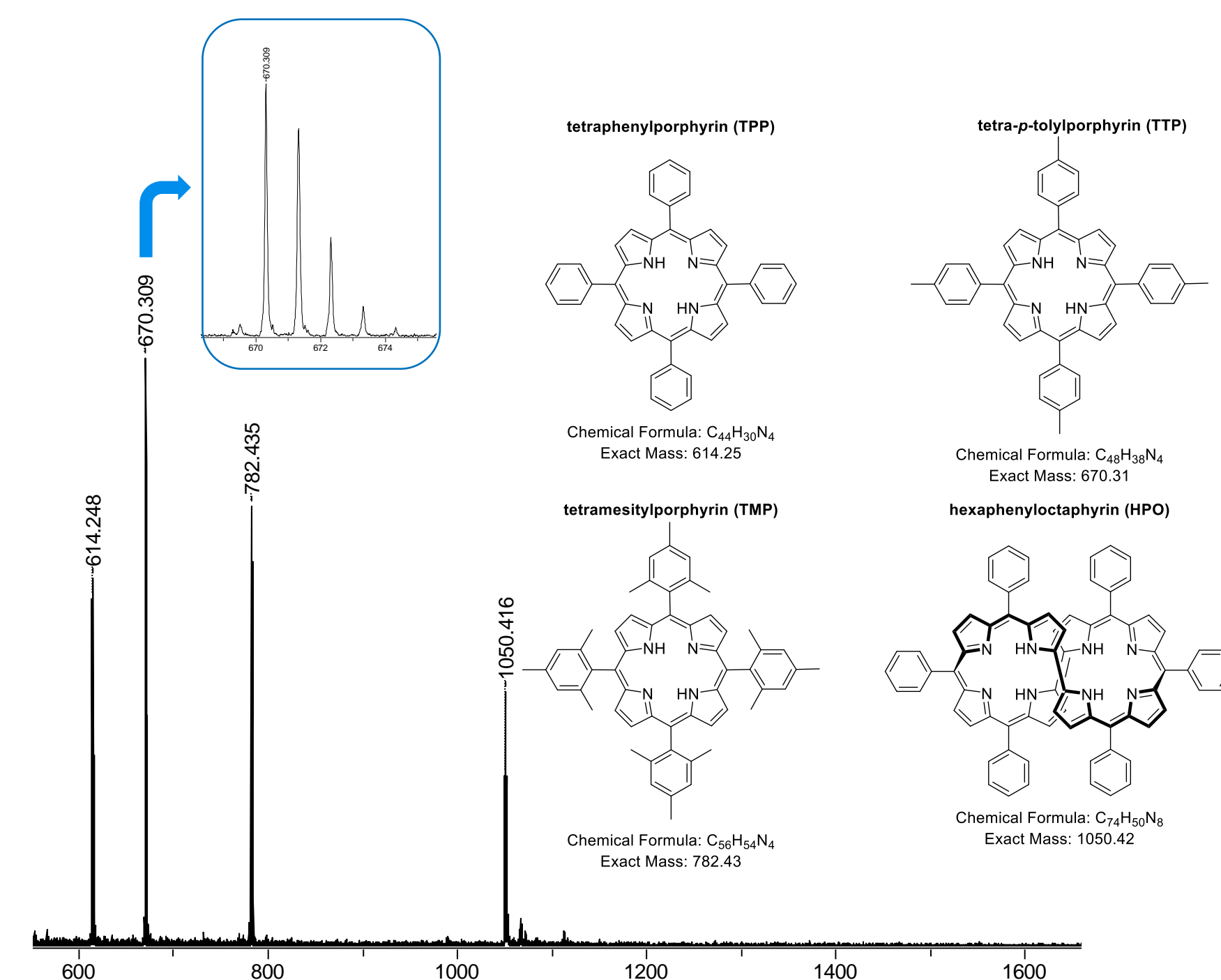


Fig. 1 Matrix-free, MALDI-TLC spectrum of a porphyrinoid mixture used for mass calibration.

The method has been applied to an impure reaction mixture of porphyrins. The porphyrins were separated by TLC into closely eluting bands (Figure 2).

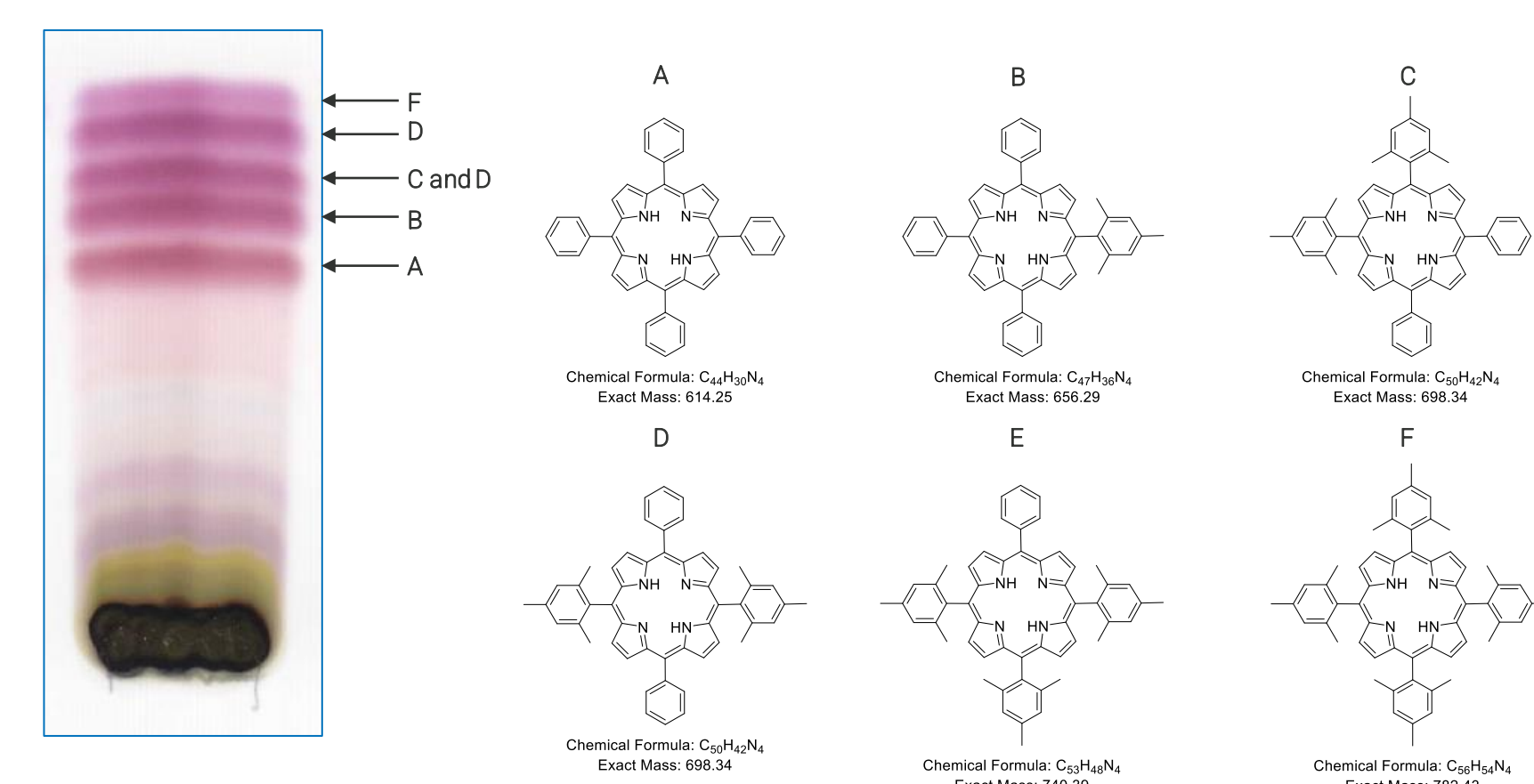


Fig. 2 Image of the developed TLC plate from a porphyrin mixture derived from the reaction of pyrrole with benzaldehyde and mesitaldehyde.

Each band containing porphyrin was analyzed by matrix-free, MALDI-TLC, and spectra consistent with the expected component of each band were obtained (Figure 3).

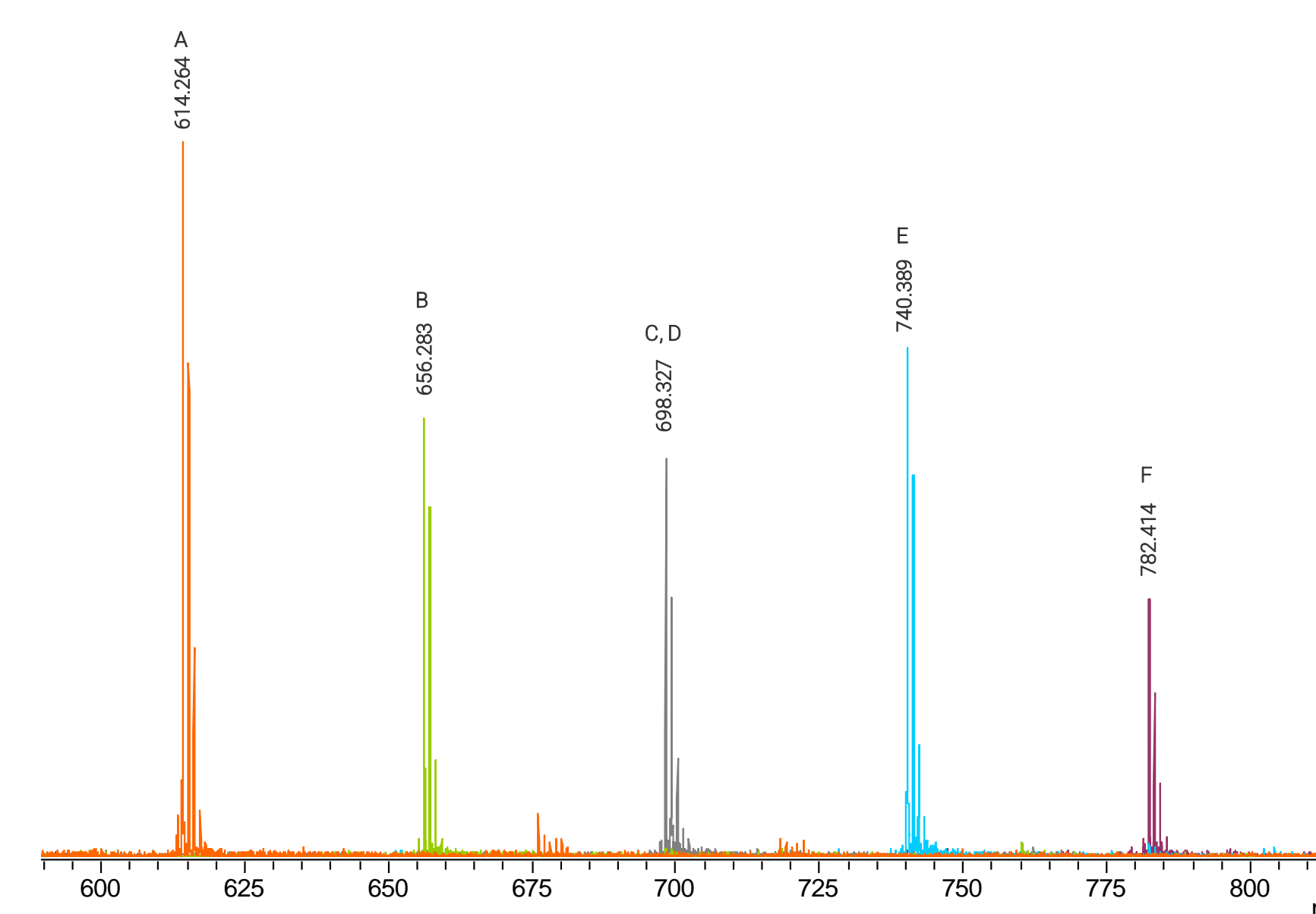


Fig. 3 Matrix-free, MALDI-TLC spectrum of a porphyrin mixture derived from the reaction of pyrrole with benzaldehyde and mesitaldehyde.

To further explore the scope of the matrix-free, MALDI-TLC methodology, a mixture of calixphyrins was prepared from the reaction of pyrrole with pentafluorobenzaldehyde and acetone. Calixphyrins differ from other porphyrins by the presence of one or more sp³-hybridized bridging carbon atoms that disrupt the conjugation of the macrocycle. Thus, calixphyrins can be more difficult to desorb and ionize, and they can produce fragments due to loss of alkyl groups at the sp³-hybridized positions.

Matrix-free, TLC-MALDI afforded the detection of peaks consistent with co-eluting phlorin and cis-porphodimethene (Figure 4). In addition to the molecular ions, peaks were detected due to the loss of one or more methyl groups, as well as a sodium adduct.

Peaks were not detected from other bands derived from calixphyrins with less conjugation.

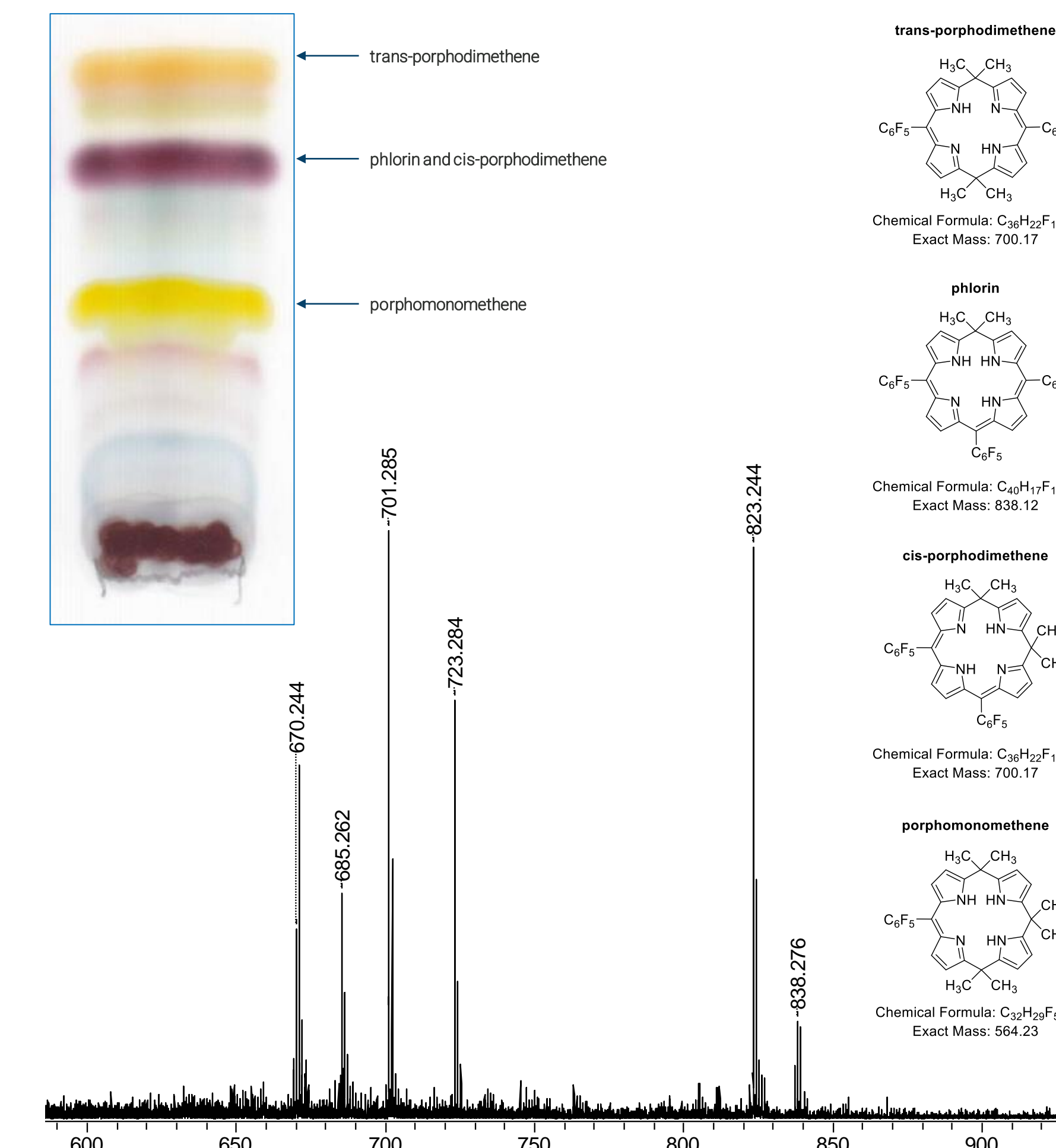


Fig. 4 Matrix-free, MALDI-TLC spectrum of the band containing phlorin and cis-porphodimethene.

Conclusion

- TLC-MALDI is a promising method for the matrix-free analysis of porphyrin mixtures.
- Direct analysis from TLC plates is simple, and scraping and extraction of spots is not necessary.
- The spacial resolution of analytes in closely eluting bands is excellent.

Acknowledgement

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