

Combining TLC Separation with MS Detection - A Revival of TLC

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It is very likely that every natural scientist has at least heard about thin-layer chromatography (TLC) because its fundamentals are taught in all basic courses of chemistry and biochemistry. TLC is widely used in preparative organic chemistry for a first check of a reaction mixture to monitor how many different products were generated. One typical application in biochemistry is the separation of plant dyes; this is particularly simple because the analytes are coloured and, thus, no staining step is necessary.

From these examples it is already obvious that TLC is a common (particularly in lipid and carbohydrate research) and relatively inexpensive method which offers many advantages [1]. Some of them are mentioned below

- TLC enables the simultaneous investigation of several samples.
- Since a new stationary phase is used for each sample, memory effects can be completely excluded.
- The application of specific dyes to detect selected compounds is very simple.
- The amount of necessary solvents is moderate. This makes TLC environmentally friendly.

Despite these clear advantages, liquid chromatography (LC) is nowadays more frequently used to analyze complex mixtures. This is - among other factors - surely deriving from the fact that LC can be easily combined with mass spectrometry (MS): the solvent stream coming from the LC column is directly introduced into the mass spectrometer. This coupling (particularly when combined with MS/MS) gives therefore detailed information about the compositions of all individual fractions. This is (at least at the first glance) much more difficult when TLC is used [2]: of course, it is possible to re-extract the analytes of interest from the stationary phase (often unmodified silica gel) and to characterize them independently by MS [1]. However, this approach is tedious and time-consuming particularly when many different fractions and/or samples have to be analyzed. Additionally, it cannot be excluded that some compounds stick very tightly to the silica and cannot be quantitatively re-eluted [3]. This "effect of chromatography" is particularly well known in the lipid field [4].

In order to make the sample re-elution process faster and more convenient, a plunger based extraction interface (now commercially available as the "TLC-MS Interface" from CAMAG) combined with an HPLC pump was shown to provide good results for quantitative TLC/ESI (electrospray) MS from silica gel plates [5]. Despite the good results achievable and the wide applicability, this is, nevertheless, an indirect (off-line) approach since previous extraction of the sample of interest is required.

Fortunately, there are different MS desorption techniques available which enable the analysis of compounds directly from a solid surface - such as the surface of a TLC plate. Desorption electrospray ionization (DESI) MS [6] and matrix-assisted laser desorption/ionization (MALDI) MS [7] are so far the most established techniques. Both methods can be used to analyze compounds directly from a TLC plate whereby our prime interest is on MALDI MS because MALDI mass spectrometers are already available in many laboratories. Direct

TLC/MALDI MS analysis is possible for both, (phospho)lipids [8] as well as carbohydrates [9], although the application in lipid research is much more established [10].

The most simple way to combine TLC with MALDI MS is to stick the TLC plate (with aluminium back because electric conductivity is required) subsequent to matrix deposition onto a standard MALDI target. However, this approach has two disadvantages: on the one hand, in order to know where the matrix has to be applied spots of interest must be previously identified by staining. This is normally done by applying the same sample two times on the same TLC plate: the first set of samples is stained while the second set of samples is used for subsequent TLC/MS. On the other hand, the achievable mass accuracy is quite poor under these conditions since the TLC plate protrudes above the MALDI target. Nowadays, specially designed adapter targets are available as well as dedicated software which allows the automated screening of the entire TLC plate [11].

In addition to this hardware and software progress, there were recently also significant improvements of the TLC plates: MERCK KGaA, for instance, now offers dedicated "MS grade" TLC plates which give two important advantages: first, these TLC plates were optimized regarding the content of MS-detectable impurities, i.e. these plates are characterized by a very low MS background. Second, the silica layer thickness has been decreased and is now only 100 µm (in comparison to 200 µm on standard TLC plates) [12]. This confers the advantage that the analytes are located closer to the surface. Since UV irradiation (the majority of commercially available MALDI mass spectrometers are equipped with a UV laser) does not penetrate deeply into the silica layer [13], this leads to a higher sensitivity and/or a reduced matrix background. This is illustrated in Figure 1.

However, what about quantification? When performing LC/MS internal standards (IS) are mandatory if quantitative data is required. Therefore one dedicated (¹³C or ²H labelled) lipid of known concentration per lipid class is normally used.

For TLC MALDI MS the shot-to-shot reproducibility of the MALDI measurements is a weak point which makes quantitative data analysis challenging. This particularly applies when common crystalline matrices (such as 2,5-dihydroxybenzoic acid (DHB)) are used since the co-crystals between matrix and analyte are never absolutely

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homogeneous [14]. The use of infrared (IR) lasers seems beneficial to overcome such quantitative concerns because glycerol can be used as IR matrix. Glycerol is a high vacuum stable liquid and mixes, thus, more homogeneously with the analyte. It has been convincingly shown that quantitative data about lipid concentrations can be obtained under these conditions [15] by using directly the peak intensities.

The approach with internal standards has as well weaknesses regarding TLC MALDI MS: depending on the length of the fatty acyl

residues for example, slightly different "ratio of front" (Rf) values are obtained - even if normal phase TLC is used [16]. Thus, different mass spectra are acquired depending on the position where the laser beam hits the TLC plate. The diameter of a typical MALDI laser spot is about 30 μm and, thus, the internal standard is not detectable in all spectra. This is illustrated in Figure 2.

In a nutshell, the possibility to combine TLC separation with MS detection has significantly increased the interest in TLC during the last years. This combined technique is fast and convenient and has the particular advantage that no additional dedicated (expensive) devices are required but a common MALDI MS device is sufficient. Although the quantitative analysis of the mass spectra recorded directly from a TLC plate is still a challenging issue, we are confident that this problem can be solved in the future.

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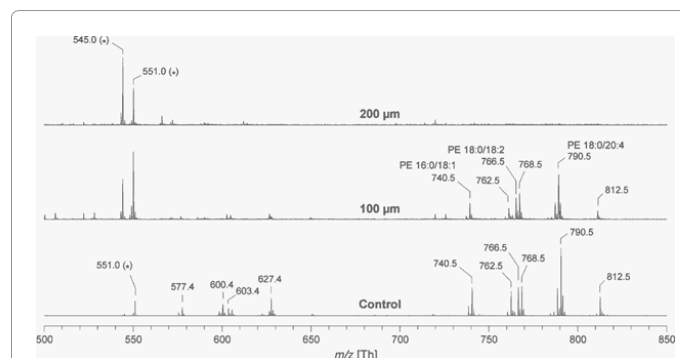


Figure 1: Positive ion MALDI-TOF mass spectra of the phosphatidylethanolamine (PE) fraction from hen egg yolk recorded directly from TLC plates with different thicknesses (indicated in the figure) of the stationary phase. The spectrum labelled with "Control" was desorbed directly from a conventional stainless steel MALDI target. Abundant PE signals are directly assigned in the figure (in this x:y nomenclature, x denotes the number of carbon atoms and y the number of double bonds per fatty acyl residue) and matrix peaks (DHB, 2,5-dihydroxybenzoic acid) are marked by asterisks. Note that reasonable peak intensities can be exclusively achieved in the case of a reduced silica gel thickness. Reproduced from Griesinger et al. [12] *Anal. Biochem.*, 451, 45-47, 2014 with permission from Elsevier.

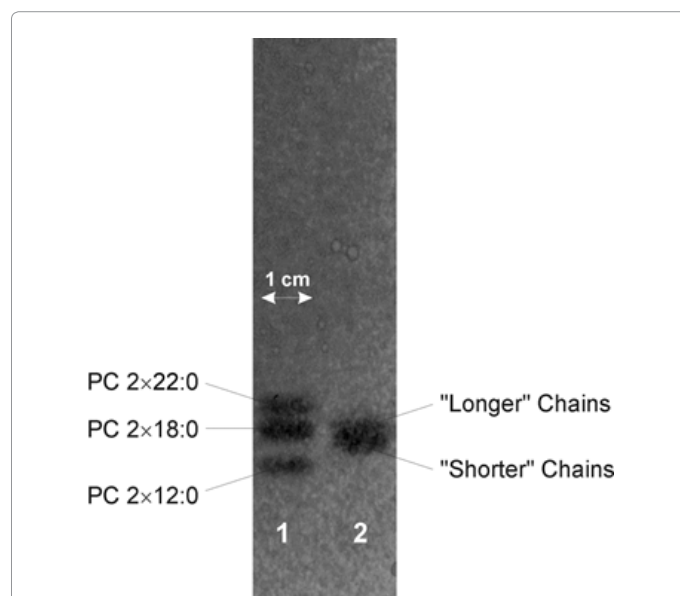


Figure 2: Image of a developed (normal phase) TLC plate subsequent to staining with primuline [8]. CHCl_3 , ethanol, water, and triethylamine (35:35:7:35, v/v/v/v) were used as the solvent system. In lane 1 an equimolar mixture of PC 2 x 12:0, PC 2 x 18:0, and PC 2 x 22:0 was used, while lane 2 corresponds to PC isolated from hen egg yolk. While the individual PC species of the artificial mixture can be clearly differentiated (due to their Rf differences), the PC species from the biological material result in a rather broad spot. However, different PC species can be detected within this spot when MALDI MS is used (spectra not shown). To our best knowledge the chain length of the fatty acyl residues has the most pronounced impact on the chromatographic properties.

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