# Bioassay-DART-MS on HPTLC plates

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## Introduction

The hyphenation of high-performance thin-layer chromatography (HPTLC), direct bioautographic assay (DB) and structure elucidation methods offers a promising possibility directly to screen and identify bioactive substances of complex samples. Contamination and ion suppression of MS-system is a significant challenge in hyphenation of HPTLC-DB with structure elucidation methods using elution-based TLC-MS, since most of the bioassay-matrix is eluted into the MS-system. In this context, applying direct analysis in real time mass spectrometry (DART-MS) could be more effective, since the desorption-based DART-MS method discriminates most compounds of the matrix in the desorption and ionization process. HPTLC-DB-DART-MS enable to record the mass spectra from bioactive zones after DB with low contamination of the bioassay-matrix. In this study, the possibility of Bioassay-DART-MS on HPTLC plates in case of parabens in cosmetics was demonstrated for the first time. Chemical preservatives like parabens (methyl-, ME; ethyl-, EE; propyl-, PE; butylparaben, BE) were widely utilised in food and cosmetic products due to their strong antimicrobial activity. Although they have relatively less hazardous to health in comparison to other preservatives[1], the *Bundesinstitut für Risikobewertung* (BfR) recommends, based on entire toxicological data of *in vivo* assays, that the level limit in cosmetic products be lower than 0,4 % for ME and EE and lower than 0,19 % for PE and BE. A time-saving approach to screen, characterize and identify parabens in complex samples is thus of interest.

## Results and discussion

### *Aliivibrio fischeri*-DART-MS

A 8 band (8 mm) pattern of ME, EE, PE and BE (each 3 – 960 ng/band) were applied on RP18 $WF_{254}s$ and Si60 $F_{254}$ plates and immersed into the bacteria suspension of *A. fischeri* . Each pattern was scanned by DART-MS without bioassay and one afterwards. DART interface settings were: source angle: 60° ; gas heater: 500 ° C; gas flow 3 L/min; Cap ID: 1.0 mm; and carrier movement: 0.2 mm/s. DART-MS scan showed that the background signals of Si60 $F_{254}$ plates with only applied parabens was comparable to the background after *A. fischeri* assay. Whereas the background of RP18 $WF_{254}s$ plates was lower after *A. fischeri* assay (Figure 1 a-c). Detectable substance amount without *A. fischeri* was similar between Si60 $F_{254}$ and RP18 $WF_{254}s$ plate, MS signal was a little stronger on RP18 $WF_{254}s$ plate. But there was a difference of MS signals after immersing plate into *A. fischeri* between RP18 $W_{F254}s$ and Si60 $F_{254}$ plate. MS signal suppression on Si60 $F_{254}$ plate was strong, therefore small substance amount of ME and EE could not be detected by DART-MS.

### pYES-DART-MS

pYES assay was performed on RP18 W plate. DART-MS showed that background of RP18 W plate was lower without pYES. MS signal was detectable at every application volume, but after immersing plate into pYES suspension MS signal suppression was strong.

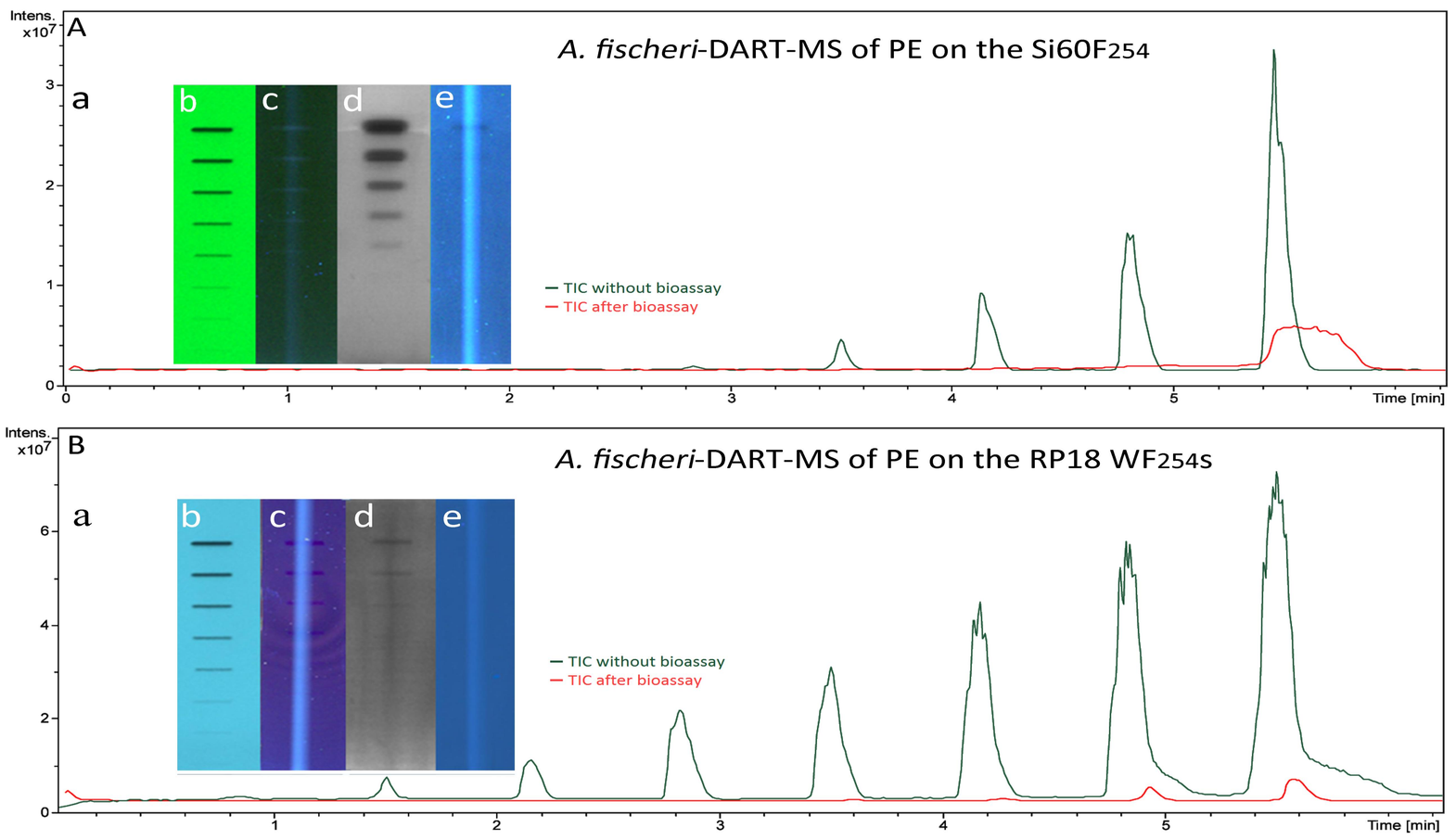


**Fig. 1** *A. fischeri*- DART-MS of PE on Si60 F254 (A) and RP18 WF254s (B) plates. DART-MS spectrum (a), natively pattern of PE on the plates at UV 366 nm (b), inhibition activity of *A.fischeri* under BioLuminizer (d). The plate after DART-MS measurement before (c)and after bioassay (e)at UV 366 nm.

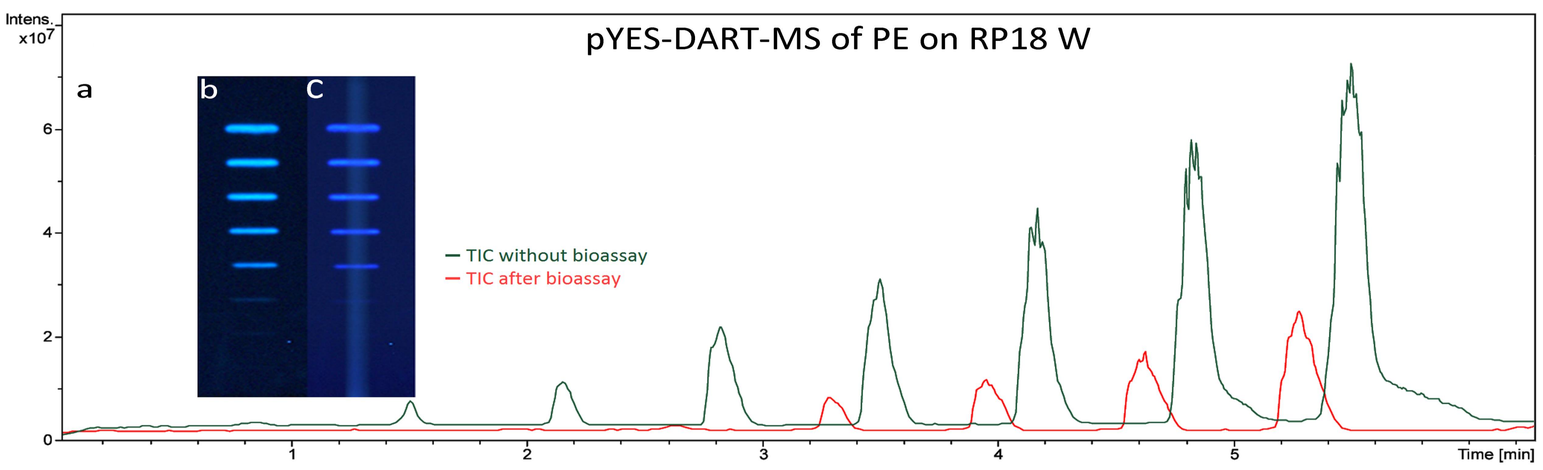


**Fig. 2** pYES-DART-MS of PE on RP18 W. DART-MS spectrum (a), natively bioactivity of PE pattern (b) and after DART-MS measurement (b) at UV 366 nm

| *A. fischeri* on Si60 $F_{254}$ | | |
|---|---|---|
| Signal area [cps] | | Remaining signal [%] |
| without bioassay | after bioassay | |
| - | - | - |
| - | - | - |
| 30850 | - | - |
| 241505 | - | - |
| 2532815 | - | - |
| 15959559 | - | - |
| 45750439 | 473128 | 0.8 |
| 113650688 | 31081785 | 19.5 |
| *A. fischeri* on RP18 $WF_{254}s$ | | |
| Signal area [cps] | | Remaining signal [%] |
| without bioassay | after bioassay | |
| 1246643 | - | - |
| 4336887 | 23170 | 0.5 |
| 14891683 | 40412 | 0.3 |
| 72853610 | 101088 | 0.1 |
| 118792156 | 168206 | 0.1 |
| 196620367 | 419802 | 0.2 |
| 298707388 | 3565439 | 1.2 |
| 447750357 | 11121570 | 2.5 |
| pYES on RP18 W | | |
| Signal area [cps] | | Remaining signal [%] |
| without bioassay | after bioassay | |
| 1246643 | - | - |
| 4336887 | - | - |
| 14891683 | 495119 | 3.3 |
| 72853610 | 1449382 | 2.0 |
| 118792156 | 11207224 | 9.4 |
| 196620367 | 30051376 | 15.3 |
| 298707388 | 62880939 | 21.1 |
| 447750357 | 122055019 | 27.3 |

**Table 1** Comparison of remaining signal of DART-MS based on signal areas of PE pattern on the different plates before and after bioassays

## Perspective

As it was shown, although MS signal suppression after different bioassays was strong, it still was possible to detect parabens by DART-MS. In future also cosmetic products should be scanned after biossay by DART-MS. This makes it possible to identify directly substances, after they showed bioactive reaction in real time.

**References** [1] M.G. Soni, S.L. Taylor, N.A. Greenberg, G.A. Burdock, Food and Chemical Toxicology 40 (2002) 1335–1373

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