ON VERSATILITY OF TLC-MS INTERFACE AND ITS ANALYTICAL IMPORTANCE

Teresa Kowalska & Mieczysław Sajewicz

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

International Symposium for Thin-Layer Chromatography, Basel (Switzerland), July 6th – 8th, 2011

Outline of presentation

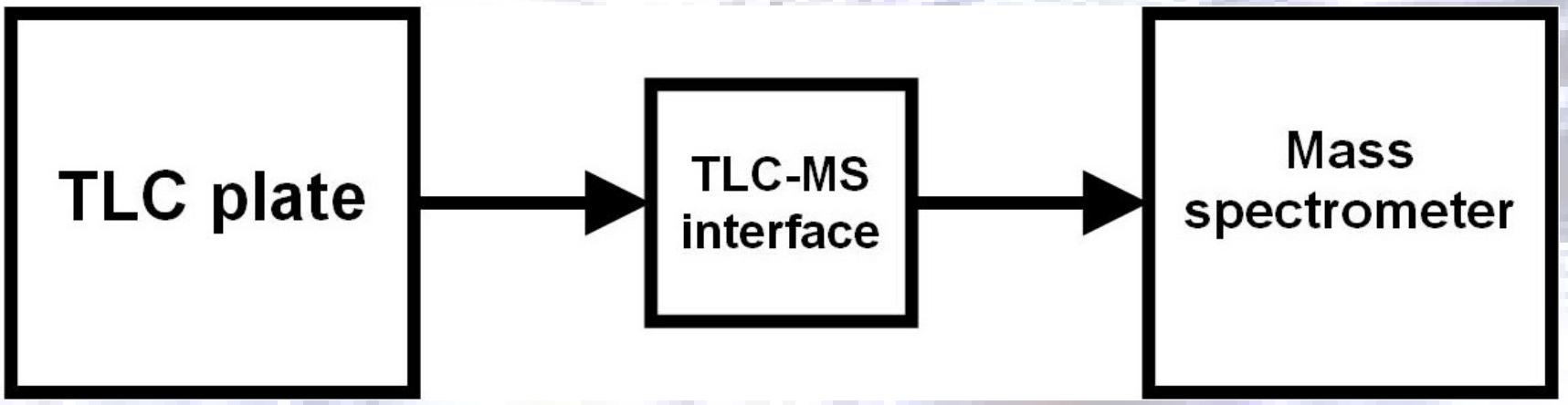
- The TLC-MS interface
- 1D TLC-MS systems
  - General scheme
  - Main advantages
  - Selected bias
  - Illustrative examples
- 2D TLC-HPLC systems
  - General scheme
  - Main advantages
  - Selected bias
  - Illustrative examples
- Conclusions

The TLC-MS interface

Manufactured by Camag, Muttenz, Switzerland

1D TLC-MS systems

General scheme of the 1D TLC-MS systems



1D TLC-MS systems

Main advantages of the 1D TLC-MS systems:

(i) A handy novel analytical mode of hyphenated TLC;

(ii) Direct coupling of a TLC plate with mass spectrometer, without any pretreatment of the separated chromatographic bands;

(iii) A possibility of couping a TLC plate with a variety of different mass spectrometer types;

(iv) A possibility of selecting individual bands of interest for MS analysis (and a possibility of skipping the others);

(v) With baseline TLC separation of a simple mixture (one chemical species per one chromatographic band), possible mass spectrometric identification of these species;

(vi) With TLC fractionation of a complex mixture (resulting in simpler mixtures per one chromatographic band), possible mass spectrometric fingerprinting of a given band and an occasional identification of individual constituents thereof;

(vii) A possibility of coupling a TLC plate with a detection system different than mass spectrometer.

1D TLC-MS systems

Selected bias of the 1D TLC-MS systems:

Generally, the same bias as with any other application of mass spectrometry.

The ionization mode

(i) The milder is the ionization mode of a given mass spectrometer, the lesser is the fragmentation and hence, the greater is the chance to trace a molecular ion of an analyte.

(ii) Consequently, the ESI-MS system seems particularly recommended as the most preservative one.

The analyte(s)

(i) Even with the relatively mild ESI-MS ionization mode, fragmentation occasionally occurs. As aromatic compounds are more resistant to fragmentation and moreover, have better defined fragmentation patterns than aliphatic compounds, the 1D TLC-MS systems are better suited for the analysis of aromatics than aliphatics.

(ii) With aliphatic analytes, the 1D TLC-MS systems provide in the first instance the fingerprint information.

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

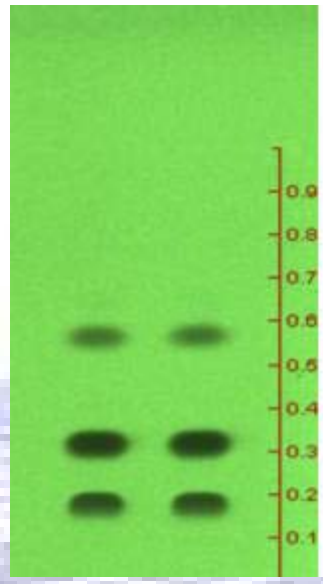
1D TLC-MS systems

Illustrative example (1):

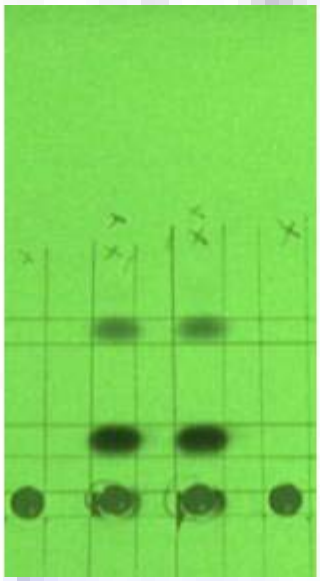
H. Luftmann, M. Aranda, H. Morlock, Rapid Commun. Mass Spectrom. 21 (2007) 3772

Analytical problem:

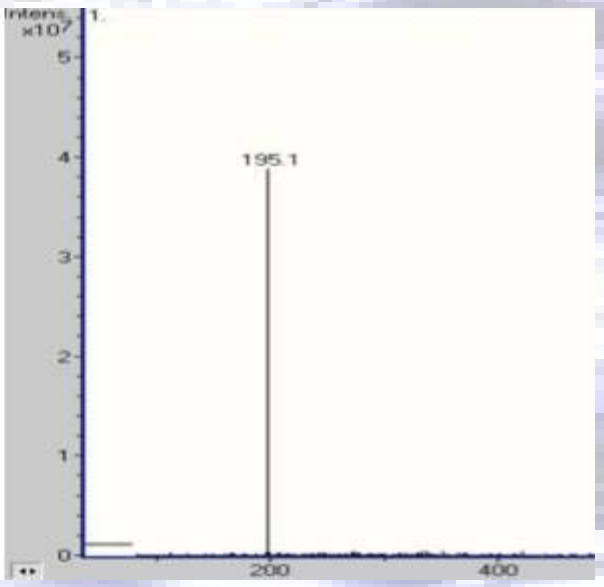
Identification of a substance in a mixture (caffeine, paracetamol, acetylsalicylic acid)



Chromatogram with 4 mm bands



Same plate after extraction of spot RF 0.15



Extracted spot identified as caffeine with MS (APCI ionisation); extraction and MS-measurement of this spot was achieved within 40 s

1D TLC-MS systems

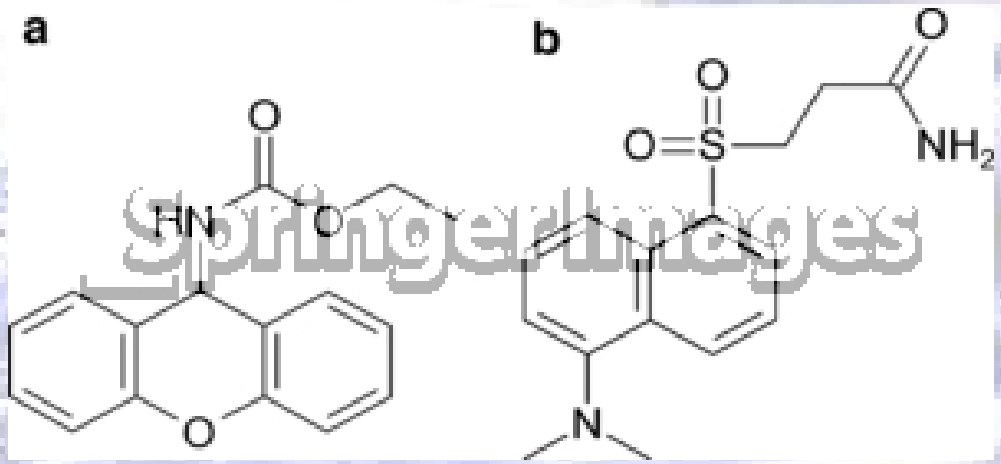
Illustrative example (2):

A. Alpmann, G. Morlock, Anal. Bioanal. Chem. 386 (2006) 1543

Analytical problem:

Improved online coupling of planar chromatographywith electrospray mass spectrometry: extraction of zones from glass plates

Test analytes:



Structure formulas of *a* xanthyl ethyl carbamate (XEC) and *b* dansyl ethylamide (DEA)

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

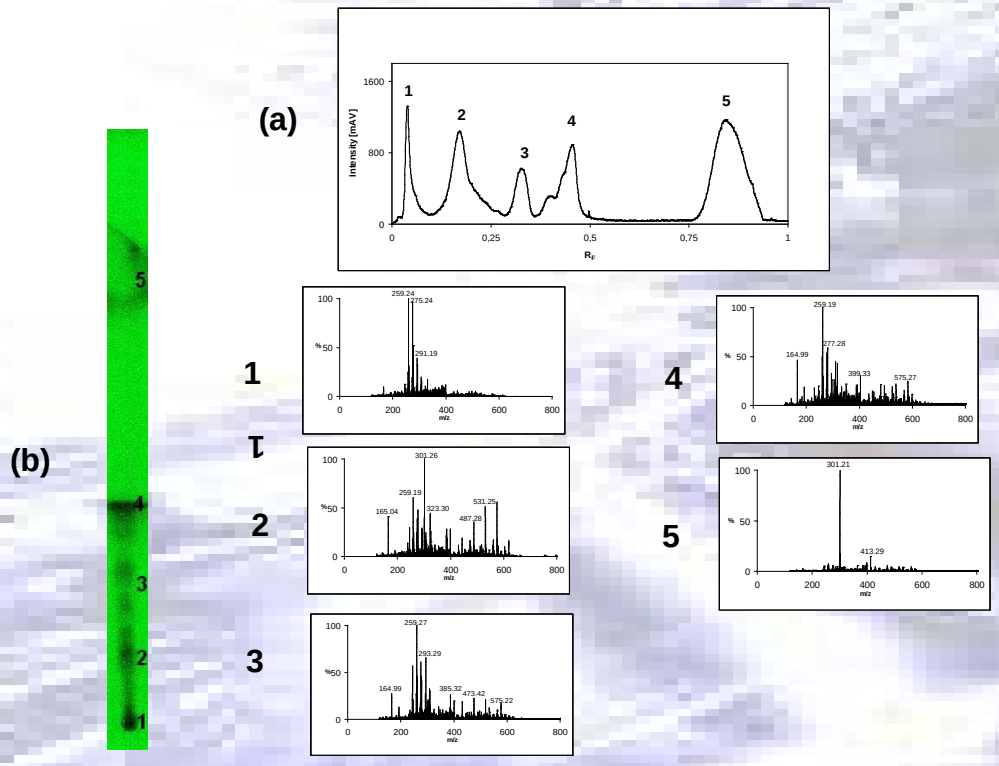
1D TLC-MS systems

Illustrative example (3):

M. Sajewicz, Ł. Wojtal, M. Hajnos, M. Waksmundzka-Hajnos, T. Kowalska, J. Planar Chromatogr. – Modern TLC, 23, 270-276 2010

Analytical problem:

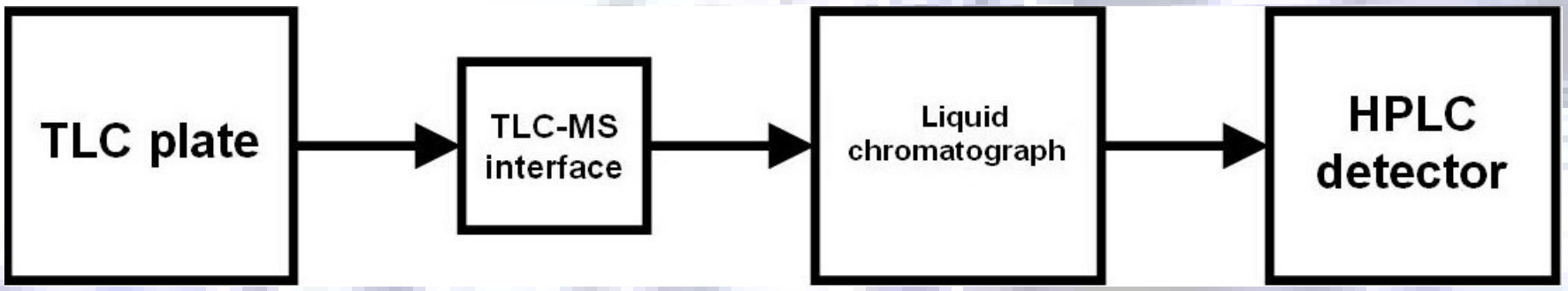
Fingerprinting of botanical material



(a) The densitogram, (b) the videoscan, and (1)-(5) the mass spectra of the five separated chromatographic bands derived from the essential oil sample of *Salvia hians*

2D TLC-HPLC systems

General scheme of the 2D TLC-HPLC systems



2D TLC-HPLC systems

The TLC-MS interface can be used as a practical and versatile building block that enables devising of different TLC–HPLC systems equipped with practically any type of HPLC detector.

Main advantages of the 2D TLC-HPLC systems:

(i) The TLC separation step can be applied to samples with a complex matrix, without preliminary purification (in that way avoiding, e.g., the SPE, or similar tedious sample preparation procedures);

(ii) With complex samples, the TLC separation step can be used as a preliminary group separation. In that way, the 2D TLC-HPLC system can be built, without a necessity to analyze each separated fraction by means of HPLC.

(iii) The 2D TLC-HPLC system can be equipped with any detector type used in HPLC (and not only with the mass spectrometric detector).

(iv) E.g., the 2D TLC-HPLC system equipped with the universal ELSD detector can prove particularly useful for the HPLC fingerprinting of selected fractions derived from herbal material.

(v) Theoretically at least, each separated band can be analyzed with use of a different HPLC detection system. Alternatively, two or more HPLC detectors can be coupled in a sequential order to provide a diverse information.

2D TLC-HPLC systems

Selected bias of the 2D TLC-HPLC systems:

Generally, the same bias as with the 1D TLC-MS systems.

Additionally, the TLC fractionation of the analytes-rich cocktails (often of a natural, e.g., a botanical, origin) cannot guarantee full separation even with use of HPLC, which results in complicated HPLC fingerprints.

In such cases, 2D TLC (or another ‘special’ TLC technique) can be involved in order to improve the result of a preliminary planar chromatographic separation.

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

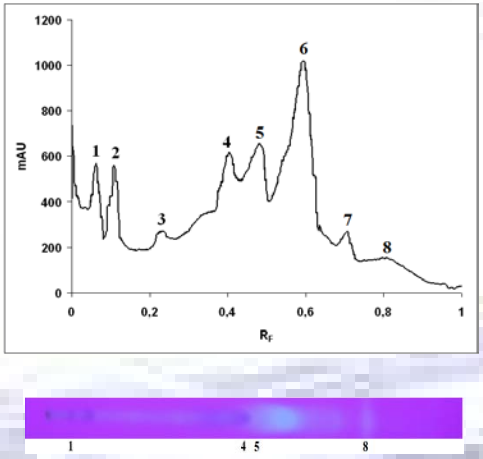
2D TLC-HPLC systems

Illustrative example (1):

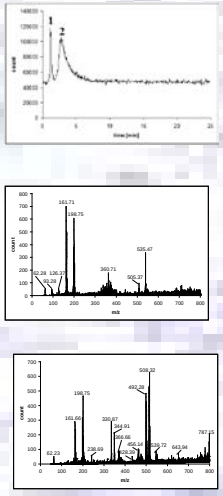
M. Sajewicz, D. Staszek, M. Natić, M. Waksmundzka-Hajnos, T. Kowalska, J. Chromatogr. Sci., (2011) – in press

Analytical problem:

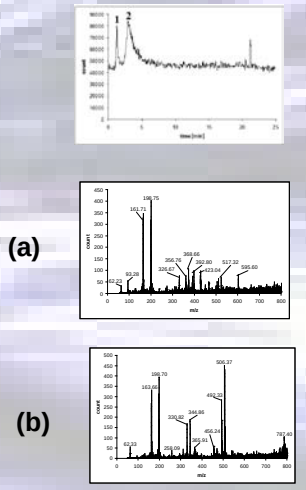
Fingerprinting of phenolic acids fraction selectively extracted from *Salvia lavandulifolia*



The densitogram and videoscan obtained from the analytical thin layer chromatogram developed at 21±0.5°C for the fraction of phenolic acids selectively extracted from *Salvia lavandulifolia*, with the bands of interest indicated by numerals 1, 4, 5, and 8



Liquid chromatogram recorded by means of the LC-MS system for band 1 eluted with use of the TLC-MS interface from the chromatographic plate, with the positions of recording the mass spectra indicated by numerals (1) and (2), and the mass spectra of (a) band 1 and (b) band (2) from the liquid chromatogram.



Liquid chromatogram recorded by means of the LC-MS system for band 5 eluted with use of the TLC-MS interface from the chromatographic plate, with the positions of recording the mass spectra indicated by numerals (1) and (2), and the mass spectra of (a) band 1 and (b) band (2) from the liquid chromatogram.

International Symposium for Thin-Layer Chromatography, Basel (Switzerland), July 6th – 8th, 2011

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

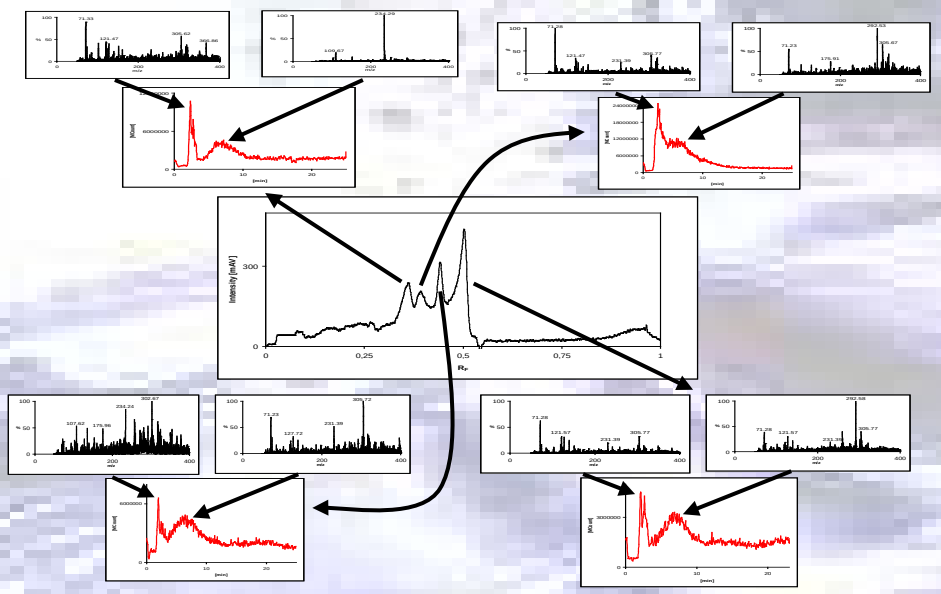
2D TLC-HPLC systems

Illustrative example (2):

M. Sajewicz, Ł. Wojtal, M. Natić, D. Staszek, M. Waksmundzka-Hajnos, T. Kowalska, J. Liq. Chromatogr. Relat. Technol., 34, 848-863 (2011)

Analytical problem:

Fingerprinting of phenolic acids fraction selectively extracted from *Salvia lavandulifolia*



The densitogram, the chromatograms, and the mass spectra of the five separated chromatographic bands derived from the essential oil sample of *Salvia triloba*

International Symposium for Thin-Layer Chromatography, Basel (Switzerland), July 6th – 8th, 2011

Institute of Chemistry, University of Silesia, 9 Szkolna Street, 40-006 Katowice, Poland

2D TLC-HPLC systems

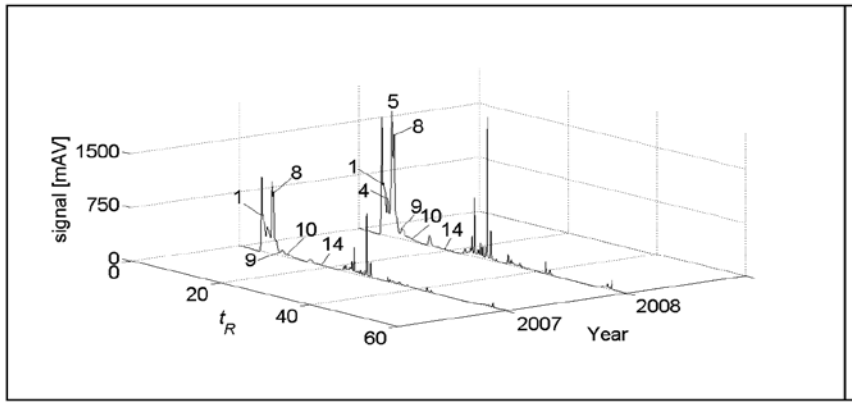
Illustrative example (3):

M. Sajewicz, D. Staszek, Ł. Wojtal, T. Kowalska, M.Ł. Hajnos, M. Waksmundzka-Hajnos, J. AOAC Int., 94 (2011) 71-76

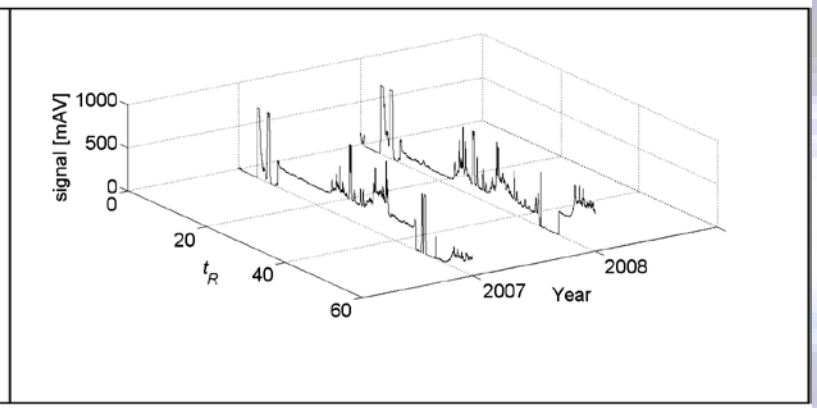
Analytical problem:

A comparison of fingerprinting result of botanical sample with use of HPL-DAD and HPLC-ELSD

HPLC-DAD



HPLC-ELSD



A comparison of the DAD and ELSD fingerprints for the methanol extract of *Salvia nemorosa*

Selected papers of Polish group

on fingerprinting of botanical material with use of 1D TLC-MS and 2D TLC-HPLC-MS systems

M. Sajewicz, Ł. Wojtal, M. Hajnos, M. Waksmundzka-Hajnos, T. Kowalska, „Low-temperature TLC-MS of essential oils from five different sage (*Salvia*) species”, J. Planar Chromatogr. – Modern TLC, **23**, 270-276 (2010)

M. Sajewicz, Ł. Wojtal, M. Natić, D. Staszek, M. Waksmundzka-Hajnos, T. Kowalska, „TLC-MS versus TLC-LC-MS fingerprints of herbal extracts. Part I. Essential oils”, J. Liq. Chromatogr. Relat. Technol., **34**, 848-863 (2011)

M. Sajewicz, D. Staszek, M. Natić, Ł. Wojtal, M. Waksmundzka-Hajnos, T. Kowalska, „TLC-MS versus TLC-LC-MS fingerprints of herbal extracts. Part II. Phenolic acids and flavonoids”, J. Liq. Chromatogr. Relat. Technol., **34**, 864-887 (2011)

M. Sajewicz, T. Kowalska, „TLC/HPTLC fingerprinting of herbal essential oil followed by liquid chromatography hyphenated with the TLC-MS Interface”, Camag Bibliography Service (CBS – “White Pages”), **106**, 11-13 (2011)

M. Sajewicz, D. Staszek, M. Natić, M. Waksmundzka-Hajnos, T. Kowalska, „TLC-MS versus TLC-LC-MS fingerprints of herbal extracts. Part III. Application of the reversed-phase LC systems with C18 stationary phase”, J. Chromatogr. Sci., 2011 (in press)

Conclusions

(i) The TLC-MS interface is a versatile buiding block of multidimensional liquid chromatographic systems, not necessarily confined to coupling TLC plates with mass spectrometer alone.

(ii) The TLC-MS interface enables easy construction of multidimensional analytical systems equipped with different HPLC detectors.

(iii) Such multidimensional systems can easily combine main advantages of TLC and HPLC (like skipping an elaborate sample preparation step, introducing preliminary fractionation of complex mixtures, selective analysis of certain separated fractions, etc.)

(iv) The TLC-HPLC systems seem particularly suitable for the separation and fingerprinting of complex mixtures (like, e.g., herbal material) and can prove a valuable tool in a high throughput quality control thereof.

Thank you so much…

…for your kind attention!