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Introduction to time-of-flight secondary ion mass spectrometry application in chromatographic analysis

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Abstract

New on-line analytical system coupling thin layer chromatography (TLC) and high selective identification unit—time of flight secondary ion mass spectrometry (TOF–SIMS) is introduced in this article. Chromatographic mixture separation and analyte surface deposition followed with surface TOF–SIMS analysis on-line allows to identify the analytes at trace and ultratrace levels. The selected analytes with different detectability and identification possibility were analysed in this hyphenated unit (Methyl Red indicator, Terpinolen and Giberrelic acid). Here, the chromatographic thin layer plays a universal role: separation unit, analyte depositing surface and TOF–SIMS interface, finally. Two depositing substrates and TOF–SIMS compatible interfaces were tested in above-mentioned interfacing unit: modified aluminium backed chromatographic thin layer and monolithic silica thin layer. The sets of positive and negative ions TOF–SIMS spectra obtained from different SIMS modes of analysis were used for analyte identification purposes. SIMS enables analyte detection with high mass resolution at the concentration level that is not achieved by other methods.

Keywords: Thin layer chromatography; Interfacing; TOF–SIMS; Methyl Red; Terpinolen; Giberrelic acid

1. Introduction

Direct analysis of thin layer chromatography (TLC) plates with secondary ion mass spectrometry (SIMS) yields no satisfactory results. Separation process takes place in the bulk of the TLC layer whereas SIMS is a surface-analytical method. During bombardment with primary ions a solid surface with analyte emits neutral particles, and electrons as well as positive and negative secondary ions. These secondary ions are emitted mainly from the uppermost monolayers and can give structural information about the analyte deposited, often on trace or ultra trace level. First "semi-on-line" analyses in TLC–time of flight (TOF)–SIMS system have been described by Merschel [1,2]. The coupling interface was prepared by silver vapour deposition on developed chromatographic thin layer and channel formed by scrapping silica–gel off along edge of the chromatographic layer. The separated analytes (APG, alkyl polyglucosides) were eluted and concentrated by side elution with dimethylsulphoxide (DMSO) onto silver-coated chromatographic thin layer aluminium backplate. Silver layer combines minimal analyte decomposition, its preionisation at surface and high secondary ion yield during mass spectrometric analysis. A further advantage is that silver-containing cations can be observed in the spectra. To overcome the problems with silver deposition (special vacuum chamber) onto developed chromatographic thin layer and cutting TOF–SIMS targets, new procedures of TLC–TOF–SIMS on-line hyphenation were developed [3,4]. A part of chromatographic thin layer was chemically modified to form TOF–SIMS compatible and no background signal-producing interface. The different detectable analytes deposited on this substrate were TOF–SIMS analysed also in scanning mode (to form surface image) when coupling interface has not to be

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cut to small targets [3,5]. There are also other articles documenting hyphenation of TLC with surface-assisted laser desorption ionisation (SALDI) MS [6] in porphyrins analysis. A combined surface sampling probe/electrospray emitter was used for the direct readout of TLC plates by electron spray ionisation (ESI)–MS [7] in organic compounds mixtures. Two interfaces developed to connect TLC with ESI–MS on-line consisted of (1) two bound optical fibres inserted into the C_{18} bonded particles at the exit of a small TLC channel and (2) a small commercial TLC strip with a sharpened tip. Small drugs molecules were structurally analysed by using on-line TLC post-source-decay (PSD) matrix-assisted laser desorption/ionisation mass spectrometry (MALDI) [8]. The main aim of this work was to introduce newly developed analytical method that can couple TLC with TOF–SIMS in selected, model analytes identification. TOF–SIMS, with its simultaneous detection of all secondary ions with one polarity, atomic as well as molecular, and its high lateral resolution (0.2–0.4 μm) is ideally suited to search for possible correlations between organic substances and element distributions as well for analytes identification.

2. Experimental

2.1. Chemicals and samples preparation

Mercury(II) Chloride, methanol (Me–OH), acetonitrile (ACN), sulphuric acid, ethanol (Et–OH) and bromcresol green indicator (BG) were purchased from Slavus s.r.o. (Bratislava, Slovakia). Giberrelic acid (GA), Methyl Red (MR) and Terpinolen (TN) were obtained from Roth GmbH (Karlsruhe, Germany). All of p.a. purity. Redistilled water was used at stock solutions preparation. Chromatographic layers (both aluminium backed) Alugram Sil G/UV_{254} were purchased from Macherey-Nagel (Germany) and RP-18 F_{254} thin layers (5 cm × 7.5 cm) from MERCK GmbH (Darmstadt, Germany). Ultrathin monolithic silica thin layers (UTLC) were supplied by MERCK GmbH (Darmstadt, Germany). Analyte stock solutions were prepared by analyte dissolution in methanol to get concentration 10^{-3} mol/dm^3. Three microlitres of these solutions were applied with microsyringe directly onto 6 cm × 8 cm and 5 cm × 7.5 cm dimensioned thin layers. Only 0.1 μL of stock solutions was applied at monolithic silica thin layer. The spots were primary detected under UV lamp at 254 nm.

3. Methods

3.1. Chromatography

TLC analysis was performed in 10 cm × 10 cm glass developing chambers with mobile phase saturated gaseous phase. The UTLC layers were developed in the chamber 5 cm × 5 cm. Different mobile phases were used for three analytes tested separation. After the layer developing and evaporating of mobile phase at laboratory temperature, the spots were primarily detected. Methyl Red was detected visually, Terpinolen under UV light at 254 nm and Giberrelic acid was first detected with Bromcresol Green solution prepared in accordance with procedure published [9], then visualised with mixture of sulphuric acid (96%)–ethanol (5:95%, v/v) and heated at 120 °C (green–blue spots). All analyses were repeated five times each.

3.2. TOF–SIMS interface preparation

3.2.1. Aluminium backplate modified interface

Factory made, chromatographic silica gel and C_{18} based thin layers (aluminium backed) were used in modified interface preparation. Aluminium backed silica thin layers have been modified with direct application of 0.1 M Mercury(II) Chloride water solution on chromatographic thin layer channel along one side of the thin layer. The impurities from modification procedure have been removed from this new surface with redistilled water. Newly formed surface was porous, homogenous contact interface with remaining chromatographic layer. Interfacial part stores the analyte from chromatographic thin layer without loss of chromatographic integrity and identity.

3.2.2. Unmodified interface represented with ultrathin monolithic silica layer (UTLC)

UTLC monolithic layers were analysed after chromatographic analysis with TOF–SIMS in macro scan mode. There was not specific coupling interface and whole layer was analysed to detect position of analytes separated on the chromatographic layer and focus them to analysis with higher resolution.

3.2.3. The analytes deposition onto interface

Chromatographic thin layer containing integrated TOF–SIMS coupling interface and the analytes separated was developed in second dimension, by side elution with methanol or water. Methyl Red indicator, playing analyte interface transfer marker role has been applied [10]. Quality of analyte interfacial deposition can be controlled with mobile phase velocity measured at interface [10]. Interface with deposited analyte has been then cut out chromatographic layer and fractionated to the small (5 mm × 7 mm) TOF–SIMS targets. Every interface part, characterised with average R_f value, was then analysed with TOF–SIMS separately. By scanning mode has been analysed whole uncut interface as one unit and the images of whole interface were reconstructed from raw data.

3.2.4. Instruments

The TOF–SIMS analyses were carried out with an ion TOF instrument developed at the University of Muenster. It consisted of Ar^+ primary ion-gun. This gun is pulsed with repetition rate of 10 kHz. The primary ion beam was rastered

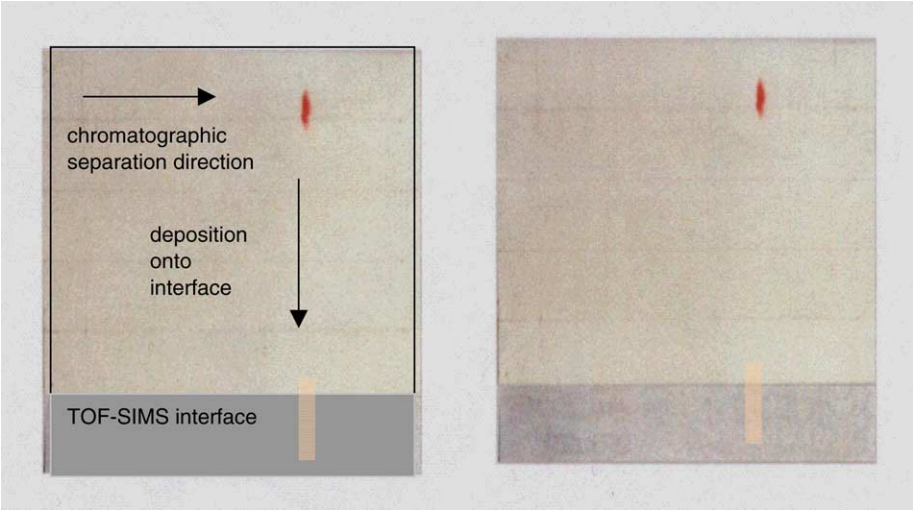

Fig. 1. Principal function of new TLC TOF-SIMS coupling interface. Analyte separated at chromatographic layer is deposited on-line onto TOF-SIMS active interface by side elution.

over a field of 75 mm × 75 mm with an average current of about 0.6 pA. The acquisition time for each measurement was 200 s, which makes up a primary ion dose density of about 1.33×10^{13} cm^{-2}. For charge compensation the electron flooding system with an electron energy of 20 eV has been used. The generated secondary ions are accelerated to 1.8 keV, mass separated in a field free flight path and then detected with ion–electron–photon conversion system.

4. Results and discussion

4.1. Results from Methyl Red TOF–SIMS analyses at aluminium backplate interface

The coupling of TLC with SIMS has one important requirement: analyte separated deposition to uppermost surface and its adsorption at declared chromatographic position to be detected with high sensitivity. The principle of new TLC–TOF–SIMS interface is shown at the Fig. 1. Methyl Red has been used as a marker to visualise analyte transfer trough interface. Seven regions of the thin layer ($R_F = 0.1 - 0.85$) were analysed. Overlayed Methyl Red spectra obtained from real chromatographic interfacial analyses and on interface directly applied analyte have been already published [11]. There Methyl Red can be characterised with positive ions: tropylium (C_6H_5–CH_2^+, $m/z = 91$), acylium (RCO^+, $m/z = 105$), $C_3H_2O^+$ ($m/z = 54$), azatropylium (106), which loses HCN to form the important $m/z = 79$ ion. Four muliplets at masses 54, 79–81, 91, 107–109 confirm presence of aromatic organic compound with nitrogen atoms and carboxylic group. Ion with mass 27 belongs to aluminium ion, 39–43 are probably the aluminium–nitrogen (like AlN^+ or CH_2Al^+) fragments. Molecular or quasi-molecular ion (269) was present with very low intensity and associated with peaks that occur at the highest mass. At classical mass spectrometric spectrum of Methyl Red is present very intensive peak with nominal mass 120 and 148. Molecule is broken not at diazo group but between aromatic cycle and second nitrogen of diazogroup. Here, in TOF–SIMS spectrum, we found out low intensive peak with 119 and 135 nominal masses. Molecule is broken symmetrically, at diazo group. Ion 119 belongs to one part of molecule $C_6H_4N(CH_3)(CH_2)^+$ and ion with $m/z = 135$ to carboxylic group containing part. TOF–SIMS spectrum without Al^+ very intensive peak gives detailed structural in-

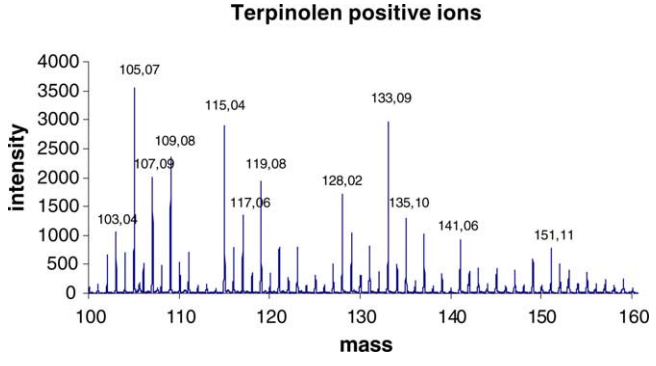

Fig. 2. TOF-SIMS positive ions spectrum of Terpinolen. Precision fragment masses as analyte identification tool.

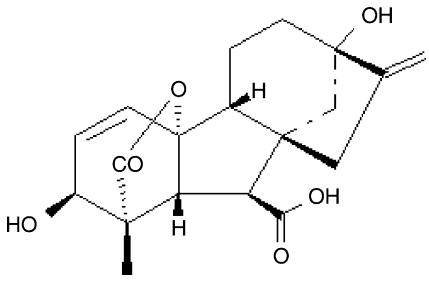

Fig. 3. Structural formula of Giberrelic acid.

formation. Overlayed spectrum of Methyl Red in negative ions region [11] does not offer source data for analyte successful identification however ion with nominal mass 123 belongs to the anionic form of the fragment from Methyl Red indicator molecule.

4.2. TOF–SIMS analyses of terpinolen at aluminium backplate interface

Terpinolen positive secondary ions spectrum showed maximum intensities of emitted ions with masses $m/z = 41$

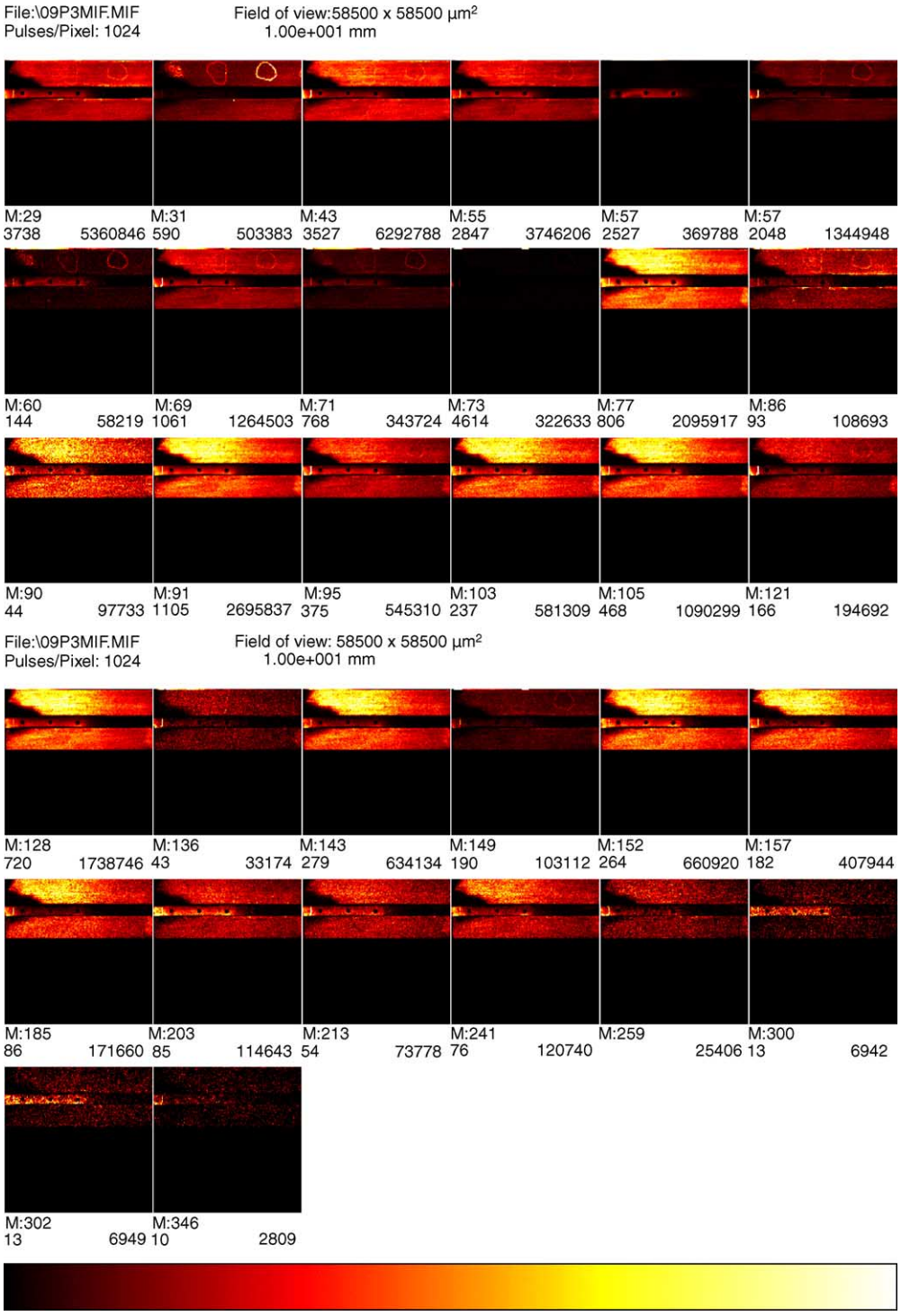

Fig. 4. TOF-SIMS positive ions image of Giberrelic acid at aluminium modified interface (fragment ion mass present when white/yellow colourised surface). Under every picture is nominal mass value. Characteristic yellow coloured spots at masses: 31, 57, 60, 69, 71, 77, 86, 128, 143, 152, 157.

($C_3H_5^+$), 43 ($C_3H_7^+$), 91 ($C_7H_7^+$), 77 ($C_6H_5^+$) 93 and 105. Masses distribution confirms the presence of terpenoic cycle in the structure. In the area over $m/z = 105$, the peak intensities were lower (Fig. 2). More intensive is peak at $m/z = 133.075430$ belongs to fraction containing Na+ or Al^{3+}. Quasi-molecular ion with characteristic masses 135.125620 was detected with very low intensity. A lot of the secondary ions emitted fallen into the background produced with modified interface. The spectrum showed that several chemical species were present at the position probed on TLC and interface. The high selectivity of TOF–SIMS measurements is based not on the peaks nominal masses principle. This method offers to measure fragmentation ions intensity and so characteristic fragmentation masses can be applied for identification purposes. If the surface being analysed is completely unknown this can really only be accomplished by comparing the major peaks in the spectrum with a library. Whilst enormous libraries are now available in conventional mass spectrometry, they are only appearing for SIMS.

4.3. TOF–SIMS results from Giberrelic acid analyses aluminium backplate interface

Identification of Giberrelic acid is more complicated than the others analytes (structure at Fig. 3). There is no sufficient primary detection and then unknown analyte position in chromatographic system. TOF–SIMS spectrum of Giberrelic acids supplies a lot of secondary ions in the region of low masses (27.955939–43.035618) and from this reason the TOF–SIMS imaging mode has been chosen as the first analytical identification tool. The positive ions macro scan on aluminium modified interface (Fig. 4) showed high intensity for characteristic masses (decreasing ions intensity from white to black colour) $m/z = 77$, 90/91, 103, 105, 128, 143, 152, 157,185. The presence of ion with $m/z = 136$ has not been detected and mechanism of secondary ions production at this modified interface is unclear and requires additional measurements. The analyte spots can be seen at pictures assigned with $m/z = 31$, 57, 60, 69, 71, 77, 86, 128, 143, 152, 157. However, none characteristic fragmentation ions have been detected at $m/z = 60$, 73, 200, 213, 259, 302, 346. This confirmed that fragmentation ions do not form high molecular clusters but low weighing secondary ions.

4.4. TOF–SIMS results from Giberrelic acid analyses on monolithic silica UTLC layer

Due to polymeric structure of monolithis silica there is no intensive background signal in TOF–SIMS spectra. Moreover, this signal can be filtered. This is a good opportunity to get primary information about separated analytes position on chromatographic surface by scanning it. The "surface photography" shows spots position and detects them. In following steps each spot can be analysed separately with higher SIMS resolution. TOF–SIMS positive ions image from UTLC monolithic silica (Fig. 5) clearly showed high intensive peaks at $m/z = 65$, 91, 105, 165 and 181 (white, white/yellow coloured). Very intensive white/yellow coloured image, signed (a) at Fig. 5 (m/z, 28), belongs to chromatographic material response.

At $m/z = 65$, 91, 105 and 165, the intensity of Giberrelic acid (b, c spots) secondary ions were the most intensive. The spots (a) and (d) belongs to the fragments from Abietic acid (a) and Methyl Red indicator (d). UTLC as depositing and

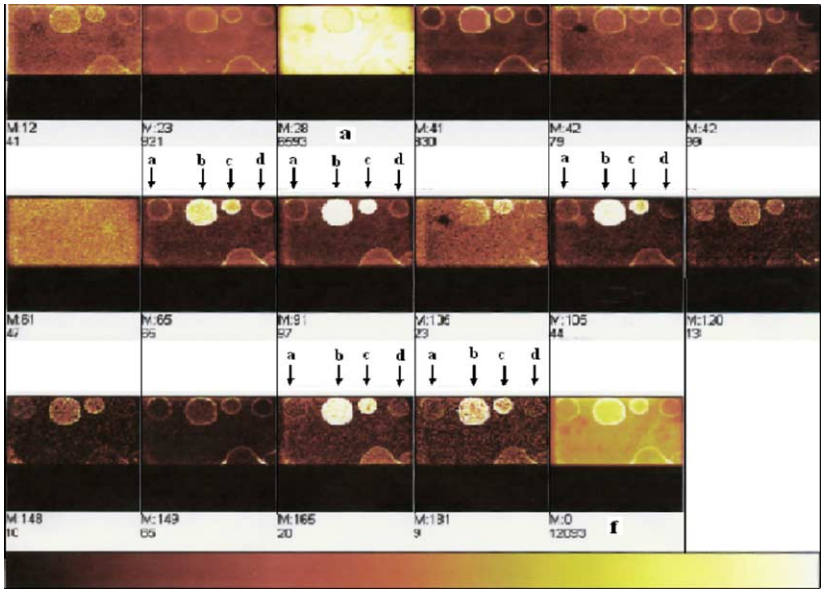

Fig. 5. TOF-SIMS positive ions image of Giberrelic acid at ultra thin monolithic silica layer. White coloured (very intensive masses) spots at: 65, 91, 105, 165, 181. a—picture with mass 28 responding to secondary ions from monolithic silica support mass; a arrow—spot of abietic acid; b—spot of Giberrelic acid; c—spot of Giberrelic acid with different concentration; d—Methyl Red indicator spot; f—picture of whole layer with analytes as "surface photography" without mass scanning.

interfacing substrate seems to be promising surface for coupling TOF–SIMS with dynamic systems as μHPLC. UTLC does not require any modification and TOF–SIMS signal is enough to identify the analytes deposited. Limit of detection (LOD) has not yet been established for the analytes tested. It depends on substrate character and analyte fragmentation. Typical detection levels for inorganic analytes were in interval 10^{13}–10^{18} atoms in cm^3 [12]. Thus, one molecule can be analysed by TLC–TOF–SIMS method.

5. Conclusions

Two interface were applied in novel hyphenated TLC–TOF–SIMS method. First interface was prepared by modification of silica gel aluminium backed thin layer. Mercury(II) Chloride has been used as modification agent. Newly modified surface interface resulted with the best interfacing and analyte mass transfer properties. Model analytes: Methyl Red, Terpinolen and Giberrelic acid were separated and eluted by side elution on interface in the same chromatographic position. It allows identification of analytes separated on thin layer. In positive ions TOF–SIMS spectrum there were found ions that can characterise analyte molecule (42, 65, 77, 91, 105, 119, 135). Removing aluminium peak from spectrum we can get very detailed TOF–SIMS spectra. In negative ions spectrum we can see only one ion that can be used for identification purposes, with nominal mass 123. Negative ions spectrum is not so complicated but information given by this is not sufficient for identification. Terpinolen supplied only low intensive peaks spectrum that can be used partially for its identification. Macro scan TOF–SIMS mode has been used at Giberrelic acid identification. Positive ions TOF–SIMS image confirmed presentation of this poorly detectable acid at depositing surface. More suitable seems to be in this case to use monolithic silica depositing substrate as production of the secondary ions was more intensive and analyte identification clearer. The introductory analyses showed that novel interface for coupling both methods works, analyte is transferred to the same chromatographic position without lose of integrity, background signal intensity is low and can be eliminated at spectra evaluation process. The main advantages of TLC–TOF–SIMS coupled method and interface developed could be assumed as it follows:

- Chromatographic separation and on-line analyte transport throughout modified interface without failure in analyte chromatographic integrity.
- Interface containing analyte can be scanned as a unit or fractionated; SIMS allows the detection of analytes in such low concentrations that they cannot be detected by other methods.
- Unit can be coupled with any dynamic system e.g. μHPLC, Raman Spectroscopy (RS) etc.
- Broad range of application in microbiological research, environmental control, biochemical focusing and analyte identification.

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