SURFACE ANALYSIS BY IMAGING MASS SPECTROMETRY

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Dedicated to Dr. Alfred Bader on the occasion of his 85th birthday.

A review of four MS-based techniques available for molecular surface imaging is presented. The main focus is on the commercially available mass spectrometry imaging techniques: secondary ion mass spectrometry (SIMS), matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), desorption electrospray ionization mass spectrometry (DESI-MS) and laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS). A short historical perspective is presented and traditional desorption ionization techniques are also briefly described. The four techniques are compared mainly with respect to their usage for imaging of biological surfaces. MALDI is evaluated as the most successful in life sciences and the only technique usable for imaging of large biopolymers. SIMS is less common but offers superior spatial lateral resolution and DESI is considered to be an emerging alternative approach in mass spectrometry imaging. LA-ICP ionization is unbeatable in terms of limits of detection but does not provide structural information. All techniques are considered extremely useful, representing a new wave of expansion of mass spectrometry into surface science and bioanalysis. A minireview with 121 references.

Keywords: Secondary ion mass spectrometry (SIMS); Matrix assisted laser desorption ionization (MALDI); Desorption electrospray ionization (DESI) and laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS); Surface imaging; Mass spectrometry; Desorption ionization.

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1. INTRODUCTION

This minireview discusses mass spectrometry (MS) methods available for imaging analysis of molecular species on surfaces. The analysis of surfaces by desorption ionization (DI) MS-based techniques is important in many areas ranging from materials engineering to fundamental fields of science and its development spreads over many past decades of research[1,2]. The long-term interest in obtaining information about two-dimensional (2D) distribution of analytes on surfaces (a methodology commonly referred as "MS surface imaging" or briefly "MS imaging") has been recently combined with growing interest in mass spectrometry of biological surfaces. The result of this merge is an armory of surface desorption ionization MS techniques that are currently available for MS imaging of biological surfaces (e.g. tumor/healthy tissues[3], cells[4], cell cultures[5]). The presented minireview cannot cover all topics in surface MS. This paper is thus not meant to be a comprehensive tutorial of the surface mass spectrometry techniques. Rather, the available most common 2D imaging techniques are described and evaluated with the general reader outside the MS community in mind, not the author's peer group. The main focus is on the MS imaging techniques that are available commercially and usable for imaging of biological surfaces. Only a very brief overview and a historical perspective of surface desorption ionization techniques are presented first and no attempt is made to review the operation of mass analyzers. The current state of MS instrumentation can be studied in modern mass spectrometry monographs[6–8].

The field of ion desorption techniques can be described without hesitation as elderly. Most likely it starts with Groves's 1853 classic report on cathode erosion by gaseous ions in a discharge source[9]. The milestone in investigation of the ion desorption phenomena was Thomson's 1910 well-known experiment in which he observed "the secondary *Canalstrahlen* produced when primary *Canalstrahlen* strike against a metal plate"[10]. The development of the modern secondary ion mass spectrometry (SIMS) was initiated mainly by Herzog's report[11] and later continued through successful practical applications. SIMS has undoubtedly developed into one of the most sensitive and successful surface analytical techniques. Interestingly, SIMS "culturally" separated from the rest of the mass spectrometry (*"Vendors of SIMS instrumentation do not strive for an active presence at major*

MS meetings [...], mainstream MS vendors do not attend SIMS meetings for much of the same reasoning")[12] and became an important island within the archipelago of all other surface techniques, where the events are not always in direct touch with the old MS continent. Meanwhile on the continent, other desorption techniques continued to evolve. Beckey is most credited for the development of a focusing field ionization ion source[13]. His field ionization and desorption (FI/FD) concepts were the first soft desorption ionization techniques. They had its flourishing period to the mid-1980's and were superseded only by fast atom bombardment (FAB)[14] and later by current soft ionization MS techniques[6–8]. The development of desorption ionization (DI) methods amplified the impact of MS many fold because at that time the inherent volatility restrictions of electron and chemical ionization limited applications of MS [15]. The most logical categorization of classic vacuum surface DI techniques is based on primary ionization and desorption agents. The incident energy can be supplied by ions or ion clusters – usually in the keV region (SIMS), atoms in the keV region (FAB), lasers – with matrix involvement (MALDI) or without matrix involvement (LDI), ions from fission fragments in the MeV region (plasma desorption, PD) or strong electric field (FD/FI). In some experiments, charged droplets were used as primary beams in vacuum[16,17]. A special category is formed by techniques that utilize simple thermal desorption with post-ionization of desorbed species. Thermal desorption with atmospheric pressure chemical ionization (TD/APCI) was the first DI technique performed outside the vacuum region of a mass spectrometer. According to van Berkel the basic TD/APCI approach can be traced back to the mostly forgotten work of Horning in 1970's[18,19]. In future, the situation might be judged very differently, but by the present-day standards it is reasonable to state that introduction of a very versatile and elegant desorption electrospray ionization (DESI) in 2004, and its swift gain in popularity, brought a reincarnation of this approach and caused a significant change in the whole field of desorption ionization[20]. Since DESI, and its partly competitive technique, direct analysis in real time (DART)[21], the new category of ambient DI techniques emerged in mass spectrometry. The ambient DI-MS was reviewed by Venter[22] and more recently by van Berkel[18], who suggested using a new term for DI-MS techniques where the desorption ionization step takes place outside the instrument vacuum chamber – *atmospheric pressure surface sampling ionization techniques*.

Technically, any surface DI technique could be used for 2D imaging because scanning the surface in *x* and *y* directions is a mechanical engineering problem that can be solved relatively easily. However, due to several practi-

cal considerations (sufficient spatial resolution and sensitivity, integration into the instrument user interface), mass spectrometry imaging is currently commercially available only for SIMS, MALDI (and other compatible LDI techniques) and DESI (and compatible ambient DI techniques) (Table I). These three molecular ionization techniques are supplemented by laser ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS), which is an atomic ionization technique designed for elemental analysis by MS, which can be used in imaging mode as well.

TABLE I
Characteristics of ionization techniques: SIMS, MALDI and DESI

Ionization technique	Abbreviation	Ionization agent	Desorbed highest mass	Spatial resolution
(Liquid) Secondary ion mass spectrometry	(L)SIMS	ion beams	1–10 kDa	under 1 μm
Matrix assisted laser desorption ionization	MALDI	laser pulses	~1 MDa	~25 μm
Desorption electrospray ionization	DESI	charged droplets	20–66 kDa	200–500 μm

2. SECONDARY ION MASS SPECTROMETRY (SIMS)

As was already discussed, SIMS has a long history and has been widely applied to surface analysis in materials science, geology, archeology, biology, metallurgy and even arts[23–26]. In SIMS, projectile (primary) ions strike the surface at well defined energies in the range 1–30 keV and eject secondary ions from the surface (Fig. 1) that are subsequently analyzed by the mass analyser[1,27]. The exact mechanism of SIMS has been debated for many years; a comprehensive fundamental review is available from the 1980's[28].

SIMS is usually divided into two classes depending on the primary ion intensity. Static SIMS uses low-intensity projectile primary beams ($<10^{13}$ ion/cm^2), which minimize sample damage and makes the method mostly nondestructive. Dynamic SIMS, on the other hand, employs intense primary beams ($>10^{13}$ ion/cm^2), which results in surface sputtering, damages the sample but also allows depth profiling from few nanometers to several hundreds of

micrometers[29,30]. Both static and dynamic SIMS can be used for imaging but static configuration provides better spatial resolution[31].

A reason for the SIMS popularity is its ability to analyze all elements of the periodic table including hydrogen in solids, liquids and frozen surfaces[32,33] although such experiments are difficult due to the attention that has to be paid to the preparation of experiment, especially to vacuum environment. In contrast to SIMS, X-ray photoelectron spectroscopy, XPS, which is considered by many as the number one principal surface analysis technique, especially for elemental quantitative analysis, is not able to detect hydrogen and helium[1]. Another praised feature of SIMS is the above mentioned ability of dynamic SIMS to obtain an analyte distribution in the third dimension. For example, Ullrich et al. analyzed trace components of silicon surfaces and investigated the dependence of the SIMS signal of sputtered atoms of boron, phosphorus or antimony on the type of primary ion beams (cesium or oxygen ions), its energy (5 and 15 keV), the tilt angle of the sample (40 and 80°) and the analysis depth[34]. The ability of SIMs to perform 3D imaging was recently reviewed by Gillen et al.[35,36].

One of the most exciting recent developments in molecular secondary ion mass spectrometry concerns the use of polyatomic species or ″clusters″ as projectiles (e.g. charged gold clusters or fullerene ions) to desorb the analyte molecules from the investigated surface[37–39]. An overview of the mechanism and the explanation why larger cluster projectile ions enhance desorption yields can be found in specialized reports[40,41]. Comparison with traditional SIMS and laser desorption is also available in the literature[42].

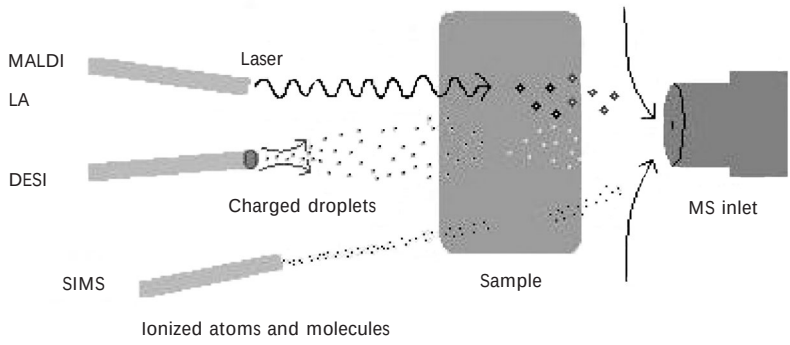

FIG. 1
Techniques used for MS surface analysis: SIMS, MALDI, LA and DESI

Matrix effects need to be considered carefully in SIMS but they can also be utilized in the technique (although this approach is sometimes compared to alchemy[43]). When an organic surface was coated with a thin layer of silver or gold and bombarded with primary polyatomic ions, the yield of sputtered secondary ions was enhanced by the matrix effect of the coating[44]. Jones et al. analyzed mixtures of two or three compounds and observed either suppression or enhancement of the emitted secondary ions due to the chemical environment[45]. For instance, signal of pure 2,4,6-trihydroxyacetophenone was abundant both in positive and negative ion mass spectra, but after mixing the analyte on the surface with tripeptide glycylglycylhistidine in the 1:1 w/w ratio, the signal was suppressed in the positive ion mode while it could still be seen in the negative mode. Thus, the matrix effects in SIMS are rather complex phenomena and the presence of matrix does not necessarily mean the improvement of ionization efficiencies[46] because the relationship between concentrations and signal intensities depends strongly on the sample composition[46,47]. Utilization of liquid matrices results in the so-called liquid SIMS (LSIMS), which has been used for a long time in order to achieve softer ionization and to extend the SIMS mass range. LSIMS generally produces spectra identical with those obtained by neutral beams in FAB. Differences in internal energy of ions sputtered from liquid and solid matrices have been evaluated by Cooks and Chan long before the current era of soft ionization[48].

SIMS allows probing the surface with spatial resolution less than one micrometer, which makes it very suitable for 2D imaging experiments. Although SIMS is used mainly in semiconductor industry, nanotechnology and related areas, it has been already used for imaging of biological tissues. Nygren et al. imaged the distribution of Na^+, K^+ and phosphocholine[49] and cholesterol in kidney tissue[50]. The thin slice of kidney was covered with a nanometer layer of silver and sputtered by 30 keV Ga^+ primary ion beams. It was found that the silver-cationized cholesterol was more abundant in membranes[50]. Chandra et al. reported the 3D imaging of glioblastoma and cells, which were harvested from isotopically enriched media[51–54]. The tissues were cut into thin slices, 2D-imaged, and the 2D images were reconstructed back into objects. Nygren et al. reported SIMS imaging of phosphocholine, Na^+ and K^+ in three-dimensions in the thyroid tumor cell without any sectional treatment of the cell. The cell was 2D-imaged with Bi_3^+ primary ion beam (lateral resolution 300 nm). Then the already scanned layer was removed (sputtered) by C_{60}^+ ions, which are able to penetrate deeper into the sample. Then a consecutive layer was 2D-imaged. This

process was repeated until the complete 3D image was obtained[55]. The resulting 3D image can be seen in Fig. 2.

3. MATRIX ASSISTED LASER DESORPTION IONIZATION (MALDI)

Together with electrospray ionization (ESI), matrix assisted laser desorption ionization (MALDI) has become the soft ionization technique of choice for the analysis of large biomolecules. In MALDI a laser beam is aimed at a surface (in vacuum), which is absorbed by ionization matrix (matrix is a carefully chosen compound added to the sample which serves as a primary acceptor of the laser energy). In the subsequent chain of events the analyte is desorbed and ionized by a combination of different mechanisms, which are still largely debated. MALDI has been introduced in the 1980's; and Tanaka received the 2002 Chemistry Nobel Prize for its first successful application in the area of protein MS[56]. The breakthrough experimental approach was based on usage of an inorganic metal-based matrix[57]. However, current MALDI users usually take advantage of an alternative type of matrices (organic compounds co-crystallizing with the analyte) developed mostly by Karas and Hillekamp[58]. The analyzed compound is mixed with a matrix, usually an aromatic acid, which absorbs the energy of primary laser pulses and

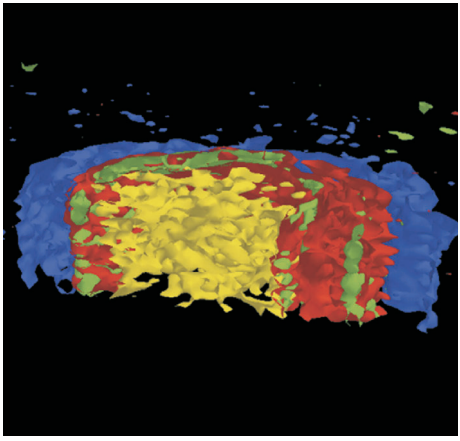

FIG. 2
3D image of a cell obtained by dynamic SIMS imaging. The image shows distribution of Na^+ (blue), K^+ (green), phosphocholine (red) and its metabolite (yellow). Reprinted from Nygren et al.[55] with permission of John Wiley & Sons, Inc.

ionizes the analyte[8]. The simplified scheme of MALDI is in Fig. 1. Molecules desorbed from the sample are typically singly protonated even-electron ions or adducts with alkali metals[59,60]. In 2003, several tutorial papers that fundamentally discussed MALDI mechanism in full details were published in *Chem. Rev.*[61–64].

There is also a new variant of the standard MALDI, an atmospheric pressure MALDI (AP-MALDI), which was first described by Laiko[65]. Analysis of proteins/peptides by atmospheric pressure and standard vacuum MALDI has been reviewed[66–70]. The main difference is in the speed of collisional cooling (AP-MALDI is softer and ions generated by AP-MALDI generally undergo less fragmentation)[67,71].

MALDI is often used for 2D imaging of molecules on biological surfaces[72–78] and there are now many examples of MALDI imaging of drugs[79–81], peptides/proteins[82–84], sphingolipids[85], phospholipids[86,87] in tissue sections. For instance, Khatib-Shahidi et al. reported the distribution of proteins and of a pharmaceutical substance olanzapine and its metabolites in a rat tissue section[81]. Homogeneous distribution of the matrix deposition on a tissue section is one of several necessary steps for obtaining a successful MALDI image[87,88]. Leinweber et al. increased the protein signal in the 25–50 kDa range by the application of the sandwich method illustrated in Fig. 3. The tissue section was placed on a drop of sinapic acid (SA) in 90% ethanol (EtOH) with Triton X-100. The slice was then dried under vacuum and subsequently covered with matrix drops[89]. Unlike SIMS, MALDI does not allow direct depth profiling in the third dimension, but the tissue can be cut successively and the 3D image reconstructed from several 2D distributions of the analyte[90,91].

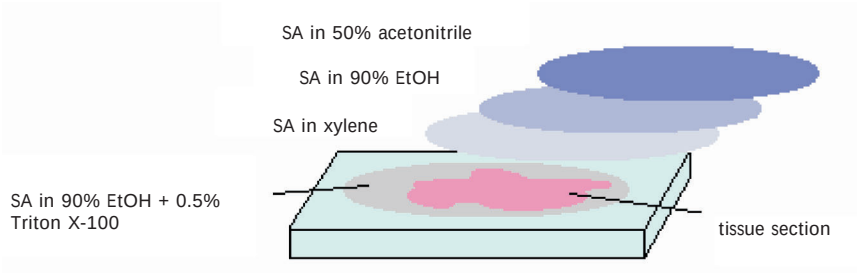

FIG. 3
The sandwich method in MALDI – tissue section between two SA matrix layers[89]

4. DESORPTION ELECTROSPRAY IONIZATION (DESI)

DESI, introduced in 2004 [20], is the youngest of the commercially available imaging MS techniques. It is also the technique that initiated the interest in ambient DI-MS techniques, which allow for direct analysis of surfaces in the open atmosphere of the laboratory or even in natural environment[22]. In DESI, a pneumatically-assisted ESI source aims at the surface to be analyzed (Fig. 1), which is usually mounted on a movable stage for proper sample positioning. A high voltage (2–5 kV) is applied to the same emitter as in standard ESI and together with solvent and nebulizing gas flow create an electroaerosol that consists of high-velocity charged microdroplets (detailed properties of the droplets used in DESI experiments can be found in a report by Venter et al.[92]). The spray is directed at the surface, where it desorbs the analyte and transports it into the mass spectrometer inlet. DESI has been thoroughly reviewed in several tutorial papers[18,22,93]; its mechanism has been investigated by computer simulation[94,95] as well as in experiments[92,96].

DESI can be coupled to all mass spectrometers with an open-atmospheric pressure inlet. The instruments most utilized for DESI are probably Thermo LTQ linear ion traps but less common mass spectrometers can be used as well. Bereman et al. and Havlíček et al. reported a potentially powerful coupling of DESI with high-resolution Fourier-transform ion cyclotron resonance mass spectrometers (FT-ICR-MS)[97,98].

DESI can be used to analyze solids, liquids, frozen samples or adsorbed gases and also small and large biomolecules[99–101]. It produces very similar spectra to ESI and the multiple-charged ions obtained by DESI are thus amenable to electron capture dissociation (ECD) analysis[98]. The distribution of ion internal energy in DESI was compared to those in ESI and electrosonic spray ionization (ESSI) and it was concluded that initial differences in energy during ionization have only a small effect on final ions[102]. However, there are redox processes that make DESI spectra very different from ESI in the case of some selected analytes[103].

DESI is able to analyze wide selection of analytes up to small proteins. It was used for instance to analyze explosives on common surfaces (glass, paper, plastic, metal etc.) without any sample pretreatment or to identify chemical warfare agents[104–106]. Zhang et al. utilized DESI for analysis of pyrolytic products[107]. Anabolic steroids have been analyzed by DESI-MS in native urine and desorbed from a solid-phase microextraction (SPME) fibre[108]. Utilization of DESI in chiral analysis has also been reported[109] and Jackson et al. used DESI to monitor the metabolites of *E. coli*[110].

Imaging of surface distribution of molecules of interest with DESI is an especially interesting application. It was used mainly to profile phospholipids in different tissue sections[111,112] but other uses, e.g. analyte bands in TLC [113] or inked lettering and imaging on paper[112,114,115] were also reported. Lateral spatial resolution of DESI imaging is usually estimated to be <500 μm but a 250 μm resolution has been achieved by DESI at a signal/noise ratio of 10:1[103]. According to a single report a resolution much below 100 μm could be achieved (40 μm)[116]. An interesting application of DESI imaging was presented by Kertesz and van Berkel[117] who have shown its ability to quickly image the spatial location of propranolol in thin tissue sections from the whole body of mice dosed at pharmacologically relevant levels. However, this non-selective β-blocker, used in the treatment of hypertension, ranks among compounds that are relatively easy to ionize and determine by DESI. For more difficult analytes the limit of detection and sensitivity of DESI need significant improvement before it can be a practical tool.

5. LASER ABLATION INDUCTIVELY-COUPLED PLASMA MASS SPECTROMETRY (LA-ICP-MS)

Inductively-coupled plasma mass spectrometry (ICP-MS) together with its sibling optical technique inductively-coupled plasma atomic emission spectrometry (ICP-AES) are now the best and dominant techniques in the field of inorganic elemental analysis, which was once ruled by atomic absorption spectrometry and earlier by different electrochemical techniques. The mass spectrometry variant of ICP achieves much better limits of detection but the optical ICP generally tolerates harsher sample matrices (e.g. salts). Coupling of ICP-MS with laser ablation allows this ultrasensitive technique to take advantage of the direct sampling from surfaces. Thus, the technique can be also used for 2D imaging applications. As a consequence of the techniques of superior sensitivity, the use of LA-ICP-MS in biological studies results in interesting findings[118,119]. As far as the life sciences applications are concerned, ICP imaging is naturally limited to probing distribution of metals in different biological environment. But such investigation can be fruitful even without structural information. The technique was used, e.g. to image toxicologically relevant distribution of uranium in mouse brain[120]. Determination of copper distribution in thin tissue section of human brain is another example[119].

6. CONCLUSION: COMPARISON OF SURFACE DESORPTION IONIZATION IMAGING TECHNIQUES

SIMS, MALDI, DESI and LA-ICP represent modern approaches to surface imaging analysis of organic, inorganic and biological materials.

SIMS is one of the oldest and classic ionization techniques while DESI is one of the youngest ionization approaches, which corresponds with the different levels of trust that the MS community currently has in these techniques. A detailed comparison of SIMS, MALDI and DESI imaging has been recently presented by a specialized surface analysis laboratory[121]. In brief, SIMS is the most expensive and demands most complex instrumentation and vacuum environment. It is a sophisticated technique and one has to be aware of huge matrix effects. In exchange it provides superior lateral spatial resolution (it is the only technique that can under optimal conditions achieve subcellular resolution), allows for depth profiling and due to its long existence there are experienced operators and well-established protocols. It is thus surprising that it has had only limited success in the biological imaging arena[12]. Naturally, the major limitation of SIMS is the upper molecular weight range limit that prohibits imaging of macromolecules, e.g. larger peptides and proteins. However, many MS imaging applications are focused on low-molecular-weight analytes (e.g. lipids, peptides, drugs) and imaging of large proteins by MS techniques is rather rare. Separation of the SIMS community, which has been long dominated by physicists and physical chemists, has been thus given as another reason for the lack of biological applications of SIMS imaging[12].

MALDI and DESI are in many ways comparable techniques for imaging applications. It can be argued that MALDI provides much better lateral spatial resolution than DESI (by a factor of roughly 2–10); however, neither technique currently achieves resolution at a cellular level, which makes the true relevance of this difference questionable. The great advantage of MALDI is its better limit of detection and the fact the biological MS community is accustomed to use MALDI. It is also the only technique that allows desorption and ionization of large biomolecules and thus only MALDI can be used for protein imaging. Due to these advantages and despite the disadvantageous fact that necessary addition of the ionization matrix changes the property of the surface, MALDI is currently the MS imaging technique number one. DESI needs to resolve sensitivity and resolution issues but the technique holds the promise of soft, local-atmospheric DI technique, which could fulfil, the desire of many people in the life science community, namely the wish to perform imaging experiments away from

the vacuum environment. LA-ICP is chiefly an inorganic analysis technique, which limits its use to analytes that contain a characteristic element that differentiates it from the background signal. The inability to provide structural information separates LA-ICP from other three DI techniques but its great advantage is the superior limit of detection.

The usefulness of the reviewed mass spectrometry imaging techniques will certainly be judged by their applicability to real scientific problems in the near future. However, one can still point out that the combined performance of all four commercially available DI imaging techniques represents a great surface-imaging tool and a dramatic extension of mass spectrometry into surface science and biological imaging, which would be probably considered fantastic just a generation ago.

7. LIST OF SYMBOLS

2D	two-dimensional
3D	three-dimensional
AP	atmospheric pressure
APCI	atmospheric pressure chemical ionization
DART	direct analysis in a real time
DESI	desorption electrospray ionization
DI	desorption ionization
ECD	electron capture dissociation
ESI	electrospray ionization
ESSI	electrosonic spray ionization
FAB	fast atom bombardment
FD	field desorption
FI	field ionization
FT-ICR-MS	Fourier-transform ion cyclotron resonance mass spectrometry
LA-ICP-MS	laser ablation inductively-coupled plasma mass spectrometry
LDI	laser desorption ionization
LSIMS	liquid secondary ion mass spectrometry
PD	plasma desorption
MALDI	matrix assisted laser desorption ionization
MS	mass spectrometry
SA	sinapic acid
SIMS	secondary ion mass spectrometry
SPME	solid-phase microextraction
TD	thermal desorption
TLC	thin layer chromatography
XPS	X-ray photoelectron spectroscopy

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