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Mass spectrometry made easy: The quest for simplicity†

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Mass spectrometry (MS) is generally viewed as a highly complex and demanding technique, full of difficulties and apprehensions. Ease and simplicity have been infrequently used descriptors of MS but a series of revolutionary developments is turning a complex technique into a model of simplicity, making MS easier than ever. Focusing on spray-based ambient desorption/ionization techniques, we discuss how previously unthinkable goals for MS – (1) to bring it to a real world open atmosphere environment; (2) to perform fast, selective, and highly sensitive chemical and biochemical MS analysis with ease while (3) avoiding pre-separation and sample work-up for samples in their natural environment and therefore, at the end, (4) to make MS accessible in wherever MS is needed and by whoever needs it – have become feasible. Without compromising the unique combination of high speed, selectivity, sensitivity and separation competences, simplicity has become a new MS attribute – a fifth 'S' in the unique 5 S set of MS trademark features. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: mass spectrometry; ionization techniques; portable mass spectrometers; drug analysis

Introduction

Simplicity, when used to qualify a technique, is normally associated with those involving undemanding tasks capable of being accomplished with ease, posing no difficulty, or requiring little effort or endeavor from the analyst, free from anxiety, trouble, or pain. Simplicity may also be used for instruments which are less demanding and more readily obtainable. Mass spectrometry (MS) is currently one of the most powerful and broadly used analytical techniques, but its success has been associated with a unique combination of three major attributes of which simplicity is excluded. In the 3 S trademark of MS, the first 'S' stands for high sensitivity now going beyond the femtomol level; the second, for selectivity via mass, isotope distribution, and fragmentation checking; and the third, for speed which exceeds hundreds of spectra per second. Throughout its centenary history[1] however, it is indeed hard to find simplicity in use as an attribute of MS.

Where simplicity is concerned, MS has always been considered to lie on the other side of the pitch, being generally viewed as a highly complex and demanding technique, full of troubles and worries, particularly for the non-expert. Mass spectrometers are considered very expensive, complex, and hard to master, thus requiring skilled operators. We may reminisce on earlier days in our MS careers being assigned to operate a classical and complex mass spectrometer – those *giant* double-focusing magnetic sectors instruments with scary oily diffusion vacuum pumps and an in-vacuum multiple parts high voltage (8000 V) electron ionization (EI) source – and recall that the first days of work were far from being 'free from worry, anxiety, trouble, or pain'. 'Readily obtainable' would also hardly be the phrase commonly used to describe most mass spectrometers when submitting grant proposals.

This overall impression of MS being a complicated and highly demanding business evolved mainly due to intricate instrumentation and demanding ionization techniques performed via complex ion sources and in the unfriendly high vacuum environment of mass spectrometers. Laborious extraction, derivatization, and pre-separation steps, required to simplify sample complexity or increase analyte volatility, were also often required, particularly for complex mixtures and matrices, in order to deliver pure and volatile molecules for MS ionization and mass analysis. Too much thermally unstable, too large (>500 Da) or not sufficiently volatile molecules were also out of bounds. When the time came to interpret the spectra,[2] those obtained by the 'hard' electron ionization (EI) technique were filled with a multitude of ions, sometimes lacking the intact molecular species and displaying numerous fragment ions eventually formed via complex, somewhat unexpected multi-step rearrangements and diverse dissociation reactions involving deep knowledge of chemical reactions and mechanisms. Although quite comprehensive EI-MS libraries are now available from prompt and reliable comparisons, spectral interpretation for structural assignments required in the early days has contributed to build the reputation of complexity surrounding MS.

The smaller the better – the size revolution

For instrumentation, a series of revolutionary developments in MS toward miniaturization are encouragingly incorporating simplicity among the portfolio of major MS attributes.[3,4] Many different designs of fit-for-purpose miniature, compact, hand-held, operator-friendly mass spectrometers including quadrupoles, ion traps, magnetic sectors, and time-of-flight analyzers connected to laptop computers with prices now reaching the popularization threshold of US$20 000 or less have been developed and are being

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increasingly incorporated into the MS market. Reduction in mass analyzer size and complexity has been facilitated by simplification and miniaturization of its major components (electronics, vacuum pumps, and sample introduction and ionization systems), making MS much simpler and smaller in terms of instrumentation. The area is, however, still struggling to reduce losses in resolution, detection limits, ruggedness, and reliability and to implement fully autonomous operation. That said, progress in overall performance is being achieved and fully portable and high-performance miniaturized mass spectrometers are soon to appear. Ambient desorption/ionization methods have removed the bottleneck of sample preparation for miniature MS by combining sampling, pre-concentration, and ionization in a single step. Obviously, high-performance mass spectrometers operated in clean laboratories under well-controlled conditions are still required and will be probably always be needed in cases where ultra-high sensitivity, speed, and accuracy are required for unquestionable and highly precise results, but numerous, less demanding applications in fields such as drug analysis, forensic, clinical and doping screening, and fuel and food quality control will surely benefit from the MS size revolution.

From high vacuum to the friendly ambient environment – the ambient revolution

As for ionization – a fundamental task for MS analysis – the first major revolution toward the 'simple domain' occurred with the introduction of techniques which removed the source from inside the mass spectrometer, i.e., from the high-vacuum environment, placing it where we all have long hoped for but did not dare to dream about, *thinking it impossible*: in the friendly atmospheric pressure open ambient environment of the laboratory. The Nobel laureate technique of electrospray ionization (ESI) introduced by John Fenn is an icon of such revolution. ESI[5] greatly simplified MS analysis, bringing MS from the restricted gas-phase environment of volatile, less polar and small molecules into the real world of nearly all types of molecules and bio-molecules in solution. ESI has provided a solid MS bridge[6] connecting gas phase to solution chemistry and *vice versa*. ESI also turned *hell into heaven* in terms of simplicity for MS since salts or polar or big molecules that we previously demonized for in-vacuum MS can now be dissolved and/or charged in solution becoming directly and most efficiently analyzable by ESI-MS in the ambient environment. Big molecules portrayed by John Fenn in his Nobel lecture[7] as 'elephant' molecules (such as peptides, proteins, and polymers or even massive species such as intact viruses,[8,9] bacteria,[10] and intact cells[11]) were given wings to fly from the ambient to the high-vacuum environment of mass spectrometers where mass analysis still takes place. The portfolio of such MS demonized molecules also included most polar drugs, organometallics, peptides, dyes, polymers, metalloproteins, and sugars but they were also brought by ESI to the MS domain. ESI and other soft ionization techniques that followed also contributed to mobilizing MS into the simple domain by simplifying MS spectra and their interpretation. Instead of forming multiple ions from a single molecule, arising from intact and fragment ions, ESI forms a single ion (together with its corresponding isotopologues) for each component in a chemical mixture, thus providing a 'one molecule:one ion' relationship. This direct matching greatly simplifies the analysis of complex mixtures, and has definitively added *separation* as an additional MS attribute. The fourth 'S' in the new MS trademark!

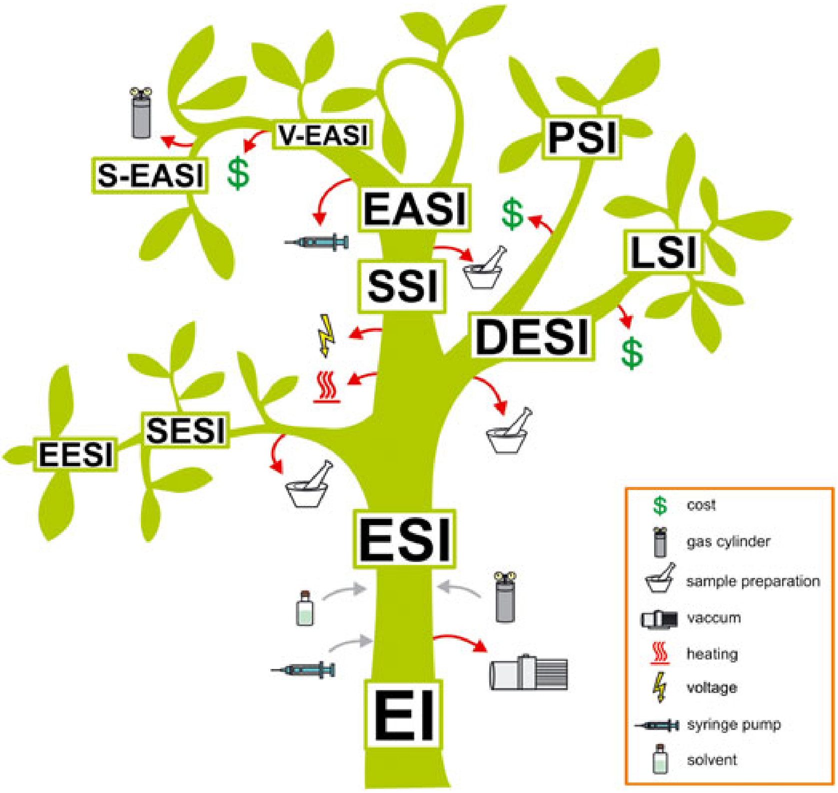

Figure 1. The 'involution tree' of spray-based ionization techniques in MS – from complexity to simplicity. Arrows indicate new demands or elimination of parts or needs when a technique evolves from its ancestor technique.

ESI is a spray-based technique; as compared to EI, it eliminated the need for a high vacuum but introduced the need for a compressed relatively inert gas, a solvent and, in most ESI sources, an electrical pumping system (Figure 1). There was therefore room for simplification, and greater simplicity has indeed been introduced via, for instance, secondary ESI (SESI,[12] Figure 1) and its sister technique extractive ESI (EESI).[13,14] These techniques eliminated sample preparation for the analysis of gases and vapors of more volatile molecules. Such vapors are analyzed by SESI or EESI via direct introduction into the ESI source where gas-phase interactions between the vapor molecules with the ESI-charged droplets promote ionization. For example, relatively volatile molecules such as heroin and cocaine can be heated to create a vapor that is transferred to the SESI chamber for ionization[15] and biomarkers can be directly detected in exhaled breath.[16]

The less voltage or heating the better – the sonic-spray revolution

Another less renowned but also revolutionary spray-based technique that helped move MS closer to simplicity was sonic spray ionization (SSI). Introduced in 1994 by Hirabaishi *et al.*,[17] SSI was unique since it launched a new concept of ionization in MS: the effective transfer of ions from solution to the gas phase without the assistance of any voltage or heating. SSI, although operating via a different mechanism, may be viewed as a simpler version of ESI, in which the need of a high voltage power supply and an electricity-demanding heater was eliminated. In SSI, the charged droplets are produced by spraying, for instance, an acidified methanol solution of the analyte at sonic speed. Charge (both negative and positive) arises not from the application of an external high voltage (required by ESI) but from a statistically imbalanced distribution of cations and anions in these very minute droplets with limited charge capacity. No heating is necessary in SSI for droplet dessolvation hence both the gaseous analyte ions $[M + H]^+$ and/or $[M - H]^-$ may arise from the charged droplets (depending on the nature of M) at room temperature. Ion formation in SSI is therefore easier than ESI (less in demand and therefore more readily obtainable) via the sole assistance of only compressed nitrogen (or even compressed air).

The less the sample preparation, the better - the ‘no sample prep’ revolution

The ‘no sample prep’ revolution has furthered the MS journey into the realms of simplicity. More recently, a series of ambient desorption/ionization techniques[18–23] has been developed, eliminating (or greatly minimizing) the need for sample preparation. The development of a large set of such techniques was triggered by desorption ESI (DESI)[24] and direct analysis of solids at room temperature (DART),[25,26] and has enabled the desorption of analytes directly from their natural matrices via a non-sample preparation procedure; all this under atmospheric pressure and at room temperature with concurrent ionization via soft ionization processes such as ESI (for DESI) and penning ionization via glow discharge (for DART). Ambient MS represents one of the most-welcomed advances in modern MS, having simplified ionization close to its uppermost limits of easiness and simplicity. Ambient MS has, therefore, as its main promise (and merit), the delivery of very simple platforms in which to perform easy, fast, and direct MS analysis favoring *in situ*, real-time, on line, high throughput and low sample-consumption protocols while requiring minimal or literally no sample pretreatments or separations. Through DESI, ambient MS has further simplified the ESI technique in respect to sample preparation (Figure 1), specifically for the analyses of solid samples and molecules resting on solid surfaces. DESI has also been further simplified in terms of part requirements (no electrical pumping) and cost, via, for instance, the disposable paper ESI technique (paper spray ionization, PSI, Figure 1).[27,28] PSI is a direct sampling approach for MS analysis of analytes in solution in which a high voltage is applied to a simple, disposable paper triangle wetted with a small volume (10 μl or less) of the analyte solution. Samples can be preloaded onto the paper, added with the wetting solution or transferred from surfaces using the paper as a wipe. Dried biofluid spots, including blood and raw urine samples, have

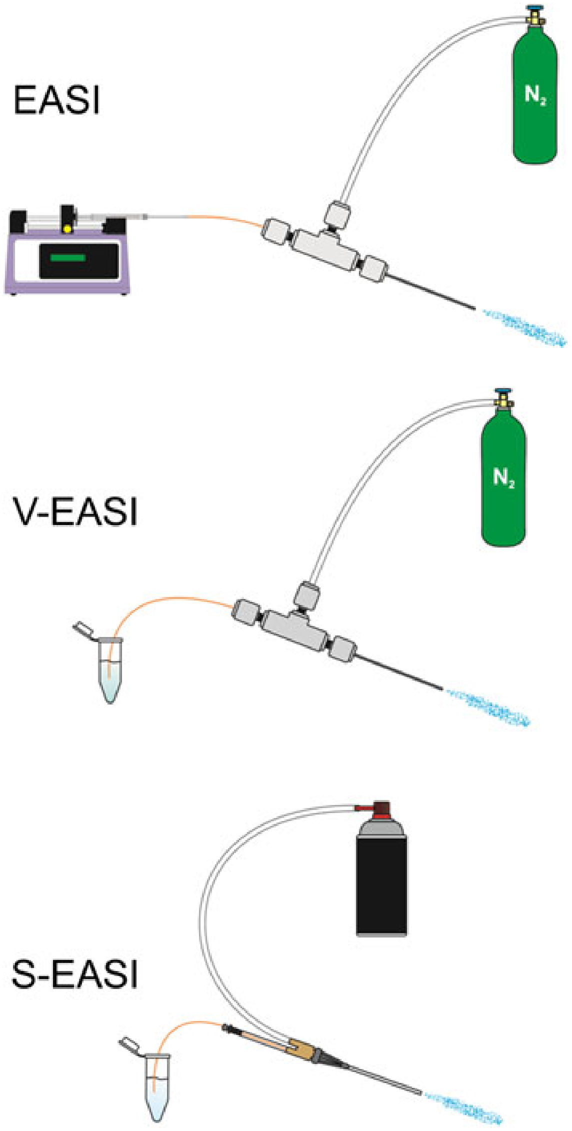

Figure 2. The involution of designs (from complex to simple) for EASI sources. EASI simplified the operation of spray-based techniques for ambient desorption/ionization by eliminating voltage and heating. V-EASI further simplified the system by eliminating the need of electrical syringe pumping, whereas S-EASI eliminated the need of cylinders of inert gases (N_2) using a can of compressed air and very simple, low cost and disposable parts.

been analyzed by PSI. Miniature mass spectrometers using PSI offer an illustrative example of simple and easy MS.[29] Other sample holders for ESI can be as simple as a needle,[30] a wooden toothpick,[31] or a simple capillary[32] placed close to the MS skimmer. Even the chemical constituents of intact plant material, including living plants, can be directly examined by a simple spray method using the leaf as the ESI emitter, viz. via leaf spray ionization (LSI, Figure 1),[33] which provides real-time information on sugars, amino acids, fatty acids, lipids, and alkaloids.

Easier than ever MS

With ambient MS, variable levels of simplicity and ease (or lack of it) have been observed for a large and rapidly growing set of techniques. Among these techniques, some have certainly offered simple, economical, easy to assemble and operate platforms, such as the PSI technique already discussed. Spartan source designs, ideally requiring no power supplies, auxiliary gases, or electrical parts such as pumps or laser or UV light sources or glow discharge devices are desirable in their ability to reduce complexity, size, and cost, simplifying manufacturing and operation, and facilitating 'in-field' analysis. The ease of operation combined with the simplicity of the parts and assembly promotes general use and servicing by non-experts such as medical doctors, nurses, police officers, and soldiers. We have introduced one of the simplest and most economical spray-based desorption/ionization techniques for ambient MS, namely, easy ambient sonic spray ionization (EASI, Figure 2)[34,35] and have demonstrated its efficacy in a wide range of applications [35-53] as a soft, low noise, reproducible and sensitive (high S/N ratio) technique inherently free of electrical or discharge interferences.

EASI can be described as de-evolving from ESI and then SSI (Figure 1). By eliminating the need for heating, voltages, lasers and/or corona discharges, EASI produces a sonic stream of very minute bipolar charged droplets of the solvent, which are used to bombard the sample surface thus causing desorption and an SSI-like ionization of the target analytes (Figure 3).

EASI and its sister techniques have been used in a variety of applications; for (1) the direct analysis of drug, pills as exemplified for sildenafil (Figure 4a), and for the characterization of TLC spots,[36] as exemplified for cocaine (Figure 4b), for the quality control and certification of geographical origin of olive oils;[37] for direct fingerprinting control of forgery in perfumes;[38] for the nearly instantaneous characterization of the composition of cationic surfactants and quality control of fabric softeners;[39] for the analysis of biodiesel and biodiesel/petrodiesel blends in combination with high performance thin layer chromatography;[40] for fingerprinting analysis of propolis;[41] for the direct analysis of fuels;[42] for the instantaneous characterization of vegetable oils via TAG and FFA profiles,[43] in combination with selective sequesters based on molecularly imprinted polymers (MIP) for the analysis of phenothiazines in urine (Figure 4d);[44] for the fingerprinting and aging of ink in forensic investigations;[45] as a rapid and secure screening method for street illegal drugs;[46,47] to characterize noble and endangered woods;[48] for the direct monitoring of known and unknown (or unexpected) drug degradation products as a function of time and manufacturing process,[49] as exemplified for enalapril maleate (Figure 4c), via thermal imprinting for the analysis of TAG profiles in meat matrices;[50] for petroleomic studies of 'untouched' crude oil samples;[51] for the forensic investigation of counterfeit money bills;[52] and, in its Venturi version, for real-time analysis of brain tissue.[53] These are illustrative of the 'whole world' (see cover picture) application of modern 'simpler' MS.

Ambient ionization techniques were originally applied mainly to solid samples or viscous liquids; liquid solutions needed to be frozen or subjected to solvent evaporation. To deal directly with solutions, indirect strategies such as a combinatory coupling with membrane introduction mass spectrometry (MIMS) have been used. Note that PSI is a convenient way to handle small amounts of solutions for ambient MS. Analyzing molecules already dissolved in proper solvents is beneficial for ambient MS since the required dissolution of the analyte is already achieved, particularly for less soluble analytes such as large proteins. To handle liquid samples, the EASI technique – which still requires a cylinder of compressed N_2 and an electrical-assisted syringe pump (Figure 2) – was further simplified via a version termed Venturi-EASI (V-EASI).[54] The V-EASI (Figure 2) technique utilizes the Venturi effect discovered more than two centuries ago by the Italian physicist Giovanni Battista Venturi. The siphon effect occurs when a high velocity fluid flows through a constricted section of pipe causing a reduction in fluid pressure which results in the self-pumping effect. V-EASI uses

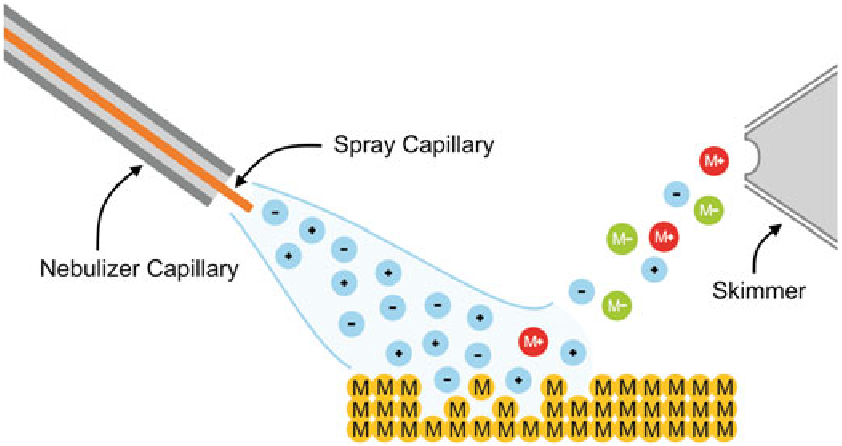

Figure 3. Schematic representation of the EASI mechanism of desorption and ionization. The bipolar stream of minute charged droplets produced by SSI bombard the surface picking up and ionizing the analyte molecules (M) in the form of either positive or negative ions, or both.

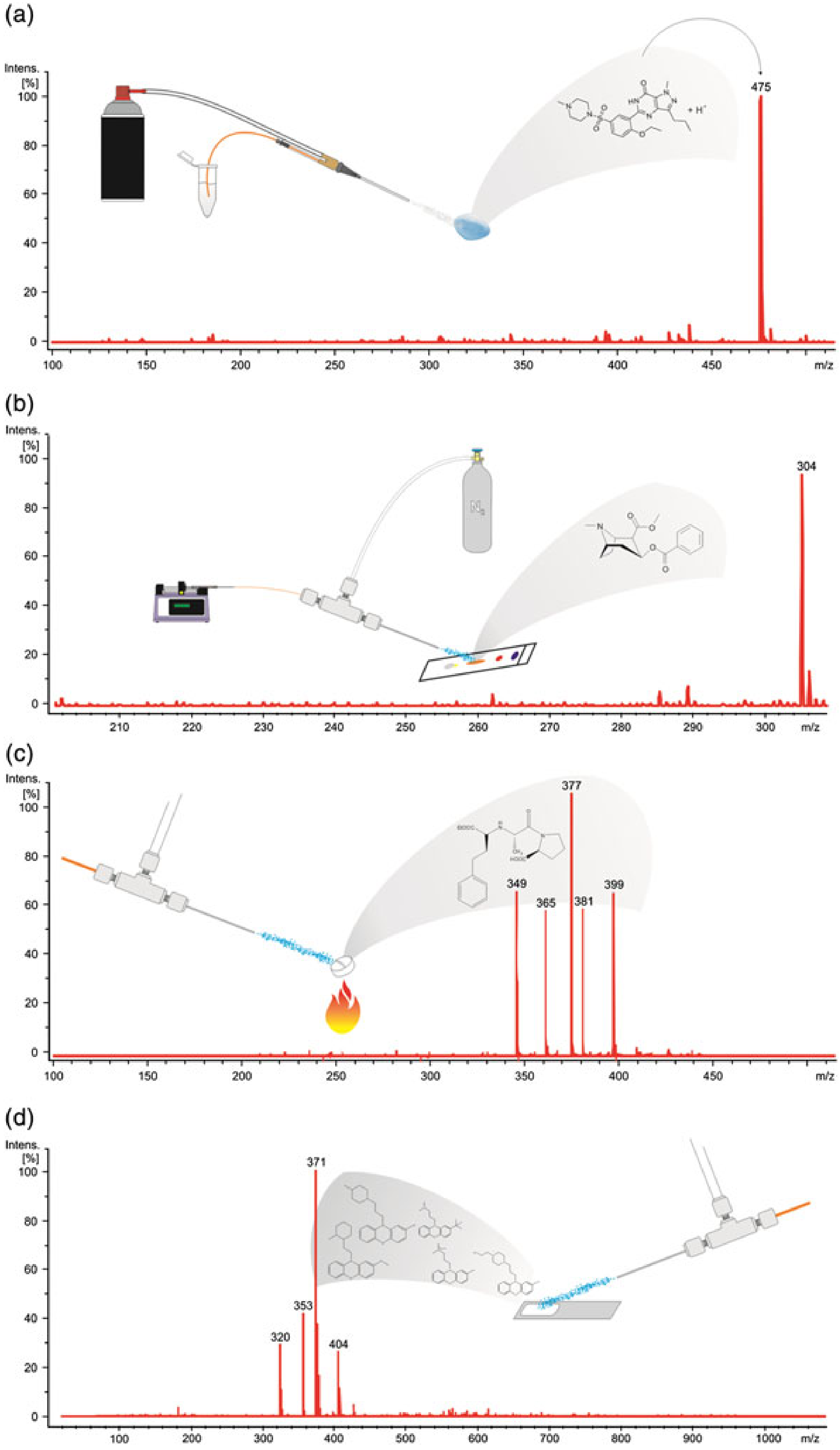

Figure 4. Set of applications of fingerprinting EASI-MS (or one of its sister techniques) for (a) the 'sample preparation free' analysis of drugs (sildenafil), (b) the characterization of the cocaine spot in a TLC plate, (c) the direct degradation monitoring of enalapril maleate, and (d) in combination with selective sequesters based on molecularly imprinted polymers (MIP) for the analysis of phenothiazines in urine.

the high velocity sonic nebulizing gas to perform two important concurrent tasks; (a) SSI and (b) Venturi self-pumping of either analyte or solvent solutions (Figure 2b). Venturi self-pumping makes the EASI apparatus even easier to assemble, operate, and handle either solid (V_S-EASI) or liquid samples (V_L-EASI) without the assistance of electrical pumping, offering a simple and flexible dual-mode ambient desorption/ionization source constructed from common laboratory parts.

More recently, a fully portable and disposable version of V-EASI that eliminates the need of laboratory gas cylinders, pure and relatively inert gases and regulators, using even simpler parts has been developed. This ‘Spartan’ EASI source (S-EASI, Figure 2),[55] we argue, seems to be one of the simplest to operate, assemble, and use; is the most economical (near zero cost) yet effective; is versatile and provides disposable platforms for dual-mode ambient desorption/ionization mass spectrometry analysis of both solid and liquid samples.

Figure 2 shows a schematic of the ‘disposable’ S-EASI source. Note the use of very simple, inexpensive, and readily commercially available parts and the ease of source assembly. The entire fully functional source is mounted using a surgical two-way catheter that functions as the T-connector. To replace the compressed N_2 cylinders and gas regulators, a disposable aerosol dust-cleaner can of compressed air was used and connected to one of the channels of the two-way surgical catheter. A fused-silica capillary was introduced into the other channel of the T-connector. A simple hypodermic needle was then connected to one of the catheter ends, allowing the silica capillary to pass through its steel capillary. To simultaneously generate proper self-pumping via the Venturi effect and efficient sonic spraying (desorption/ionization), the end of the silica capillary was positioned a few millimeters before the tip end of the needle. Pneumatic tube fittings were used for sealing. The other end of the capillary was dipped into the analyte solution for liquid samples in the V_L-EASI mode whereas for solid samples, V_S-EASI, a proper solvent such as methanol is used as the spray solvent and the tip of the hypodermic needle is manually positioned close to the MS orifice in a nearly perpendicular angulation. For V_S-EASI, an angle of *ca* 40° was used.

Despite the simplicity of the S-EASI apparatus, the source showed excellent stability, reproducibility, and reliability[27,28] and simultaneous self-pumping and SSI could both be efficiently attained by the reasonably constant gas flow provided by the dust cleaner can. No voltages are applied to promote S-EASI, hence the air/hydrochlorofluorocarbons (HCFC) mixture contained in the duster cans was found to produce no chemical interferences. Nearly constant gas flow could be sustained for 2 min with *ca* 5 min of rest, for a total of *ca* 40 min of MS acquisition. The disposable duster cans could be easily replaced, providing near continual use of the V-EASI source. The desorption/ionization ability of the Spartan V-EASI source was also demonstrated for solid samples in the V_S-EASI mode, as for instance in the analysis of intact drug pills.

Simplicity but at the cost of sensitivity?

As for most, if not all ambient MS techniques, due to the need to desorb the analytes directly from surfaces, or the direct analysis without any pre-concentration or derivatization protocols, sensitivity is not always the best attribute. But the superb ion transmission and detection efficiency of modern mass spectrometers (even for miniature versions) compensate for these limitations and therefore no serious compromises of sensitivity are normally observed. For EASI and its sister techniques V-EASI and S-EASI, as well as its most recent variation thermal imprinting EASI (TI-EASI), for instance, we have systematically observed less abundant ions (as measured by absolute ion counts) but superior results in terms of S/N ratios. This gain has been associated with the reduced charge states of the SSI droplets hence with more selective ionization. The superior S/N ratio is a welcome feature of EASI, which greatly benefits detectability in ambient MS analysis.

Conclusions and future perspectives

Portable, robust, easy to operate, low-cost fit-for-purpose mass spectrometers with simple and easy to use and assemble ionization sources are already feasible. Even disposable ion sources are available, capable of desorbing and ionizing analyte molecules of variable sizes and polarities directly from their natural environments or matrices or in simple solutions. The major task toward ‘simple MS’ still to be fully developed is the achievement of truly simple and efficient mass analyzers, and their overall commercial availability. Although most MS companies are still reluctant to follow the trend, this seems inevitable and urgent. Fit-for-purpose portable mass spectrometers that accommodate simple and inexpensive ambient MS sources are already being constructed to sizes that could be as small as those of match boxes.[8,9] Simplicity has been therefore incorporated as the fifth ‘S’ in the 5 S set of MS attributes (speed, selectivity, sensibility, separation and simplicity).

High-performance, ultra-fast, sensitive, and/or accurate MS analysis performed under well-controlled laboratory conditions are still – and perhaps will always be – required, particularly for highly precise and accurate quantitative analysis in a number of applications, as for instance of a target molecule in trace amounts in a complex matrix (e.g. a drug in plasma). These mass spectrometers have also experienced a considerable reduction in terms of size and complexity, and easiness of operation. But the previously unthinkable goal of MS – to offer fit-for-purpose portable and affordable MS systems that could be taken to the real world of open atmosphere environments so as to enable proper chemical, pharmaceutical, forensic, environmental, clinical and biochemical analyzes of samples in their natural environment and primary field location – is now fully feasible. Immediate MS can now also be performed by non-specialists with ease and simplicity using no pre-separation and sample work-up protocols. We can now offer mass spectrometry to the masses – wherever it is needed and to whomever needs it.

Acknowledgements

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