Electron ionization LC-MS with supersonic molecular beams—the new concept, benefits and applications

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A new type of electron ionization LC-MS with supersonic molecular beams (EI-LC-MS with SMB) is described. This system and its operational methods are based on pneumatic spray formation of the LC liquid flow in a heated spray vaporization chamber, full sample thermal vaporization and subsequent electron ionization of vibrationally cold molecules in supersonic molecular beams. The vaporized sample compounds are transferred into a supersonic nozzle via a flow restrictor capillary. Consequently, while the pneumatic spray is formed and vaporized at above atmospheric pressure the supersonic nozzle backing pressure is about 0.15 Bar for the formation of supersonic molecular beams with vibrationally cold sample molecules without cluster formation with the solvent vapor. The sample compounds are ionized in a fly-through EI ion source as vibrationally cold molecules in the SMB, resulting in ‘Cold EI’ (EI of vibrationally cold molecules) mass spectra that exhibit the standard EI fragments combined with enhanced molecular ions. We evaluated the EI-LC-MS with SMB system and demonstrated its effectiveness in NIST library sample identification which is complemented with the availability of enhanced molecular ions. The EI-LC-MS with SMB system is characterized by linear response of five orders of magnitude and uniform compound independent response including for non-polar compounds. This feature improves sample quantitation that can be approximated without compound specific calibration. Cold EI, like EI, is free from ion suppression and/or enhancement effects (that plague ESI and/or APCI) which facilitate faster LC separation because full separation is not essential. The absence of ion suppression effects enables the exploration of fast flow injection MS-MS as an alternative to lengthy LC-MS analysis. These features are demonstrated in a few examples, and the analysis of the main ingredients of Cannabis on a few Cannabis flower extracts is demonstrated. Finally, the advantages of EI-LC-MS with SMB are listed and discussed. Copyright © 2015 John Wiley & Sons, Ltd.

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Introduction

Liquid chromatography–mass spectrometry (LC-MS) has become an established, widely used technique. Electrospray ionization (ESI) is by far the most widely used LC-MS ionization method because of its superior sensitivity, robustness and extended sample molecular weight range, enabled by the formation of multiply charged ions. ESI is supplemented with atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI), which in some cases, show better performance with relatively small and less polar compounds. However, ESI, APCI and APPI still suffer from limitations in the ionization of non-polar compounds, are characterized by non-uniform compound-specific response and are plagued by ion suppression effects. In addition, all these atmospheric pressure ionization (API) methods are soft techniques that usually produce protonated or deprotonated molecular ion (with or without adducts), hence requiring MS-MS or high resolution accurate mass MS for analyte identification and characterization. As a result, they are expensive and do not share the benefit of gas chromatography–mass spectrometry (GC-MS) of having 70-eV electron ionization (EI) mass spectra with library based sample identification that uniquely provide automated identification with names and structures at the isomeric level. Furthermore EI provides information rich fragmentation pattern with significant amount of structural information.

The main EI-LC-MS approach that was used until about a decade ago, particle-beam (PB) LC-MS suffers from limited sensitivity, nonlinear response and adverse matrix effects. Cappiello and co-workers demonstrated significant advances in the development of their unique capillary scale PB-LC-MS and in the last 13 years in their advanced direct EI which excels with low LC flow rates while producing library searchable classical EI mass spectra. Moreover, Cappiello and co-workers gave compelling evidence for the lack of matrix related ion suppression or enhancement effects in their direct EI LC-MS and demonstrated its strength and usefulness in a few selected interesting applications.

While standard 70-eV EI is a powerful ionization method for unknown sample identification, it is not ideal. About 30% of the NIST library compounds either have a weak (below 2% relative abundance) or no molecular ion. This problem is further exacerbated for LC related compounds which are larger and more thermally labile than standard GC-MS compounds. Furthermore, LC-MS compounds are usually less volatile and therefore require
higher standard EI ion-source temperatures with the consequence of further intra ion-source degradation and lower molecular ion relative abundance. Without a molecular ion, EI based sample identification is not as trustworthy. Furthermore, for compounds that are not included in the EI libraries the absence of the molecular ion severely hampers the usability of the EI mass spectra. Thus, the ‘ideal’ ionization method should provide the informative, library searchable EI fragments combined with an enhanced molecular ion (relative to standard thermal EI), whose observation as the largest m/z mass spectral peak should be trusted.\textsuperscript{[33]}

We developed and explored the interface of GC and MS with supersonic molecular beams (SMB) and the use of a fly-through EI ion source as a medium for EI of vibrationally cold sample compounds in the SMB (hence also named GC-MS with Cold EI). We found that GC-MS with Cold EI provides enhanced molecular ions, extended range of compounds amenable for GC-MS analysis, improved sensitivity particularly for compounds that are difficult to analyze, facilitates faster GC-MS analysis and provides relatively uniform compound independent ionization yields. GC-MS with SMB (Cold EI) is reviewed in reference\textsuperscript{[33,34]}

We also explored electron ionization LC-MS and in our past publication\textsuperscript{[35–39]} we described a new method and instrumentation for the combination of LC-MS and EI through the use of supersonic molecular beams (SMB) as a medium for EI of vibrationally cold sample compounds (hence also named Cold EI). The reported new (at that time) approach of EI-LC-MS with SMB\textsuperscript{[35–37]} was based on spray formation behind a supersonic nozzle, followed by full thermal sample particle vaporization prior to sample expansion as isolated molecules from the supersonic nozzle. The supersonic free jet was collimated and formed a supersonic molecular beam that contains vibrationally cold sample molecules. These molecules proceeded axially along a dual cage fly-through EI ion-source\textsuperscript{[40]}; for obtaining Cold EI mass spectra with enhanced molecular ions. Cluster formation with the solvent vapor was practically eliminated by using a relatively large diameter and separately temperature-controlled nozzle. Vaporized solvent molecules served as the SMB carrier gas without adding another seeding gas. LC-MS with SMB was experimentally evaluated for a broad range of thermally labile and relatively large compounds in liquids\textsuperscript{[35–37]} and it generated the following main advantages: (1) Library searchable EI mass spectra are provided for positive sample compounds identification, combined with enhanced molecular ions for improved confidence level in the identification; (2) Non-polar compounds are amenable for analysis in addition to the standard range of APCI compounds; (3) Approximately uniform compound independent response was obtained which enables easier sample quantitation even for unknown samples. (4) Matrix related ion suppression effects do not exist.

However, our past approach and system suffered from a major problem of poor robustness as the use of thermally assisted spray (Thermospray, also sometimes known as ‘spray and pray’) resulted in frequent clogging of the solvent delivery tube near its exit because of sample degradation and solvent salt residue deposits in that heated place (like limestone deposits in domestic kettles). Furthermore, service to replace the clogged solvent delivery tube required full venting of the system and thus was tedious and time consuming. In addition, because all the LC solvent was exhausted into the nozzle and vacuum system the liquid solvent flow rate was limited to practically 50 μl/min depending on the vacuum pump flow rate acceptance. Thus, our research was continued to improve our EI-LC-MS with SMB approach in order to retain its benefits while eliminating its abovementioned deficiencies. Consequently, the main challenge in having EI-LC-MS with SMB is how to achieve a reliable and robust conversion of steady LC output liquid flow into gas phase (vapor) flow with the following features:

a) No cluster formation.
b) No nozzle and/or solvent delivery tube clogging.
c) In a robust and easy to maintain system.
d) For broad range of compounds.
e) With a stable spray.
f) For useful solvent flow rate range of 20–250 μl/min.
g) With acceptable helium nebulizing flow rate.
h) With minimal added cost.
i) Dual use GC-MS and LC-MS with SMB (Cold EI) in one system.

In this paper we describe our new EI-LC-MS with SMB method and system while describing how it retains the past demonstrated benefits together with the full elimination of its past downsides to provide an effective and useful new type of LC-MS.

Experimental—the EI-LC-MS with SMB system

In order to design an optimal EI-LC-MS system we started with having a strategy and list of goals for instrument development. Accordingly, our system and its components are based on the following novel concepts and considerations:

1. Use of a supersonic molecular beams (SMB) interface and its fly-through EI ion source in order to obtain enhanced molecular ions and extended range of compounds amenable for analysis. This range should be bigger than that of the particle beams LC-MS or GC-MS (equivalent to that of APCI plus the non-polar compounds).
2. Use of a quadrupole MS for lower cost because the ability to identify sample compounds with the library implies that high cost high resolution MS is not essential.
3. Use exactly the same SMB interface and fly-through ion source as in GC-MS with SMB in order to have the option for both GC-MS with Cold EI and EI-LC-MS with SMB in the same system with an easy interchange procedure.
4. Use of triple quadrupole MS for the experimental system to have MS-MS capability in order to explore the option to analyze samples in complex matrices via much faster flow injection MS-MS instead of lengthy LC-MS (in view of absence of ion suppression effects).
5. Employ high pressure nebulization (1–1.5 Bar) as in APCI for improved robustness and spray reliability as well as easy vaporization chamber service without venting.
6. Perform the spray vaporization inside a standard GC liner for easy and low cost replacement service in case of liner contamination.
7. Connect the vaporization chamber to the SMB nozzle with a short heated deactivated fused silica capillary transfer line (neutral funnel) in order to reduce the nozzle backing pressure to suppress and eliminate sample cluster formation with the solvent vapor.
8. Use of a larger diameter nozzle (0.3-mm ID instead of 0.1-mm ID) for lower pressure supersonic jet expansion to suppress cluster formation while retaining the SMB vibrational cooling.
9. Design the heated sample vaporization chamber with maximum axial temperature gradient at its entry side for best spray stability (eliminate onset Thermospray spray instabilities).
10. Use helium as the nebulization gas for improved nozzle-skimmer jet separation and higher signal.
11. Develop an optimal pneumatic spray head that will produce stable spray at wide range of solvent flow rates and composition at minimal helium flow rate.
12. Develop a vaporization chamber split valve that will enable the use of pneumatic spray with increased LC solvent flow rates given the limit of total (helium and solvent) flow rate acceptance of the turbo molecular pump of the SMB interface vacuum chamber.
13. Design a spray head axial positioning device for its optimal temperature and spray stability positioning inside the heated vaporization liner.
14. Design an axial positioning device for the solvent delivery tube for its placement at the optimal position inside the pneumatic spray head.
15. Design the whole EI-LC-MS spray vaporization device as a one unit from solvent delivery tube up to the supersonic nozzle in order to enable GC-MS with Cold EI and EI-LC-MS in a one system that can be interchanged via the change of the vaporization chamber unit with the GC-MS transfer line only.

Having the above design considerations in mind we shall now describe the experimental EI-LC-MS system with some emphasis on how it achieves these goals. The description will begin with the SMB-interface, ion source and MS system and after that the EI-LC-MS vaporization chamber and interface will be described. The EI-LC-MS with SMB apparatus is schematically shown in Fig. 1. It is based on our modified home-made GC-MS with SMB (GC-MS with Cold EI) system that was previously described in details.\(^{[34]}\) The base MS system is a Varian 1200 L triple quadrupole MS system that was previously described in details.\(^{[136]}\) The base MS system is a Varian 1200 L triple quadrupole MS system that was modified to include the SMB interface and fly-through ion source, and we named it 1200-SMB.

In the 1200-SMB the supersonic nozzle expands 60–90 ml/min mixture of vaporized solvent, vaporized sample compounds and helium nebulization and make up gas into a nozzle (SMB formation) vacuum chamber that is differentially pumped by a Varian Navigator 301 turbo molecular pump (Varian Inc., Torino Italy) with 250 l/s pumping speed. The pressure at this vacuum chamber is about 6 μBar. While most of the helium flow arrives from the pneumatic nebulizer some low helium flow rate (~5 ml/min) can be separately provided directly to the nozzle, and it can be mixed (via an opening of one valve) with perfluorotributylamine (PFTBA) for periodic system tuning and calibration. The sample compounds seeded in the helium and solvent vapor expand from a 300-μm diameter supersonic nozzle. The supersonic expansion vibrationally cools the sample compounds, and the expanded supersonic free jet is skimmed by a 0.8 mm skimmer and collimated in a second differentially pumped vacuum chamber, where an SMB is formed. The second vacuum chamber is pumped by a Varian 400/300 split turbo molecular pump that pumps both the second vacuum chamber (400 l/s) and main MS vacuum chamber (300 l/s). The SMB seeded with vibrationally cold sample compounds flies through a dual cage El ion source\(^{[40]}\) where these beam species are ionized by 70-eV electrons with 1–2-mA emission current. The ions are focused by an ion lens system, deflected 90° by an ion mirror and enter a radio frequency (RF)-only hexapole ion transfer optics (Q0 of the original Varian 1200 system). The 90° ion mirror is separately heated and serves to keep the mass analyzers clean from sample induced contaminations. The ions are further transferred through an ion lens into the MS vacuum chamber and are analyzed by a quadrupole MS-MS mass analyzer system. It consists of two quadrupole mass analyzers (Q1 and Q3) and a collision cell (Q2). As in any quadrupole MS-MS system, it can operate in SIM or full scan mode, as well as in all common MS-MS scan modes. Because Q2 is a 180° curved RF-only quadrupole ion transfer system in the 1200 L, a head-on ion detector is positioned directly in the path of ions exiting Q3. Its entrance is biased at 5 kV, serving as an efficient ion to electron converter. In this configuration we already achieve several goals from the above list of 15 goals. The SMB interface and its fly through ion source provide Cold EI mass spectra with enhanced molecular ions, and this is the same system as used in GC-MS with Cold EI. The use of big nozzle with 300-μm ID serves to reduce its operational pressure hence suppress and practically eliminate sample cluster formation with the solvent vapor.

In Fig. 2 we show the EI-LC-MS sample vaporization chamber and its interface with the supersonic nozzle. Going from left to right in

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**Figure 1.** Electron ionization LC-MS with SMB system outline. The liquid is introduced either from HPLC system after its column or a syringe pump to the heated vaporization chamber through a pneumatic nebulizer. The helium nebulization gas enter the SMB interface through a nebulization gas line, sheath gas line and nozzle make up gas line (indicated). The SMB interface is separately pumped by a turbo molecular pump and the vibrationally cold sample molecules fly through the ion source while the produced ions followed 90° deflection into the Varian 1200 L triple quadrupole MS.
Fig. 2 we shall now describe the EI-LC-MS vaporization chamber along the liquid flow path. Samples were introduced using a HPLC (model 1100, Agilent Technologies, Waldbroon Germany) (or model ProStar LC, Varian Inc, Walnut Creek CA).

In some cases samples were injected using a 5-μl injection loop, directly into a liquid transfer line without a separation column. The liquid transfer line was typically made of fused silica capillary with 75-μm ID and 190-μm OD provided by PolyMicro Technologies (Phoenix Arizona, USA). Methanol and water were used as the solvents of choice at flow rates in the 20–250 μl/min range while acetonitrile, hexane, iso-propanol, ethylacetate and other solvents were also used. Acetonitrile required the use of special rhenium filament wire as it etched the tungsten ribbon filaments. The solvent flow in the capillary entered the EI-LC-MS system through the Z-Axis spray probe tune device that enabled the careful positioning of the solvent delivery capillary at the pneumatic spray nozzle. We found that axial positioning within 0.2 mm from its optimum place is desirable for having the most stable spray for the broadest solvent flow rates range and with relatively low helium nebulizing gas flow rate. The solvent delivery capillary further entered the liquid nebulization chamber via a Z-Axis spray probe tune device that can mechanically adjust the position of the pneumatic spray nozzle from 6 mm before the entrance to the heated vaporization oven up to 20 mm inside the vaporization oven. The purpose of this Z Axis tuning device is to optimize the position of the spray head in a location that is not yet hot, in order to suppress onset of Thermospray related spray instabilities, while emitting spray that touches the heated walls of the vaporization chamber at surface points which are sufficiently hot to vaporize the sample without any peak tailing. As a result, the spray formation and vaporization chamber is designed according to the concept of maximum axial temperature gradient. A glass liner serves as the heated vaporization chamber, and it is air cooled at its entrance by aluminum cooling block (Cooling unit), that despite being close within 10 mm from the heated vaporization chamber that is maintained at typically 300 °C it is at about 40 °C only. The glass liner (standard GC glass liner) which is a very good thermal insulator directs the Nozzle Spray head into the heated vaporization chamber. As a result, because of the maximum axial temperature gradient the liquid output from the LC is retained as liquid without premature formation of bubbles which mark the onset of Thermospray and produce undesirable spray instability. Typical spray nozzle head location is about 6 mm inside the heated vaporization chamber. The spray nozzle head has a unique design that is based on our modification of the concept of ‘flow focusing’ nebulizer.[16,41] In this spray nozzle the solvent delivery capillary is centered at a narrow neck that also enables the flow of nebulizing helium gas. The solvent delivery capillary ends at about 0.1 mm from the exit of the spray head tube with 0.5-mm ID that ends with 0.25-mm ID cup. We found that this structure provides a stable spray that is directed forward at a narrow angle so that the spray touches the surface of the heated vaporization chamber in a place where it is fully heated to its set temperature. The vaporization chamber is based on a standard GC liner which is a deactivated glass. We use a liner of Agilent 7890 GC with 78-mm length, 6.35-mm OD and 4-mm ID. We found that the Jennings cup liner seems to be the most suitable liner as it does not require glass wool that can exhibit surface activity yet it prevents the penetration of unvaporized particles into the capillary transfer line and ensures full vaporization at the inert glass liner. The vaporization chamber is separately heated and temperature controlled. The liquid solvent is pneumatically transformed into a spray, the sprayed solvent is vaporized and the emerging sample particles are fully vaporized, similarly to their vaporization in APCI or APPI. Helium is used for nebulization at typically 150 ml/min from which 130 ml/min serves for nebulization and about 20 ml/min as a sheath gas to prevent back migration and tailing of sample compounds. One electronic flow control stabilizes the helium flow rate which is split with flow restrictors into the flow rates as above. The heated sample vaporization chamber also includes a split out tube and valve for the gas after the liner exit, in similarity to the split gas line in GC injectors. Consequently, the vaporization chamber pressure is mechanically adjusted via a valve knob to provide about 45–60 W turbo pump power consumption which emerges from flow rate of about 60–90 ml/min. This split valve allows the use of sufficient helium flow rate as needed for achieving stable pneumatic spray as well as liquid flow rate up to 250 μl/min without overloading the turbo pump. Typical operation is at 1.5 Bar with split ratio of 1:1.5. After the sample is fully vaporized at the heated liner it is swept with the helium nebulizing gas and solvent vapor into a separately heated and temperature controlled deactivated fused silica capillary transfer line with 0.25 mm ID. This capillary that separates the high pressure (1–1.5 Bar) vaporization chamber and the nozzle which is maintained at about 0.15 Bar via its large diameter (0.3 mm ID) serves to optimize the sample vaporization process and enables...
its service at high pressure yet to maintain the nozzle at its optimal backing pressure to provide good vibrational cooling without sample and solvent vapor cluster formation. Thus, the transfer line capillary serves as flow impedance between the vaporization chamber and nozzle. At the length of 22 cm and at 300 °C it delivers about 28 ml/min helium flow rate at ambient 1 Bar pressure in the vaporization chamber (open split valve). The whole structure of solvent delivery capillary and its axial adjustments devices, spray nozzle, heated vaporization chamber and its split line, heated transfer line and nozzle are mounted as a one unit into an XYZ adjustment mechanism on the nozzle vacuum chamber. Thus, the nozzle can be accurately positioned in front of the skimmer at the optimal distance of about 12 mm from the skimmer. The dual cage fly-through ion source is placed a few millimeters after the skimmer. As explained above, with this design all our 15 goals as described at the beginning of this chapter are achieved.

The nozzle diameter is an important parameter that is linked to the useful liquid flow rate range. It must be sufficiently large to eliminate post expansion cluster formation yet small enough to enable effective vibrational cooling. Cluster formation of the sample with the solvent molecules depends on three body collisions, hence on P^3, while the vibrational cooling depends on PD, where P is the pressure behind the nozzle and D is the nozzle diameter. Thus, for a given liquid solvent flow rate, the tripling of the nozzle diameter (from its GC with Cold EI diameter) leads to the reduction of the vaporized solvent pressure by a factor of 9 hence to the reduction of cluster formation by a factor of 729 (=9^3). Meanwhile, such tripling of the nozzle diameter has only a minor penalty factor of 3 on the efficiency of vibrational cooling, that is far superior to that of pure helium in view of methanol or water being a heavier molecule without velocity slip.

Initially, we assumed that achieving the fastest possible sample vaporization would lead to the softest sample vaporization because of minimizing the time during which the sample can degrade. However, in view of our more recent experience with the analysis of thermally labile compounds in GC-MS, we came to the opposite conclusion that it is better to employ slower vaporization at cooler conditions because temperature has a greater effect than time on the promotion of thermal degradation. As a result, we now feel that the use of larger diameter glass liner with 4 mm ID as a vaporization chamber is preferable, and certainly it is more robust in terms of requiring less frequent internal cleaning. We note that as a result, in comparison with typical APCI or APPI our sample thermal vaporization is slower yet performed at lower temperatures such as 300 °C instead of 400 °C. From our experience our conditions lead to softer thermal vaporization than in most current APCI vaporizers.

Results—cold EI mass spectra, features and selected applications

In Fig. 3, the Cold EI mass spectrum of Trinitrotoluene (TNT) in methanol solution is shown in the left trace, and compared with its NIST 08 EI library mass spectrum shown in the lower left trace. Note the similarity of the library mass spectrum to the Cold EI MS obtained with the EI-LC-MS with SMB apparatus. All the major high mass fragment ions of m/z 210, 193, 179, 164, 149 and 134 as well as the lower mass ions are exhibited in Cold EI the same as in standard EI, and thus good library search results are enabled with NIST library matching factor of 846, reversed matching factor of 859 and 89.5% identification probability in the TNT identification.
increases our confidence level in the correct sample identification but also because it enables the confirmation or rejection of NIST library identification. Furthermore, while a quadrupole mass spectrometer with unit resolution is used, elemental formula information can be obtained with the TAMI software that is based on isotope abundance analysis of the molecular ion group of isotopomers which require the availability of abundant molecular ions.

An additional important beneficial feature of EI-LC-MS with SMB is that its response is linear unlike that of the Particle Beam. In a previous publication we demonstrated over 4 orders of magnitude linear dynamic range (LDR) for pyrene with linear correlation coefficient $R = 0.9993$ (as shown in Fig. 9 in that publication) and in our current system our LDR is five orders of magnitude from the limit of detection which is about 1 pg in SIM mode to about 1 μg and the main limitation is in the proper adjustment of the ion detector gain within its limited dynamic range. We note that this high Cold EI LDR is higher than that of typical ESI LDR.

Another important feature of the EI-LC-MS with SMB system is that electron ionization in contrast to all the API ionization methods (ESI, APCI and APPI) is an in vacuum ionization method without any ion molecule reactions. Consequently and as demonstrated in the literature, EI does not exhibit ion suppression or enhancement effects that plague the various API ionization methods. Cold EI, like EI, is an in vacuum ionization method, and furthermore the ionization is performed in collision-free molecular beam environment; hence it is completely free from any ion suppression or enhancement effects. In Fig. 4 we demonstrate the absence of ion suppression or enhancement effects in the three consecutive flow injection analysis of 1 ppm Pyrene (SIM mode on its molecular ion $m/z = 202$) that are followed by three consecutive flow injection analysis of 1-ppm Pyrene that was spiked with 1000 ppm each caffeine (basic drug) and ibuprofen (acidic drug). As demonstrated, the 1000 times higher levels of added ‘matrix’ did not affect the Pyrene response. We note that Pyrene has negative proton affinity and is difficult

![Figure 3. Cold EI mass spectra of TNT, Sulfamethoxazole and Haloperidol as obtained with the EI-LC-MS with SMB system and its comparison with NIST library classical EI mass spectra of these compounds. Flow injection of these compounds in methanol was employed with a 5-μl loop and methanol flow rate of 50 μl/min.](image-url)
to ionize by ESI hence it is expected to be highly subjected to ion suppression effects. Thus, while Fig. 4 provides only suggestive information it can serve as an additional proof that Cold EI is free from any ion suppression or enhancement effects.

Because EI-LC-MS with SMB has no matrix related ion suppression effects, it does not always require LC for sample separation from the matrix, and thus a new approach is enabled for achieving ultra-fast flow injection (FI) EI-MS-MS with SMB analysis. Recently, the topic of flow injection MS-MS as an alternative to lengthy LC-MS analysis received significant attention in the world of pesticides analysis in agricultural products.\(^{[49-53]}\)

In these and in other experiments with electrospray ionization,\(^{[49-54]}\) the sample extracts were diluted about 100–200 times to reduce or eliminate ion suppression effects and analyzed by flow injection, electrospray ionization MS-MS. However, as known, electrospray cannot serve for the ionization of all pesticides, especially with 100 times dilution, and its sensitivity is not sufficient for a few groups of pesticides, particularly those that currently require GC-MS analysis such as the organo-chlorine pesticides. Our EI-LC-MS with SMB system can serve without any extract dilution, in its flow injection (FI) mode, to supplement and complement ESI in the development of fast and universal method for pesticide analysis. In Fig. 5, we show FI-EI-MS-MS with SMB analysis of 100 ppb (also 100 ng/g) diazinon in a QuEChERS mixed produce extract (provided by S. J. Lehotay from the USDA). The first injection peak shows a fruit matrix spiked with Diazinon at 100 ppb (also 100 ng/g) concentration. The second injection peak is of the unspiked matrix, while the third injection peak is of pure 100 ppb of Diazinon in acetonitrile. The liquid flow rate was set to 15 μl/min acetonitrile. The measured signal to noise ratio is 80, and the estimate LOD is ~10 ppb based on the matrix equivalent signal.

In Fig. 5, we show FI-EI-MS-MS of Diazinon insecticide in a fruit and vegetable matrix is shown without ion suppression or enhancement effects. The MS-MS signal of Diazinon is at its molecular ion \(m/z = 179\). The first injection peak shows a fruit matrix spiked with Diazinon at 100 ppb (also 100 ng/g) concentration. The second injection peak is of the unspiked matrix, while the third injection peak is of pure 100 ppb of Diazinon in acetonitrile. The liquid flow rate was set to 15 μl/min acetonitrile. The measured signal to noise ratio is 80, and the estimate LOD is ~10 ppb based on the matrix equivalent signal.

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times lower than expected based on uniform response. The lower relative response of Agidol 40 is attributed to the decline of the quadrupole mass spectrometer transmission at high masses. We note that the Pyrene EI mass spectrum shows almost no fragment ions below \( m/z = 101 \) and thus can start above \( m/z = 100 \) (as in Fig. 6) resulted in almost no fragments based TIC losses (unlike with other compounds); thus higher relative response was achieved.

This close to uniform response observation represents an important attribute of EI as an ionization method because in contrast to APCI or ESI, its ionization efficiency is independent of the sample compound polarity and identity. While the ionization yield is uniform and compound independent the system response uniformity is somewhat eroded because of quadrupole (and its ion detector) ion transmission decline with mass and the fact that low mass ions below 70 u are not collected. Furthermore, the response uniformity holds true only for pure compounds or simple mixtures. On the other hand, unlike in standard EI, in GC-MS with Cold EI no ion source peak tailing affects the response uniformity. We estimate that despite these imperfections the response uniformity allows us to know (with a factor of 2) how much we have from each compound without the tedious and time consuming process of full identification, synthesis and the performance of compound specific calibration curve.

In Fig. 7 the EI-LC-MS with SMB analysis of four simple compounds is shown. Dimethylphthalate, Diethylphthalate, Ethylparaben and Butylparaben were analyzed using gradient elution from 50% water/methanol to 75% methanol.

The upper trace shows the obtained total ion mass chromatogram, while the bottom traces show the obtained Cold EI mass spectra of diethylphthalate and ethylparaben and their respective NIST library mass spectra (inverted bottom MS). As demonstrated, very good similarities between the Cold EI and NIST library mass spectra are visibly observed, and some enhanced molecular ions are also shown. For diethylphthalate the NIST library search

| Table 1. Relative abundance of peak areas of the four indicated compounds |
|----------------|-------|---------|
|                | RT [min] | Area     | Percent |
| OFN            | 0.67    | 8.84E + 08 | 24%     |
| Pyrene         | 0.842   | 1.54E + 09 | 42%     |
| Agidol 40      | 1.489   | 3.60E + 08 | 10%     |
| Cholesterol    | 2.067   | 8.66E + 08 | 24%     |

Figure 6. OFN, Pyrene, Agidol 40 and Cholesterol analysis by EI-LC-MS with SMB. Sample concentration was 20 ppm and C18 Phenomenex Luna column was used with 3-\( \mu \)m particles, 50-mm length and 1.0-mm ID, using 5-\( \mu \)l sample loop. The LC method in this experiment was isocratic 100% methanol at a flow rate of 70 \( \mu \)l/min.

Figure 7. EI-LC-MS with SMB analysis of dimethylphthalate, ethylparaben diethylphthalate and butylparaben in order of their elution, at 50 ppm each. The HPLC gradient used was from 50% water/methanol to 75% methanol with a solvent flow rate of 35 \( \mu \)l/min, in a 50 mm x 1.0 mm C18 Phenomenex Luna column with 3-\( \mu \)m particles.
matching factor was 883, reversed matching was 887 and identification probability was 77.6%. For ethylparaben the NIST library search matching factor was 933, reversed matching was 940 and identification probability was 80.4%. Thus, Fig. 7 demonstrates the applicability of EI-LC-MS with SMB for the analysis of these compounds with effective library based identification. In addition, Fig. 7 also demonstrates the feature of response uniformity via the similarity of the TIC peaks of all the four compounds despite having two different classes of compounds of paraben drugs and phthalate diesters.

Cannabis and its main ingredients analysis became recently an area of growing importance as while it is required for law enforcement it is also required for the optimization of its growth as tool for product control in the increasing number of legal markets for cannabis plant products. Mass spectrometry and in particular EI-LC-MS with SMB can provide an effective and legally defensible analysis method for the detection and quantification of plant compounds and metabolites. In Fig. 8 the fast EI-LC-MS with SMB analysis of the three main ingredients of Cannabis is shown (TIC at left) together with the obtained Cold EI and its identification results with the NIST library.

As shown, Cannabidiol (CBD), Cannabinol (CBN) and Tetrahydrocannabinol (THC) at 20 ppm are analyzed in under 2-min analysis and exhibit uniform compound independent TIC peak areas. The Cold EI mass spectra are highly characteristic and the Cold EI MS of CBD and THC are very different despite the fact that they are isomers that cannot be differentiated by ESI and/or MS-MS.[55] As shown, the NIST library provided unambiguous identification with names and structures at the isomer level with high (indicated) identification probabilities.

While these compounds are among the major components of Cannabis and thus high sensitivity is not required in Cannabis analysis, high sensitivity is always a feature of importance. Thus, while we evaluated the sensitivity of the EI-LC-MS with SMB system a few times in the past and found it to be with around 1 pg LOD[36] we decided to further evaluate it with additional compounds in our new EI-LC-MS with SMB system. In Fig. 9 we demonstrate the system sensitivity in the single ion monitoring (SIM) analysis of CBD and THC as in Fig. 8 but now at 40-ppb concentration levels (200 pg on column with 5-μl sample loop) while monitoring the molecular ion m/z = 314 of CBD and THC in SIM mode.

**Figure 8.** Cannabidiol (CBD), Cannabinol (CBN) and δ9-Tetrahydrocannabinol (THC) analysis by EI-LC-MS with SMB; 20 ppm each compound was injected through a 5-μl loop into a C18 Phenomenex Luna column with 3-μm particles. The flow rate was set to 60 μl/min, and the gradient was from 50% to 80% methanol in water in 1 min.

**Figure 9.** Sensitivity evaluation of the EI-LC-MS with SMB system in the single ion monitoring analysis of Cannabidiol (CBD) and δ9-THC analysis; 40 ppb was injected through a 5-μl loop to a C18 Phenomenex Luna column with 3-μm particles. The gradient was from 60% methanol and 40% water to 100% methanol in 1.5 min. The MS was monitoring at m/z = 314, and the Varian software calculated signal-to-noise ratio (peak to peak) is 293 for CBD and 737 for δ9-THC which translates to linearly extrapolated LOD of 0.7 pg and 0.3 pg.

B. Seemann et al. J. Mass Spectrom. 2015, 50, 1252–1263

As demonstrated, clean mass chromatogram is obtained, and the Varian software determined the signal to noise ratio for THC as 737 in peak to peak calculation. Thus, the linearly extrapolated LOD is 0.3 pg. The peak of CBD is about half of that of THC because of its lower relative abundance of its molecular ion. We note that the measured LOD can be different from an extrapolated LOD which is optimistic most of the times.

In Fig. 10 we show the analysis of Cannabis flower extract. A small portion of dry Cannabis flower was crushed and extracted by pure methanol under 20-min sonication, followed by centrifugation and filtration. The extract was injected as is into a Varian ProStar LC equipped with a 15-cm column with 1-mm ID (Phenomenex Luna, using 5-μm C18 particles) and separated with gradient elution at 60 μl/min solvent flow rate from 50% water/methanol mixture to 100% methanol. As demonstrated, the results were initially surprising as we found that the two biggest peaks had the same Cold EI mass spectra that were identified by the NIST library as of THC. The explanation for this experimental observation is that the second peak is of THC-A meaning Tetrahydrocannabinol acid which was separated from THC on the LC column, but dissociated at the heated vaporization chamber into THC.

We analyzed four Cannabis flower samples, and as shown in Table 2 in each one of them we found the THC-A to be highly or significantly changed from sample to sample. This finding which is well known[56] demonstrates the importance of LC-MS analysis over GC-MS (or GC-FID) and the effectiveness of EI-LC-MS with SMB that enables direct quantification of the ratios of Cannabis main ingredients.

Discussion and advantages

A new type of electron ionization LC-MS was developed, based on the use of supersonic molecular beams for LC and MS interface and as a medium for electron ionization of vibrationally cold sample molecules in a fly-through ion source. The structure of this new EI-LC-MS with SMB system, and its main components is described, and its various unique benefits were demonstrated in selected examples and applications. The new EI-LC-MS with SMB system provides the following major benefits and advantages over currently used API based LC-MS:

1. **Library based identification.** Library searchable EI mass spectra are provided, unlike with ESI and/or APCI. This is an important advantageous feature, shared with Particle Beam LC-MS and 'Direct EI' that enables fast, automated, legally defensible and reliable molecular identification with sample names and structures at the isomer level.

2. **Enhanced molecular ions.** High quality Cold EI mass spectra are provided with enhanced molecular ions and isomer structural information for further increased confidence level in the sample identity, particularly versus its degradation products and homologous compounds.

3. **Provision of elemental formula.** While quadrupole mass spectrometer with unit resolution is used, elemental formula information can be obtained in our system with the TAMI software that is based on isotope abundance analysis of the molecular ion group of isotopomers,[48] and the similarity of the measured masses.

4. **No ion suppression.** Ion suppression and/or enhancement effects that plague API based ion sources are fully eliminated.

5. **Fast LC-MS.** Faster LC-MS analysis is enabled through the availability of automated deconvolution software and particularly in view of the absence of ion suppression effects under coelution conditions. Consequently, full LC separation is not essential and partial co-elution can be tolerated that facilitates much faster analysis.

6. **Flow injection MS-MS.** Fast flow injection MS-MS can sometimes replace lengthy LC-MS analysis because of the elimination of ion suppression or enhancement effects.

7. **Non-polar compound analysis.** EI-LC-MS with SMB can serve for the analysis of both semi-polar and non-polar compounds and unlike ESI and/or APCI based LC-MS it can analyze completely non-polar compounds including saturated hydrocarbons and polycyclic aromatic hydrocarbons (PAHs).

8. **Uniform response.** Electron ionization provides uniform ionization yield (response) for all compounds that enter the ion source for improved quantitation without the need for compounds specific calibration and it can serve for the analysis of both semi-polar and non-polar compounds and unlike ESI and/or APCI based LC-MS it can analyze completely non-polar compounds including saturated hydrocarbons and polycyclic aromatic hydrocarbons (PAHs).

9. **No nitrogen generator.** The bulky heavy and expensive nitrogen generator and its air compressor (noise, service, added price and added lab or extra room place) that are used in Electrospray LC-MS are not required plus the rotary pump of the system is much smaller. Thus, unlike in standard LC-MS the EI-LC-MS with SMB system can be transportable to the hood or process on a cart with a small helium cylinder.

### Table 2. The relative abundance of THC-A, THC and CBN in four Cannabis flower samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>THCA</th>
<th>THC</th>
<th>CBN</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>79%</td>
<td>19%</td>
<td>2%</td>
</tr>
<tr>
<td>b</td>
<td>91%</td>
<td>8%</td>
<td>1%</td>
</tr>
<tr>
<td>c</td>
<td>42%</td>
<td>51%</td>
<td>7%</td>
</tr>
<tr>
<td>d</td>
<td>89%</td>
<td>9%</td>
<td>2%</td>
</tr>
</tbody>
</table>
10. **Reasonable LOD.** Reasonable LOD of around or just below 1 pg in SIM mode were obtained for several compounds including OFN, Agidol 40, Cholesterol and Pyrene. While these LODs are not as good as obtained with ESI-LC-MS they are about the same for all compounds that are amenable for EI-LC-MS analysis hence better than those of ESI-LC-MS for non-polar compounds.

11. **Large linear dynamic range.** High linear dynamic range (LDR) is provided of five orders of magnitude.

12. **GC-MS and LC-MS with SMB in a one system.** GC-MS with Cold EI (SMB) and EI-LC-MS with SMB system replacement could be obtained through a change of a single flange with the GC-MS transfer line or LC-MS vaporization chamber and transfer line hence the system can have dual GC-MS and LC-MS usage.

13. **Low cost of goods.** The feature of library based identification implies that a simple low cost single quadrupole mass spectrometer can be used, the same as in GC-MS, because the library based identification reduces the need for costly high resolution MS.

Cold EI facilitates the provision of a new and useful LC-MS system that supplements and complements ESI-LC-MS. The EL-LS MS with SMB excels especially in the analysis of unknown compounds and/or for the analysis of synthetic organic compounds in which the uniform response enables the provision and optimization of chemical reaction yields. The EL-MS MS with SMB can also serve for flow injection MS-MS to supplement and complement FI-ESI-MS-MS as a fast alternative to lengthy LC-MS analysis. Our vision is that Cold EI with its unique benefits will serve as a dedicated low cost EI-LCMS system, with high performance for the analysis of small molecules.

It is worthwhile to briefly compare our Cold-EI with SMB approach to the Direct EI approach of Cappiello and co-workers.[20–32] In Direct EI, the LC and MS interface is as simple as it gets, and the MS of a GC-MS can be used with only minor added EI ion-source and interface modifications. However, the LC column flow rate is restricted to below 1 μl/min which necessitates the use of packed capillary Nano LC columns with its resulting lower sample loading ability and loss of concentration sensitivity. We feel that the major advantage of our Cold EI approach is the higher quality mass spectra obtained which adequately compensates for the added instrumental complexity.

The EI-LS-MS with SMB system and approach require further investigation and exploration of further applications for its establishment as a useful technique. We also intend to combine it with other more modern MS system such as the Agilent 5977 MSD.

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## References


Electron ionization LC-MS


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