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LC–MS with electron ionization of cold molecules in supersonic molecular beams

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Abstract

A new approach is described for the combination of electron ionization and LC–MS based on sample ionization as vibrationally cold molecules in a supersonic molecular beam (Cold EI). Cold EI of sample compounds in liquid solutions (methanol, acetonitrile, water, etc.) is achieved through spray formation, followed by soft thermal vaporization of the sample particles prior to their supersonic expansion and direct electron ionization of the sample compounds while they are contained in a supersonic molecular beam (SMB). Cold EI mass spectra were demonstrated to combine an enhanced molecular ion and improved mass spectral information (in comparison with standard EI), plus all the library searchable fragments. Cold EI enables the ionization of a broad range of compounds, including the full range of non-polar samples. Four orders of magnitude linear dynamic range is demonstrated and a detection limit of 2 pg was achieved for a 774 amu compound in single ion monitoring mode at $m/z = 774$. The method and apparatus are under continuous development and we feel that it can excel particularly in the analysis of unknown samples, while enabling fast LC–MS analysis through automated mass spectral deconvolution of coeluting LC peaks. In addition, the same MS system can also serve as an advanced GC–MS with supersonic molecular beams.

Keywords: Supersonic molecular beams; LC–MS; Electron ionization; Cold EI

1. Introduction

Liquid chromatography–mass spectrometry (LC–MS) experienced significant growth in recent years and has become an established, widely used technique. Electrospray ionization (ESI) [1–3] is by far the most widely used LC–MS ionization method due to its superior sensitivity and extended mass range, enabled by the formation of multiply charged ions. However, ESI is supplemented with atmospheric pressure chemical ionization (APCI) [4,5] which, in some cases, shows better performance with relatively small and less polar compounds. Recently, atmospheric pressure photo ionization (APPI) [6–8] was introduced, which may further help in the analysis of certain weakly or non-ionized compounds in LC–MS. However, ESI, APCI and APPI still suffer from limitations in the ionization of non-polar compounds, and are also characterized by non-uniform compound-specific response. More important, all of these atmospheric pressure ionization (API) methods are soft techniques based on ion reaction chemistries that usually produce only the protonated or deprotonated molecular ion (with or without adducts), hence requiring MS–MS or high resolution accurate mass MS for further analyte characterization. As a result, they are not well suited for the analysis of true unknown compounds as routinely performed in gas chromatography mass spectrometry (GC–MS).

Particle beam LC–MS (LC–PB–MS) [9–16], with its electron ionization (EI), is thus far the only LC–MS method that enables library searchable EI mass spectra. This type of identification can be achieved automatically by non-experts, can be legally defensible and it contains structural information that helps with unknown compound identification [17]. Thus, it has been argued that despite its lower sensitivity and smaller range of compounds, LC–PB–MS is still a vital and useful technology [16]. However, recent PB-MS research is sparse,

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performed mostly by Cappiello et al. [18–21] on the combination of capillary and nano LC with EI-MS.

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 amplified for LC related compounds that are larger and more thermally labile than standard GC–MS compounds. Furthermore, they are usually less volatile hence, require higher EI ion-source temperatures with the consequence of further intra ion-source degradation and weaker molecular ion production. Without a molecular ion, EI based sample identification is not as trustworthy. 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 highest mass spectral peak should be trusted [22,23].

In a preliminary communication [24] 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 named Cold EI) [25–29].

The new approach of LC–(Cold-EI)–MS with SMB [24,30] is based on spray formation at high pressure, followed by full thermal sample particle vaporization prior to sample expansion as isolated molecules from a supersonic nozzle. This first step is similar to sample vaporization in APCI, however, instead of the next APCI step of Corona discharge for inducing chemical ionization, the sample according to our method expands from the supersonic nozzle. Then, the supersonic free jet is collimated and forms a supersonic molecular beam that contains vibrationally cold sample molecules. These molecules proceed axially along a fly through Brink type EI ion-source for obtaining Cold EI mass spectra with an enhanced molecular ion. In the preliminary report [24], the problem of vaporization of intact thermally labile compounds was addressed through achieving fast sample vaporization followed by supersonic expansion cooling. The issue of liquid solvent load on the vacuum pumps was addressed by differential pumping. Cluster formation 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.

The purpose of this paper is to describe our recent results with emphasis on the unique features and improved MS information content provided by Cold EI.

2. Experimental-liquid sampling EI-MS with SMB

The LC–MS with supersonic molecular beam (LC–SMB–MS) apparatus, which is schematically shown in Fig. 1, is based on our modified home made GC–MS with supersonic molecular beam system that was previously described [27–29,32]. Samples were introduced using a model 1100 HPLC (Agilent Technologies, Waldbroon Germany). In most cases samples were injected with a HPLC

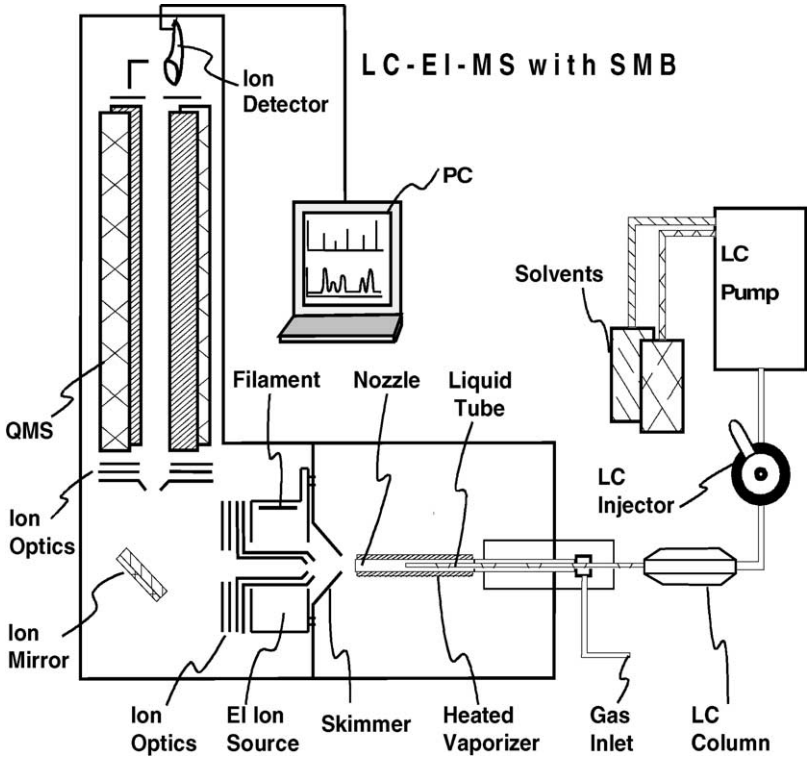

Fig. 1. The supersonic LC–EI-MS apparatus.

injector (model 9725i, Rheodyne, Rohnert Park CA, USA) using a 5 μl injection loop, directly into a liquid transfer line without a separation column. A 600 μl home-made injection loop was also used for the feeding of liquid for the study of Cold EI mass spectra and its dependence on various experimental parameters. The liquid transfer line was typically made of fused silica with 110 μm i.d. and 170 μm o.d. provided by PolyMicro Technologies (Phoenix Arizona, USA). Methanol (HPLC grade, J.T. Baker Philippsburg NJ, USA) was used as the solvent of choice at flow rates in the 50–250 μl/min range. Acetonitrile (HPLC grade, J.T. Baker Philippsburg NJ, USA), water (Chromatography grade, Merck Darmstadt, Germany), heptane, benzene, cyclohexane (all Spectroscopy grade, Merck, Darmstadt, Germany) and mixtures of methanol and water or acetonitrile were also used. Helium gas was provided in some cases through a 0.53 mm i.d. standard GC transfer line capillary at low flow rates in the 0–40 ml/min range (nitrogen could equally be used). The reason for restricting the He gas flow rate was that our system is based on full direct discharge of the vaporized HPLC liquid flow into the first nozzle vacuum chamber and thus each one ml/min added gas flow reduced the upper acceptable liquid flow rate by about 1 μl/min.

The sample and its solvent were vaporized inside the soft thermal vaporization nozzle (STVN) chamber that is described below. The gaseous mixture of solvent, sample and He (if added) expanded through the supersonic nozzle into the first vacuum chamber that was pumped by a turbo molecular pump having 250 l/s pumping speed (Navigator 301, Varian Inc. Torino, Italy). The SMB was collimated by a skimmer with 0.8 mm diameter. This pumping capability and skimmer diameter are identical to what we have in our supersonic GC–MS system [31]. After skimming, the collimated SMB entered a second vacuum chamber pumped by two Balzers 63 mm diffusion pumps with 135 l/s pumping speed each and total net pumping speed of 210 l/s (after considering the gate valve's gas conductivity). The two diffusion pumps were backed by the nozzle chamber turbo molecular pump which was backed by a single 200 l/min Edwards RV12 rotary pump (Edwards, Crawley, UK).

The SMB sample compounds were ionized in our home made dual cage design "fly-through" EI ion-source [32], and the ions were deflected 90° through an ion mirror (deflector) and analyzed by an Extrel 2000 amu mass range quadrupole mass analyzer. The mass analyzed ions were detected by a channeltron ion detector (DeTech, Palmer MA, USA), and the data was processed for identification and quantification using the Merlin software of Extrel and NIST 98 mass spectral library.

The "heart and soul" of our system is the soft thermal vaporization nozzle (STVN) chamber. The STVN accepts the liquid flow from the LC or flow injection liquid transfer line tube, and converts it into a supersonic molecular beams of vibrationally cold undissociated sample compounds that are amenable for EI and mass analysis.

In Fig. 2 the STVN used in our third generation unit is schematically pictured. The liquid sample solution enters from the liquid transfer line tubing that is connected with a union to a heated liquid transfer line for obtaining thermally assisted spray as in thermospray [33,34] and thermally assisted particle beam [35]. The thermally assisted spray is formed via direct current heating of a metal liquid transfer line tube (Upchurch Scientific Oak Harbor WA, USA, part number U-121) or through the direct current heating of a stainless steel tube of 0.53 mm i.d. and 0.77 mm o.d. (Restek, Bellefonte PA, USA) containing fused silica capillary tubing inside the metal oven tubing. In this latter device, about 5 W power was enough to vaporize 20–60% of the solvent (while

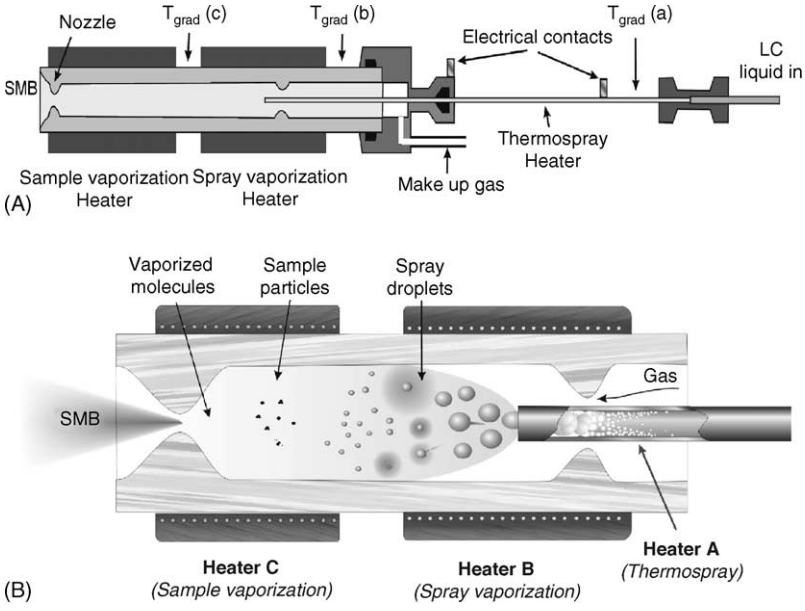

Fig. 2. The soft thermal vaporization nozzle (STVN).

inside the capillary tubing), which was sufficient for inducing a stable spray.

The spray vaporization chamber was made from fused silica tubing with 1.4 mm i.d. and 3 mm o.d., and it terminated with a home-made nozzle (using glass blowing tube size reduction) of 0.3–0.35 mm diameter. The STVN chamber itself was separately heated by two heaters made from Kanthal AF wire (Kanthal Hallstahmmar Sweden) (0.42 mm diameter) coiled around alumina ceramic tubing that matched the outer diameter of the STVN fused silica chamber. The rear heater was used for spray droplet vaporization followed by particle vaporization while the front heater was used for the final soft particle vaporization into intact unionized sample molecules and to establish the optimal nozzle temperature for cluster-free vibrational cooling. The rear heater power was typically 20 W, which attained external temperatures of about 500–800 °C. The rear heater length was 30 mm and the total STVN heated length with its two heaters was 50 mm. According to Covey and coworkers [36] the rear heater in APCI should preferably be heated up to 900 °C, above the onset of the Leidenfrost effect [37], so that the spray droplets would self-vaporize during their approach towards impacting the oven surface. The vaporization rate should be sufficient to form self-repulsion of the spray droplets without adsorption to the walls that may lead to peak tailing and sample degradation. We similarly found that the use of a hotter rear heater is beneficial, but in our case, the use of two separate heaters was essential for the proper separate control of the supersonic nozzle temperature which is critical in order to eliminate post-expansion clusters and/or induce effective sample vibrational cooling. We observed that the internal STVN walls (as well as the entire heater) were significantly cooled by the spray which could lead to STVN-induced peak tailing, depending on the specific design, and this tailing was noticeably lower with 1.4 mm i.d. STVN tubing than with 0.9 mm i.d. tubing. Another unexpected observation was that the spray droplets and sample particles experienced backward scattering, thus the helium gas was used (up to 40 ml/min) mostly to block this backward scattering and not to help the nebulization process. We attribute this backward scattering phenomenon to an increased pressure at the heated zone due to the vaporization of the liquid droplets. The nozzle was never clogged in our studies and our major reliability (robustness) problem was in the occasional clogging of the solvent delivery tubing at its outer edge that was placed a few mm inside the rear STVN heated zone. A major reason for such clogging was a sudden stop of the LC flow for various reasons (such as replacement of a solvent or a change of LC flow parameters, etc.). Consequently, we used a separate thermocouple located at the thermospray heated tube for controlling a circuit breaker in case the LC liquid flow stopped and as a result the thermospray heater temperature exceeded a certain predetermined temperature.

The nozzle diameter is an important parameter that is linked to the useful liquid flow range. It must be sufficiently large to eliminate post-expansion cluster formation [38], 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^3D, 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 solution flow rate, the doubling of the nozzle diameter leads to the reduction of the vaporized solvent pressure by a factor of 4 hence to the reduction of cluster formation by a factor of 32. Meanwhile, such doubling of the nozzle diameter had only a minor factor of two penalty on the efficiency of vibrational cooling, that was far superior to that of pure helium in view of methanol being a heavier molecule without velocity slip [39,40]. As a result, our STVN possesses three separately heated thermal zones and three temperature gradients between them, and these temperature gradients must be appropriately included and considered in the STVN design. We note that the temperature gradients between the heated zones were also important in preventing premature solvent vaporization that could cause sample condensation on relatively cool surfaces and eventually to clogging of the transfer tube. Similarly a fourth radial temperature gradient between the heated tube and inner solvent delivery tube was also important to suppress the clogging of the solvent delivery tubing.

We estimate that the thermally assisted spray was formed in the STVN chamber at about 0.1 atm with a methanol flow rate of 120 μl/min and as will be shown below. Under these conditions, appropriate vibrational cooling was achieved combined with practical elimination of clusters of sample compounds with solvent molecules.

Initially, we assumed that achieving the fastest possible sample vaporization would lead to the softest sample vaporization due to minimizing the time during which the sample can degrade [24]. However, in view of our recent results with experiments aimed at improving the analysis of thermally labile compounds in GC–MS, we came to the opposite conclusion that it is better to employ slower analyses at cooler conditions since temperature has a greater effect than time on the promotion of thermal degradation [41]. As a result, we now feel that the use of larger diameter STVN chamber is preferable and certainly it is more robust in terms of requiring less frequent internal cleaning.

Our LC–EI-MS approach can be perceived as a synthesis of a few current and past approaches, but in fact, it is a combination of a few of these approaches plus its own unique ingredients. The initial step of spray formation relates to thermospray [33,34] or to the ThermaBeam particle beam system of Willoughby and coworkers [35], but unlike thermospray our spray is only thermally assisted, formed at non-vacuum, relatively high pressure and without the step of thermospray ionization. Unlike the ThermaBeam [35] and all other particle beam systems, our approach is characterized by complete sample particle vaporization prior to its supersonic expansion. Our approach seems more akin to APCI in that it involves full sample vaporization at relatively high pressures, but unlike typical APCI, our spray is fully thermally assisted without using any gas for pneumatic nebulization.

Furthermore, no high pressure CI is involved as our Cold EI is a full in-vacuum ionization method. On the other hand, to a first approximation our ability to handle thermally labile compounds is similar to that of APCI since both methods share similar high pressure full thermal sample vaporization. However, in contrast to APCI, our Cold EI can ionize all the sample compounds that were vaporized, regardless of their polarity, and no ion molecule reaction (collision free molecular beam) can interfere in the obtained mass spectra. While our approach uses EI as the ionization method, our EI is Cold EI (in contrast to the particle beam methods) with the ionization of sample compounds while they are vibrationally cold in the SMB, without any scattering from interior surfaces of the EI ion-source. Consequently, our EI ion-source is a unique fly-through dual cage EI ion-source aimed at obtaining Cold EI mass spectra without vacuum background [32] and with enhanced molecular ion and mass spectral information.

3. Results — Cold EI mass spectra

In Fig. 3, the Cold EI mass spectrum of corticosterone in methanol solution is shown in the upper trace, and is compared with the standard NIST 98 EI library mass spectrum shown in the lower trace. Note the similarity of the library mass spectrum to that obtained with the SMB apparatus. All the major high mass ions of *m*/*z* 227, 251, 269 and 315 are with practically identical relative intensity and thus good library search results are enabled with NIST library matching factor of 829, reversed matching factor of 854 and 86.5% confidence level (probability) in the corticosterone identification. In addition, the molecular ion at *m*/*z* = 346 is now clearly observed while it is practically missing in the library (very small in the shown mass spectrum and absent in the other three replicate mass spectra). The relative abundance of the high-mass ion at *m*/*z* = 328 is also enhanced. On the other hand, low mass

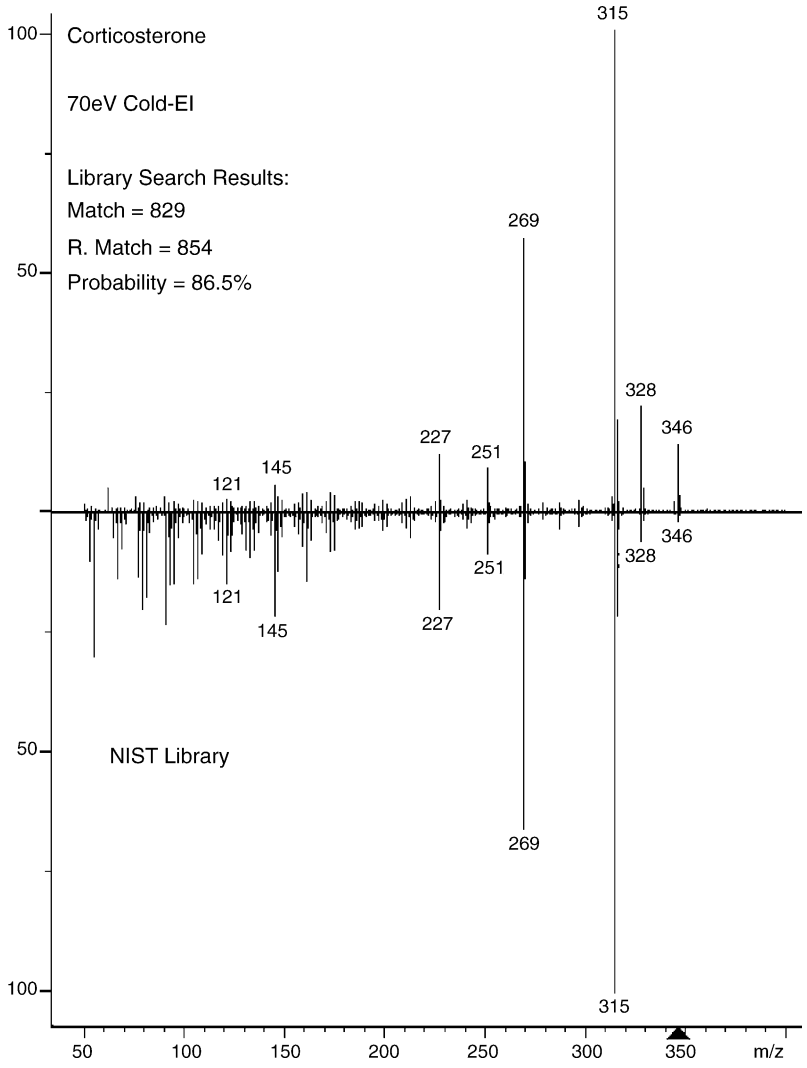

Fig. 3. A comparison of cold EI mass spectrum of corticosterone obtained with the supersonic LC–EI-MS system and its fitted NIST library mass spectrum, including the NIST library matching factors and probability of identification. Note the enhanced molecular ion (*m*/*z* = 346) exhibited. The NIST library matching factors and probability of identification are included. Corticosterone was flow injected using 200 μl/min methanol solvent flow rate with 1 ng/μl corticosterone sample concentration.

fragments are suppressed and the overall effect of vibrational cooling on the appearance of the Cold EI mass spectrum is of shifting intensity from low mass fragments to high mass fragments and in particular to the molecular ion. Overall, the obtained NIST matching factors are high but usually are not as high as obtained with standard EI. However, the probability factors and confidence level in the identification is actually higher with Cold EI as found in the analysis of many pesticides [22]. These higher probabilities and superior confidence level in sample identification emerge from three main reasons: (a) The ion-source temperature is typically higher in standard EI, such as with particle beam LC–MS, than the ion-source temperature used to obtain the NIST mass spectra, since in real analyses the ion-source temperature must be raised to prevent peak tailing of the least volatile sample compound and maintain ion-source cleanliness. Thus, at increased ion-source temperature the degree of molecular ion fragmentation is increased due to greater intra-ion vibrational energy content. As a result, while in Cold EI the molecular ion is enhanced, in standard EI it is suppressed in comparison with its relative abundance in the NIST library, resulting in reduced identification probabilities with standard EI compared with Cold EI. (b) Thermally labile compounds such as corticosterone can degrade in the hot ion-source in particle beam LC–MS or GC–MS. (c) The availability of the molecular ion is of critical importance for correct sample identification with high confidence level. For example, in the NIST hit list other candidates appear after corticosterone, such as corticosteroneacetate ($m/z = 388$), which is listed as number two with 10.8% probability, since its standard EI mass spectrum is almost identical to that of corticosterone. The reason for this is that the MS of both compounds exhibit no molecular

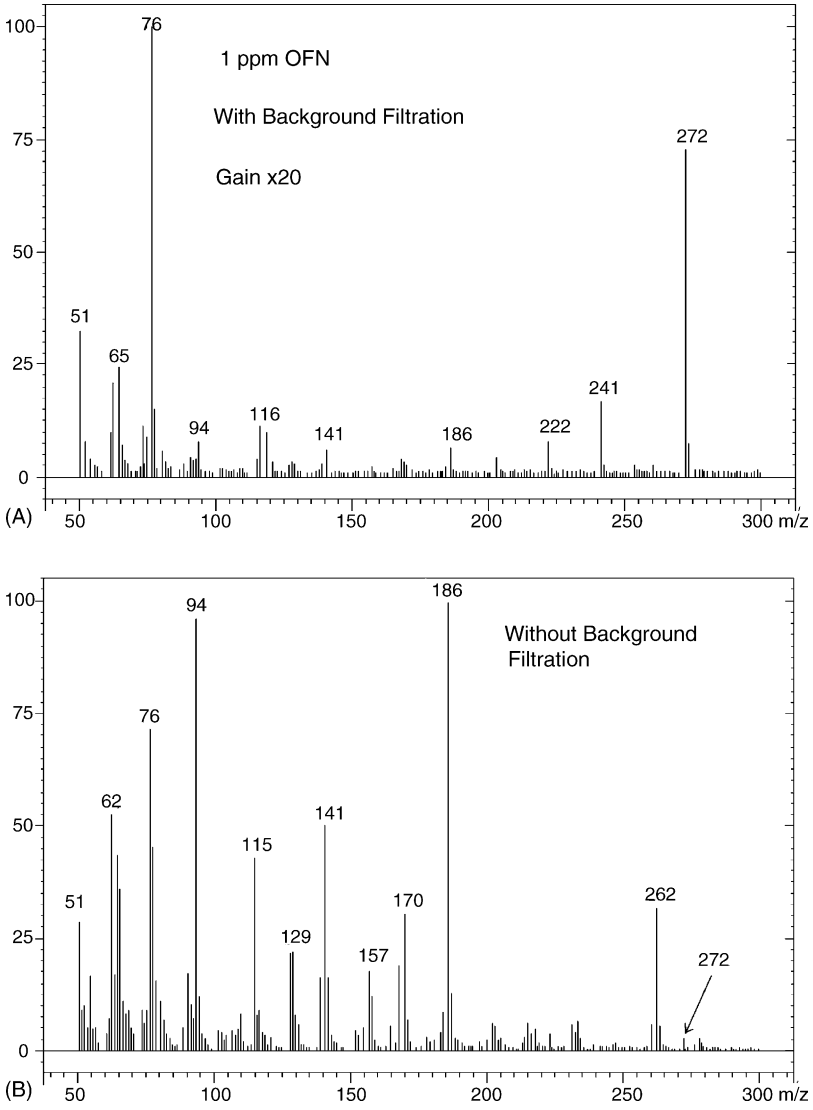

Fig. 4. Vacuum background filtration with the supersonic LC–EI-MS. The upper MS trace A is the octafluoronaphthalene (OFN) mass spectrum obtained with background filtration voltage of about 0.6 V while the bottom MS was obtained under the same condition but without any filtration retarding voltage. The vacuum chamber was relatively dirty soon after pump down. OFN was flow injected using 120 μl/min methanol solvent flow rate with 1 ng/μl OFN sample concentration.

ion while having exactly the same fragments (and structure) after the molecular ion fragmentation of $m/z = 315$ and even a small $m/z = 328$. This observation is typical in a series of homologous compounds and well known for large aliphatic hydrocarbons that all show mostly $m/z = 43$, 57, 71 and 85 fragments. Consequently, having both a clear molecular ion peak at $m/z = 346$ and lack of a molecular ion peak at $m/z = 388$ unambiguously indicates that the sample compound is corticosterone and not corticosteroneacetate. Similarly, several other candidates in the NIST hit list are eliminated resulting in almost 100% probability that the sample is corticosterone or one of its isomers (1.2% probability). Thus, Fig. 3 demonstrates the usefulness of the SMB-liquid sampling-MS approach, in both the analysis of a thermally labile compound as well as in showing the benefit of a mass spectrum which shows a combination of enhanced molecular ion and standard fragments for correct sample identification.

Fig. 3 is similar to Fig. 2 in our preliminary report [24] but with an important difference in that the demonstrated mass spectral range is now m/z 50–400 instead of previously m/z 200–400. The main reason for this is that due to the current use of the dual cage EI ion-source and its enabled vacuum background filtration, we could better explore the low mass spectral range, despite its extended vacuum background noise in our dirty MS chamber system that is pumped by diffusion pumps with a service history of 14 years.

In Fig. 4 the effect of vacuum background filtration is demonstrated in the mass spectra of octafluoronaphthalene

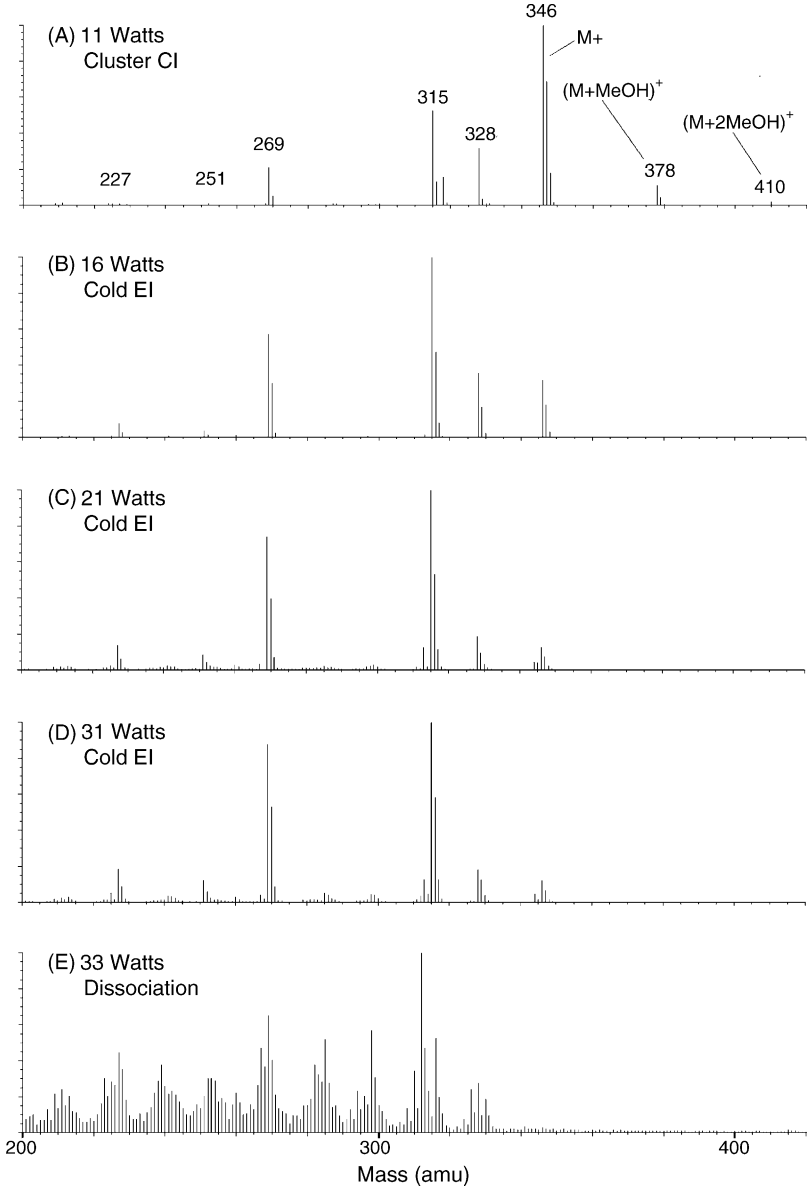

Fig. 5. The effect of total heating power of the soft thermal vaporization nozzle (with its three heated zones) on the EI mass spectra of corticosterone. While using 11 W (trace A) heating power, cluster chemical ionization is observed, in the sweet zone (traces B–D) of 15–31 W vibrationally Cold EI-MS is obtained. At and above 33 W (trace E) significant dissociation is shown. corticosterone was flow injected using 120 μl/min methanol solvent flow rate.

(OFN) that are shown with background filtration (upper trace A) and without it (lower trace B). Fig. 4 was obtained a few hours after pump down in which the vacuum system and ion-source were particularly dirty. (after a few days of pumping, the level of vacuum background became significantly lower than what is shown in Fig. 4). The dual cage ion-source is based on using an ion volume with zero internal electrical field, thus even trace B is already with reduced vacuum background (since vacuum background ions are not extracted but in part they can exit the ion-source), while in trace A, a small (0.5 V) retarding potential was added at the ion-source exit lens to prevent the transit (exit) of vacuum background ions from the ion-source. In contrast, ions formed from SMB species that had a few eV directional kinetic energy penetrated this retarding potential and were selectively analyzed.

The elimination of vacuum background is an important feature of our system since in addition to providing higher sensitivity it helps to better expose the Cold EI-MS of vibrationally cold molecules through the removal of self background of sample compounds that scattered from the vacuum chamber and ion source walls. Furthermore, the fly-through EI ion source design in combination with vacuum background filtration, eliminates ion-source related peak tailing from lengthy intra ion-source adsorption-desorption cycles that could occur with relatively low volatility compounds, since any compound that scatters from the ion-source walls will thermalize (hence become vacuum background) and even if ionized will not be detected anymore. While such ion-source related peak tailing is known to occur in GC–EI-MS and/or in particle beam LC–EI-MS, its elimination in LC–MS is relatively more

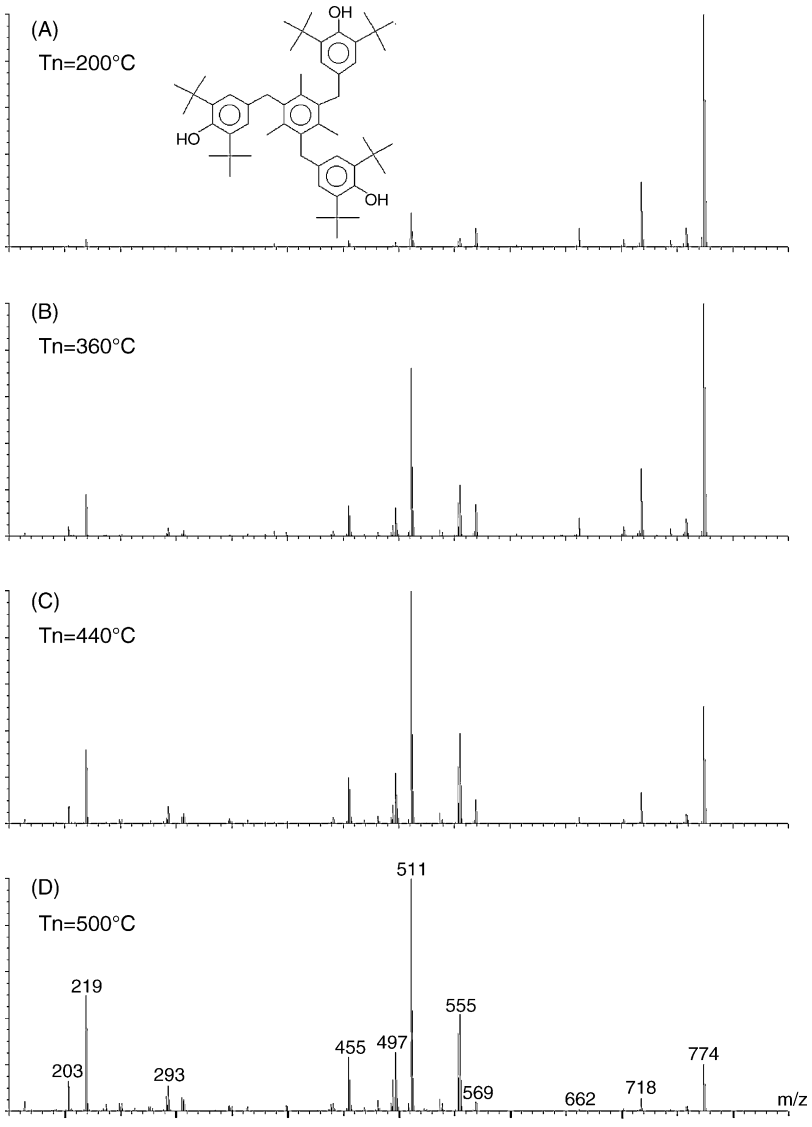

Fig. 6. Nozzle temperature effect on the vibrational cooling and cold EI mass spectra of a relatively large (774 amu) molecule. The upper EI-MS trace was obtained with 200 °C nozzle temperature demonstrating effective vibrational cooling and only little fragmentation. As the nozzle temperature is increased (while using relatively low solvent flow rate) increased EI fragmentation is demonstrated, yet the molecular ion is retained even at 500 °C nozzle temperature. The indicated compound was flow injected using 120 μl/min methanol solvent flow rate.

important than in GC–MS due to the general lower volatility of LC compounds. We note however that although the ion-source related peak tailing is eliminated in our system, it remains as a potential adverse effect in the STVN, in similarity to APCI in its thermal vaporizer. A closer look at the results shown in Fig. 4 reveals that even with vacuum background filtration the observed mass spectrum (that is unprocessed by background subtraction) shows low mass spectral peaks such as $m/z = 51, 65$ and 76 plus a few higher mass background ions that are not completely filtered (such as $m/z = 186$ and 141). These peaks originate in part from incomplete vacuum background filtration and in part from impurities in the LC solvent (methanol) used. Since EI is a universal ionization method solvent impurities posses a greater problem than in the selective API methods and very pure solvents must be used.

The effect of total STVN heating power on the obtained Cold EI mass spectra and sample-solvent cluster formation was explored and the results are shown in Fig. 5. It was found that, due to the relatively high temperature of the nozzle and its relatively low pressure (compared with supersonic GC–MS) of below 1 atm, the effect of cluster formation could be negligible. In the upper trace (A) the Cold EI mass spectrum of corticosterone is shown, obtained with 11 W total STVN heating power. Below 11 W the signal rapidly decreases while at 11 W the Cold EI is dominated by cluster CI effects [23,38]. Evidently, corticosterone forms clusters with the methanol vapor upon its expansion from the relatively cool nozzle. Following the 70 eV EI of the clusters intra-cluster proton transfer from the methanol to the corticosterone can take place, leading to protonated corticos-

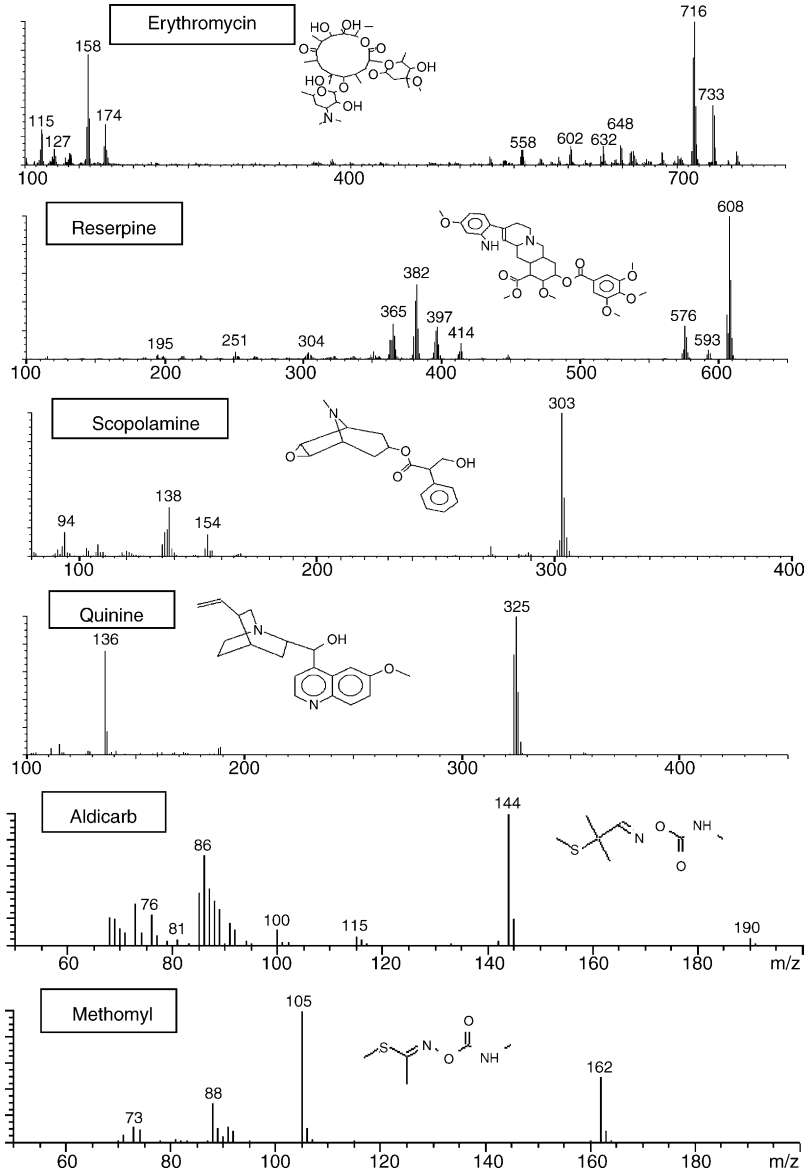

Fig. 7. Cold EI mass spectra of the indicated drugs and carbamate pesticides. Enhanced molecular ion peaks are observed together with the standard library searchable EI fragments. Drugs and carbamate samples were flow injected using 120 μl/min methanol solvent flow rate and 100 ng/μl sample concentrations.

terone molecular ion. In addition, the relative abundance of the molecular ion is increased (also compare Figs. 3 and 5) and satellite mass spectral peaks of corticosterone with one and two methanol molecules appear. The topic of cluster CI is discussed in detail in references [23,38]. From the analytical point of view we note that the presence of cluster CI can serve for the confirmation of the identity of the molecular ion and thus for obtaining increased confidence level in sample identification [23]. When the STVN heating power is increased to 16 W "clean" Cold EI mass spectrum of corticosterone is observed (similar to the one in Fig. 3). The further heating of the STVN with 21 and 31 W makes only a slight (almost unobserved) reduction of the relative abundance of the molecular ion, but as shown in trace E 33 W causes an almost complete dissociation of corticosterone. From Fig. 5 we conclude that there is a significant "sweet zone" of operation in which thermally labile compounds can be analyzed, even under less than optimal conditions.

Although we call the technique "Cold EI", the actual intramolecular sample vibrational temperature depends on the nozzle diameter, the vaporized solvent flow rate, the nozzle temperature, and especially on the molecular weight and number of atoms (vibrational heat capacity) of the analyzed compound. As a result, large polyatomic compounds that expand from a large diameter supersonic nozzle with relatively low flow rate of vaporized liquid solvent (such as 50–100 μl/min) could be relatively ineffectively cooled, and thus may exhibit some nozzle temperature effects on their de-

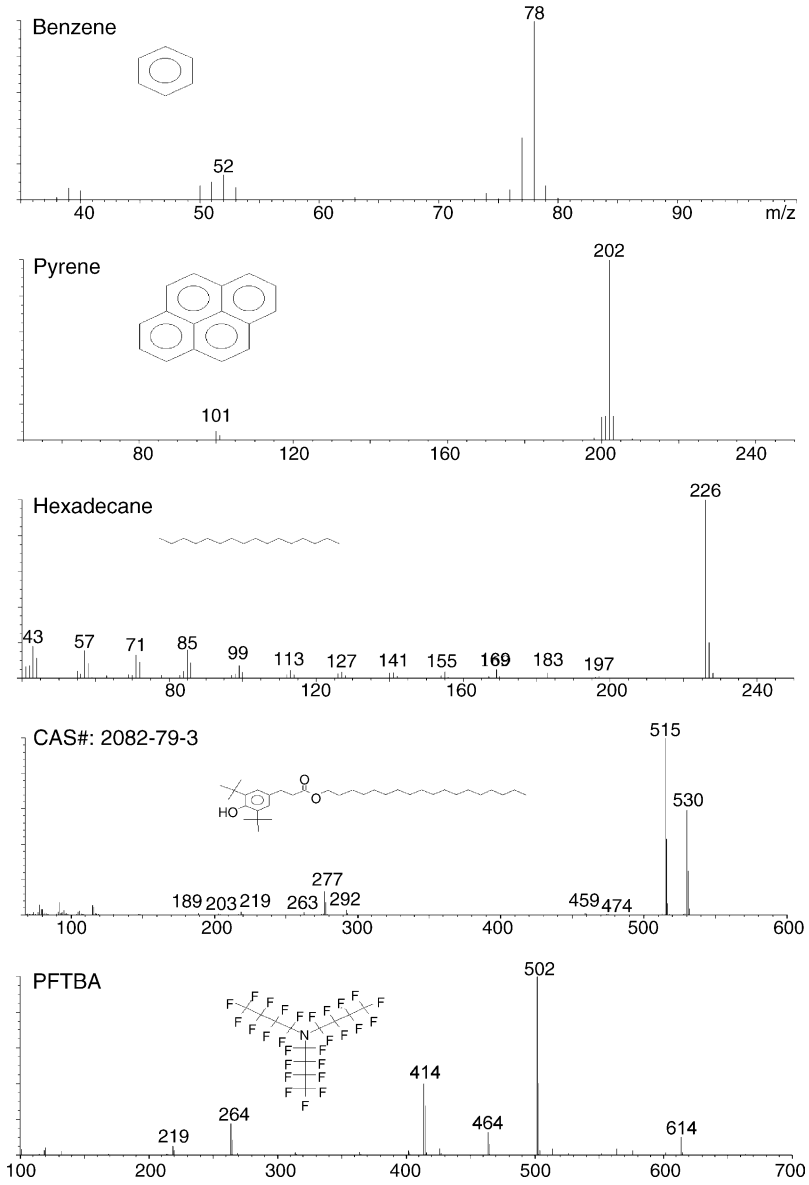

Fig. 8. Cold EI mass spectra of the indicated non-polar compounds. While these compounds are trivial with the LC–EI-MS they are difficult to analyze with APCI or ESI. Enhanced molecular ion is shown in particular in the Cold EI-MS of hexadecane. Samples were flow injected using 120 μl/min methanol solvent flow rate.

gree of fragmentation. This effect is shown in Fig. 6 for the indicated propeller shaped compound with molecular weight of 774 amu ($C_{58}H_{78}O_3$ CAS 1709-70-2). While the degree of fragmentation clearly depends on the nozzle temperature, indicating incomplete vibrational cooling, the molecular ion is always observed due to sufficient vibrational cooling, even with relatively very hot nozzle temperature of 500 °C (trace D at the bottom). On the other hand, as shown in the upper trace A the vibrational cooling effect can be very strong at a relatively low nozzle temperature of 200 °C. We note that with methanol or other vaporized solvents as cooling gas, the vibrational cooling is much more effective than with helium due to the prevalence of effective cluster formation based vibrational cooling [39,42]. For relatively small molecules with molecular weight of $m/z < 500$ this nozzle temperature effect is practically absent due to smaller sample vibrational heat capacity and lower velocity slip in the stage of initial aerodynamic acceleration [39,40].

While cluster CI that was demonstrated in Fig. 5 upper trace A can help in the confirmation of molecular weight identity, it can be a detrimental aspect to any needed isotope abundance information that could be important by itself or serve for a further independent elucidation of the sample empirical formula. The ability to obtain Cold EI mass spectra with clean and informative isotope abundance pattern, free from any cluster CI effects is demonstrated in Table 1. A few molecules were investigated and in Table 1 the relative (to the lowest mass molecular ion) isotope abundances of the molecular ion isotopomers of phenothiazine and CAS 2082-79-3 compound (molecular weight of $m/z = 530$) are given, and compared with the calculated isotope abundances using the NIST isotope calculator. Very good fits are observed between the experimental and calculated results, demonstrating the practical elimination of residual cluster CI effects as well as vacuum background interference. While cluster CI can affect the isotope abundance peak height distribution, it can be suppressed by using a relatively low solvent flow rate (120 μl methanol in Table 1). In addition, one can use solvents such as acetonitrile, or others that do not have labile hydrogen atoms (non-alcohols) and with these solvents cluster CI cannot produce protonated molecular ions, thus its adverse effect on isotope abundance analysis is eliminated. We note that the observation of such "clean" isotope abundance patterns of the "true" (non-protonated) molecular ion is unique to Cold

Table 1
Relative isotope abundances obtained with Cold EI for phenothiazine and $C_{35}H_{63}O_3$ (CAS 2082-79-3) and its favorable comparison with the calculated values using the NIST isotope calculator

	$M+1$	$M+2$	$M+3$	$M+4$
Phenothiazine, MW = 199				
Calculated (%)	15.24	5.38	0.64	0.09
SMB result (%)	15.56	5.56	0.99	0.11
CAS# 2082-79-3, MW = 530				
Calculated (%)	39.90	8.34	1.21	
SMB result (%)	39.22	8.14	1.33	

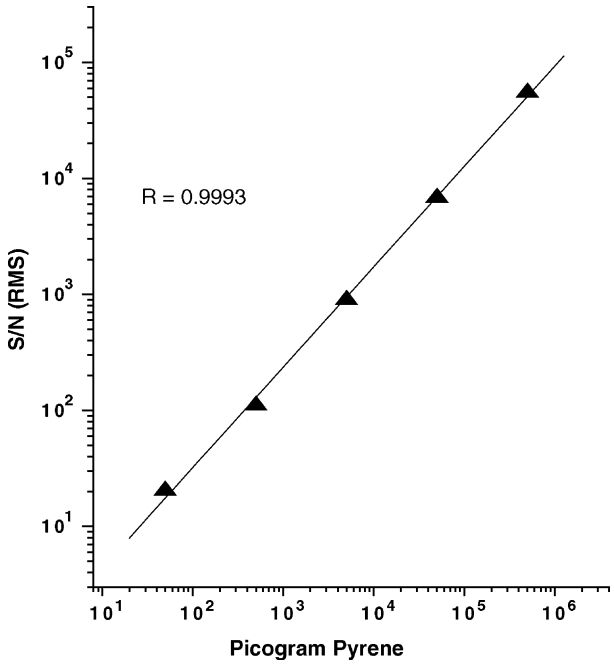

Fig. 9. Response linearity with Cold EI. Linear response of 4 orders of magnitude is shown for pyrene. Pyrene samples were flow injected using 120 μl/min methanol solvent flow rate and 5 μl samples.

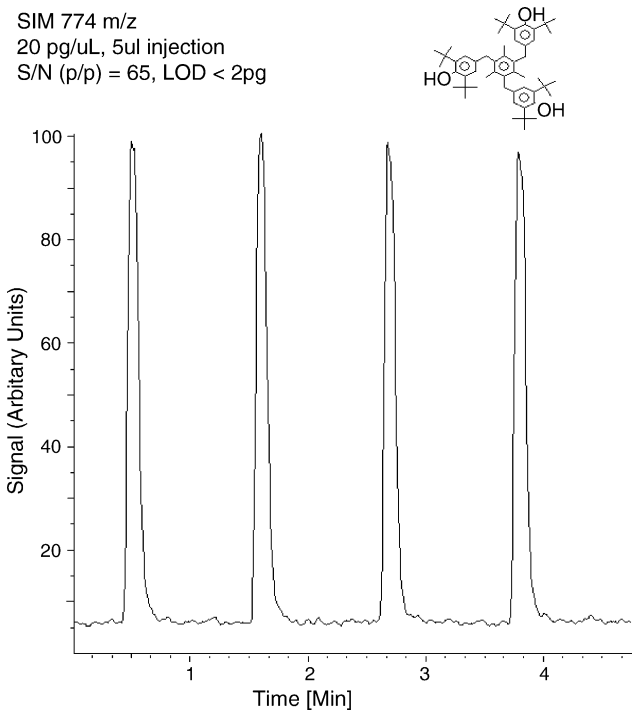

Fig. 10. Sensitivity evaluation of the supersonic LC–EI-MS system. Limit of detection (LOD) of less then 2 pg of the indicated 774 amu compound ($C_{58}H_{78}O_3$ CAS 1709-70-2) is demonstrated. Four flow injections of 5 μl each of 20 pg/μl of the indicated sample were flow injected using 120 μl/min methanol solvent flow rate.

EI. In standard thermal EI the molecular ion is missing in over 30% of the samples and residual self CI plus vacuum background effects distort the obtained pattern, especially for polar compounds (with high proton affinity) with relatively weak molecular ion. Finally, we are currently working on the development of unique software for the automatic conversion of isotope abundance patterns into elemental formulas and sample identification.

In Fig. 7 the Cold EI mass spectra of the indicated six drugs and carbamate pesticides (erythromycin MW = 733, reserpine MW = 608, scopolamine MW = 303, quinine MW = 324, aldicarb MW = 190 and methomyl MW = 162) are shown with rich and informative fragmentation patterns, as expected in EI. Good matching to the NIST library mass spectra plus enhanced molecular ion was obtained for these drugs and pesticides. For quinine we used conditions that promoted partial cluster CI for further enhancement of the molecular and protonated molecular ions (since its library MS practically do not show a molecular ion).

A relatively important attribute of EI as an ionization method is that unlike APCI or ESI, its ionization efficiency is uniform and independent of the sample compound polarity. The Cold EI mass spectra of the five indicated nonpolar compounds are shown in Fig. 8 (benzene MW = 78, pyrene MW = 202, hexadecane MW = 226, CAS 2082-79-3 MW = 530 and perfluorotributylamine (PFTBA) MW = 671). While benzene is trivial to analyze by GC–EI-MS, its LC–MS analysis with ESI or APCI is practically impossible. We found that in our system, benzene can be easily ionized and analyzed at low ppb concentrations, and this similarly applies to all other small and volatile compounds. Note that hexadecane exhibits significantly enhanced molecular ion in Cold EI as

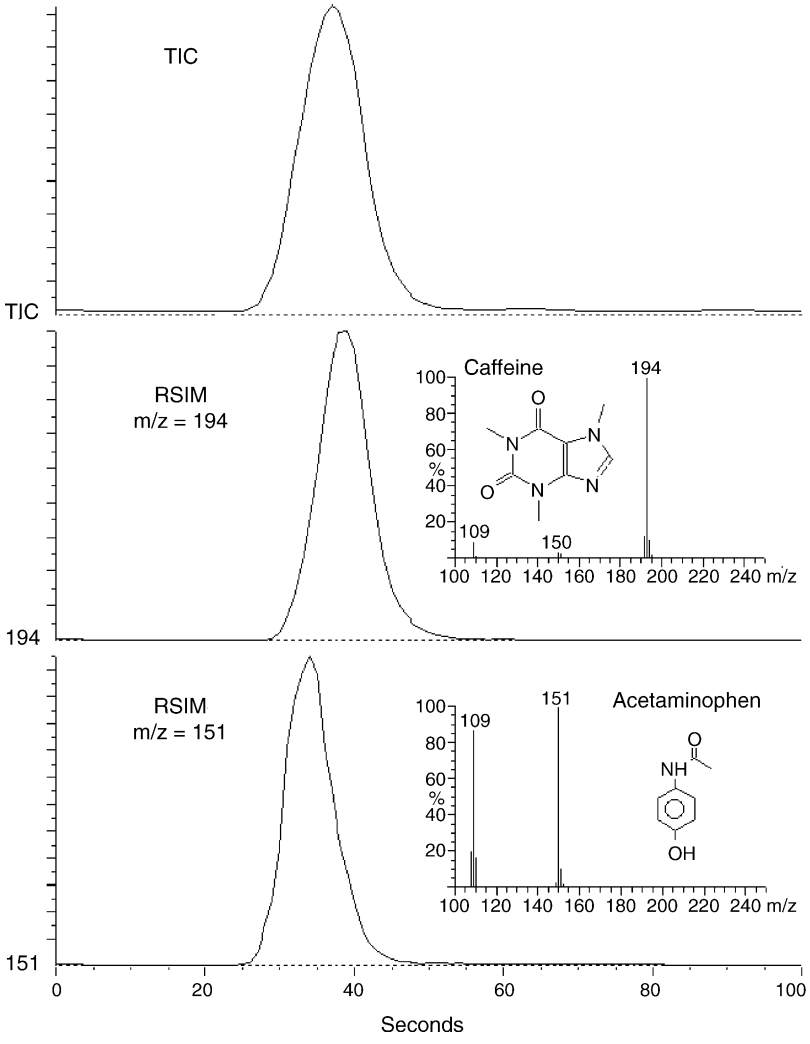

Fig. 11. Fast LC–MS analysis with the supersonic LC–EI-MS. EI provides the molecular ion together with several fragment ions which enable mass spectral deconvolution through the use of deconvolution software (such as AMDIS by NIST). The sample was injected using 5 μl injection loop into a 1 mm i.d., 16 mm long C_{18} guard column (Opti-guard, Optimize Technologies inc, Oregon City, OR, USA) with 50:50 methanol–water solvent under isocratic flow rate of 80 μl/min. The sample contained 1.5×10^{-4} caffeine and 3×10^{-4} pharacatamol concentrations. The insets show the experimentally extracted mass spectra of caffeine and acetaminophen (paracetamol).

previously reported [27] and the Cold EI mass spectrum of PFTBA is also characterized by significant enhancement of high mass fragments.

A major feature that is desirable in any analytical instrument is broad linear dynamic range (LDR). Particle beam is known to possess a limited LDR as its sensitivity sharply declines at low concentrations in a compound dependent fashion due to extended small particle loss in the momentum transfer stages. In Cold EI, full sample vaporization takes place prior to the supersonic expansion thus, a linear response is expected. Over four orders of magnitude LDR are demonstrated for pyrene in Fig. 9 and probably the LDR extends further beyond the investigated range.

A very important parameter that characterizes any LC–MS system is its sensitivity. The current sensitivity of our Liquid-(Cold-EI)-MS system using flow injection is demonstrated in Fig. 10. In this experiment, 5 μl methanol solution of the indicated compound with $m/z = 774$ ($C_{58}H_{78}O_3$ CAS 1709-70-2) at 20 pg/μl concentration was injected four times (repeatedly). The methanol flow rate was 120 μl/min and the measured average S/N is 65 (peak to peak). Thus, the extrapolated lowest amount detected (LOD) for this compound in single ion monitoring (SIM) mode on its molecular ion with Cold EI was 2 pg. This compound was kindly donated to us (Magnus Jornten Karlsson, Astra Zeneca, Lund Sweden, Private communication and kind donation of several samples) and it was claimed that it is difficult to be analyzed by either LC–ESI-MS or by LC–APCI–MS. With a simpler compound such as octafluoronaphthalene (OFN) using SIM on the OFN molecular ion $m/z = 272$, we could obtained a detection limit of less than one picogram, using 2 μl flow injection of 10 pg/μl sample with 60 μl/min methanol flow rate. We obtained with this OFN sample 6 s peak width due to injector broadening, with S/N > 20 in peak to peak. Our current signal level with OFN is ∼100 ions per picogram in SIM mode on the molecular ion ($m/z = 272$) while the noise is mostly mass independent background noise at a rate of 50 counts/s.

An interesting feature of EI is that, since its mass spectra are characterized by several ions (fragments and molecular), and it has a large size library, EI-MS enables automated mass spectral identification under coelution conditions [43–45]. Such deconvolution software named AMDIS is available "free" from the internet and it is also included in the NIST library. Clearly, if coelution of LC peaks can be allowed, much faster LC–MS analysis methods can be developed. Every factor of two lower LC resolution that can be simply obtained by using four times shorter LC column could enable four times faster analysis. As a result, EI-MS deconvolution software is advertised by multiple vendors for their GC–EI-TOF-MS products for enabling fast GC–MS and more recently also for standard quadrupole based GC–MS. For LC–EI-MS, due to the relatively broad LC peaks, fast LC–MS through MS deconvolution can be achieved with any standard quadrupole MS. The mass spectral identification of two simple coeluting compounds in our system is shown in Fig. 11. Caffeine and acetaminophen are two components of a real drug that were injected into a 15 mm guard column that served for their fast, sub 1 min LC–MS analysis. Clearly their EI mass spectra are easily identified under these fast LC–EI-MS conditions. While this is only a preliminary result the concept seems easy to realize and straight-forward to demonstrate and we intend to further explore it in the future.

4. Discussion and potential advantages

A new method and instrumentation were described for obtaining high quality, informative, library searchable EI mass spectra for a relatively broad range of samples in liquids. The presented method provides a few major demonstrated advantages:

1. A library mass spectral search is enabled, unlike with APCI and ESI. 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.
2. Extended mass spectral information is revealed including enhanced molecular ion, accurate isotope abundance pattern and extended isomer and structural mass spectral information, which is superior to that provided by particle beam LC–MS and any other standard thermal EI.
3. A broad range of compounds are amenable for analysis with sample vaporization softness similar to APCI but without restriction on vaporized sample polarity. Thus, Cold EI can serve for the analysis of the full range of non-polar compounds as well as polar samples.
4. Mass spectral deconvolution software could further enable fast LC–MS analysis combined with automated sample identification under coelution conditions.

We feel that our Cold EI can serve as a useful LC–MS method and excel especially in the analysis of unknown compounds or for the universal analysis of a broad range of target compounds such as pesticides (and other chemical contaminants) in agricultural products. Although it can be designed as an add-on unit to existing LC–ESI-MS systems, such combination is not easy in view of ESI being an atmospheric pressure ionization method while EI is inherently an in-vacuum ionization method. Our vision is that Cold EI with its unique benefits will serve as a dedicated low cost LC–EI-MS system, with high performance for the analysis of small molecules. We feel that it can be characterized by relatively low cost in view of the following facts: (a) EI does not require costly MS–MS for sample identification thus it can operate with a single quadrupole. (b) If ESI is not included, the mass range of the MS can be restricted to 1000–1500 amu. (c) No nitrogen generator is required as the system gas requirement is relatively very low or none. (d) The same MS and vacuum system can also serve as a supersonic GC–MS [21,31] with the replacement of only one flange.

It is worthwhile to briefly compare our Cold-EI with SMB approach to the Direct EI approach of Cappiello and co work-

ers [18–21]. In direct EI, the LC and MS interface is as simple as it gets, and the MS of GC–MS can be used with only minor added EI ion-source and interface modifications cost. However, the LC column flow rate is restricted to below 0.5–1 μl/min which necessitates the use of packed capillary LC columns with its resulting lower sample loading ability (and potential 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.

Cold EI requires considerable further investigation for its establishment as a useful technique and in order to explore and expose additional unique features, and continuous research towards these goals is underway.

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