Characteristics and origins of common chemical noise ions in negative ESI LC–MS

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INTRODUCTION

Chemical background noise ions are omnipresent by-products of atmospheric pressure ionization liquid chromatography–mass spectrometry (API LC–MS), resulting from the efficient and generic nature of this soft ionization technique. In mass spectrometry, any trace of ionized contaminants and stable cluster ions, which survive under mild de-clustering conditions, can contribute to chemical background noise. The consequent drawbacks observed in qualitative as well as quantitative studies include, e.g. the contribution to the high and drifting baseline in the total ion current (TIC) chromatogram, and the effect on the limits of detection or quantification (LOD/LOQ) due to the resulting low signal-to-noise ratio (S/N) and/or isobaric ion interferences. Furthermore, poor quality MS or MS/MS spectra complicate qualitative studies for the identification of trace components, such as unknown metabolites, where the chemical background ions may completely overshadow the target analytes in chromatograms and even in mass spectra.

Chemical noise reduction and prevention have been as an important and interesting research topic as the exploration of the ionization technique itself. Neither hardware[3–5] nor software approaches[6–8] developed in the past decades could solve this problem completely due to the intrinsic soft and generic feature of the atmospheric pressure ionization. Most efforts of the hardware improvements for noise reduction were focused on either efficient ionization or transportation of ions into the mass analyzer.[3] The interference is almost unavoidable in LC–MS due to the complex natures of the origins of the chemical background interferences as well as their mechanisms of formation, even when improved interfaces for de-clustering and desorption have been developed and high-purity HPLC solvents and additives were applied. Moreover, it is known that the chemical interference varies sometimes significantly with HPLC mobile phases, matrices and MS ionization conditions. The contaminants can result from HPLC solvents as well as additives, tubing materials, MS ion sources and even laboratory air. The best solution is probably the selective removal or reduction of chemical background ions before they reach the MS-detector, rather than post-acquisition data processing.

Recently, we have reported a novel technique of chemical noise reduction in LC–MS, which is based on selective and efficient reactions of background ions with a chosen reagent such as dimethyl disulfide,[10] ethylene oxide or butadiene monoxide.[11] The involvement of chemical reactions and resolution is the key issue for the successful noise reduction, which requires the extensive knowledge of the structures of major noise ions. An earlier study[12] has shown that, in positive mode, common chemical background ions in LC–MS can be classified into three groups: (1) cluster ions...
derived from mobile phase constitutes and additives; (2) clusters from the solvation of some typical contaminants (e.g., additives and degradation products from tubing, impurities in mobile phase, etc.) and accompanied by (3) some contribution from individual contaminants. Additionally, it is concluded that there is a striking difference in structures between these chemical noise ions and (predominantly) protonated analyte ions generated using positive API. This result has contributed directly to the development of the above-mentioned noise reduction approach \[13\] since it indicates further that these two types of ions (analytes and chemical noise) may have different reactivities with a chosen neutral reagent for noise reduction.

It should be noticed that the background ions in the positive mode are very different from those in the negative mode, which points out that the discovered reagents for positive noise reduction \[10,11\] may not be suitable for this purpose in the negative mode because of the different chemistry involved. For the continuation of this development, it is very important to study the nature of background anions with regard to their structures and origins. For the purpose of an automatic identification and data interpretation, Tong et al. \[14\] have published an extensive listing of typical artifacts and cluster ions in API mass spectrometry for both positive and negative modes. Beside this, although the interference of background ions has been a serious problem even in modern mass spectrometry, \[15,16\] to our knowledge, there has not been any detailed studies about the nature of negative noise ions in LC–MS. In this study, we will present a systematic characterization of typical common negative chemical background interferences using tandem mass spectrometry by classifying them into a few major groups according to their structures. The obtained information can surely help to understand the formation of negative chemical noise in LC–MS and also contribute to develop methods for noise reduction as already carried out for the positive mode. \[10,11\]

**EXPERIMENTAL**

The unit resolution ESI-MS and ESI-MS/MS experiments were carried out using a triple quadrupole API-2000 system (MDS Sciex, Concord, ON, Canada) coupled with either a 1100 HPLC system (Agilent, Waldbonn, Germany) or an infusion pump. The turbo ionspray ionization with the heater gas assistance during the negative mode was used with the spray voltage (IS) setting to 4500 V. The ultra-pure nitrogen (5.0) without further purification was used as the curtain and collision gases and the synthetic air as the nebulizer gas. The direct infusion flow was set to 10–50 µl/min, and the flow rate of the isocratic LC–MS experiments was 200 µl/min. For a comparison purpose, the LC–MS curtain gas was operated either normally (about 25 arbitrary units) or with a lower value (10 arbitrary units). The typical representative LC eluents with different compositions and additives were investigated, which include (1) 1 mM CH₃COONH₄ and/or 0.1% CH₃COOH and (2) 1 mM HCOONH₄ and/or 0.1% HCOOH in ACN/H₂O or MeOH/H₂O (1:1, v/v) under different ionization conditions (mainly by varying the curtain gases for de-clustering), respectively. Before starting the experiments and also changing the mobile phases, the syringe and the complete tubing were flushed sufficiently until the chemical background was constant with regard to the ion intensities and types of the ions.

After inspecting the negative background ions in the full-scan mode, MS/MS precursor ion scan and product ion scan with collision energies ranging from 5 to 40 eV were acquired for the major noise ions in order to draw a structure relationship between them. In order to improve the detection of the low abundant background ions and their fragments, an average of 20–50 mass spectra was generally needed.

The accurate mass measurements were carried out on a 7.0-Tesla LTQ-FT hybrid linear ion trap Fourier transform ion cyclotron resonance (ICR) mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with an electrospray ion source. The FT-ICR was operated in full-scan mode at a 200 000 resolution (m/z 400) for m/z 50–600 to obtain an overview of the background ions. For the highly sensitive detection of some specific background masses, the mass range was selected as ±1 Da of the mass of interest. The following parameters were used: the ESI spray voltage: 4.8 kV and the tube lens offset was 87 V. The sheath gas flow was set to 3 or 5, and sweep gas flow to 0, 2 or 4 arbitrary units and the capillary temperature set to 250 °C. The same mobile phases as for the low-resolution experiments at a flow of 5 µl/min were used.

Acetonitrile and MeOH (HPLC gradient grade) were obtained from Karl Roth GmbH (Karlsruhe, Germany). Formic acid (98 – 100%, p.a.) and acetic acid (100%, p.a.) were purchased from Merck (Darmstadt, Germany). Furthermore, ammonium acetate (>99%, p.a) and ammonium formate (>99%, p.a) were obtained from Sigma – Aldrich Chemie GmbH (Buchs, Switzerland). The ultra-pure water was supplied by an in-house ion-exchange Milli-Q Gradient system (Millipore, Bedford, USA).

**RESULTS AND DISCUSSION**

The focus of this study is to investigate typical negative background ions derived from common HPLC solvents and additives as well as different interface conditions in API LC–MS. In the practice and literature, it has been evident that chemical background interferences depend slightly on different suppliers of the used chemicals. However, the intention of this study is not to compare the supplier-dependent impurities of the chemicals, but to comprehensively characterize a few typical LC–MS mobile phase conditions with regard to the negative chemical background noise. Therefore, the result may be universally valid and provides insights into the sources of chemical background interferences under the negative mode. This study is realized by investigating the products and precursors of the major background ions to find out their structure relationship using tandem mass spectrometry.

**Typical negative background mass spectra in LC–MS**

The typical negative ESIC–MS background mass spectra obtained from the two frequently used LC eluents are compared in Fig. 1. It is known that the abundances of the background ions are closely related to the de-clustering conditions of the LC–MS interface such as the curtain gas flow, the de-clustering potential, the ring focusing potential and the temperature of the heater gas on a Sciex triple quadrupole instrument. A similar observation of the variation of the background on the interface temperature with a heated capillary on other instruments is also well recognized in practice. Although the overall abundances of the negative background ions are lower than that in positive mode, \[12\] it is still a crucial interfering factor in negative LC–MS trace analysis. In order to study the very low abundant background ions, some experiments were also carried out under a relatively low curtain
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As already mentioned and shown in Fig. 1, the main abundant background ions of the investigated eluents result from the added acids and/or salts, which are used as ionization agents under the negative mode. Actually, the background spectrum remains rather similar when acetonitrile is changed to methanol due to their limited involvement. However, further clustering with water plays an important role in the generation of the background noise. Some important clusters of mobile phase components were studied using precursor and product ion scans and are discussed below in detail.

The precursor ion scan mass spectra of the two major background ions m/z 59 [CH$_3$COO]$^-$ and m/z 119 [(CH$_3$COOH)$_2$$^-$H$^-$] are shown in Fig. 2, where the clear involvement of acetic acid CH$_3$COOH (M$_w$ 60) and H$_2$O (M$_w$ 18) molecules for clustering is indicated in these partially magnified mass spectra. The ions observed here make up to the major background contribution both in intensity and entities. A summary of the possible typical clusters of mobile phase components and clusters of contaminants with mobile phase components is given in Table 1.

Only very minor clustering of the neutral molecule CH$_3$CN (M$_w$ 41) was observable even under the low de-clustering conditions (Fig. 2). For instance, this refers to the precursor ions of [CH$_3$COO]$^-$ (m/z 59) at m/z 100 (addition of 41) and 141 (addition of 82) in Fig. 2(a) and m/z 141 in Fig. 3. The product ion scan mass spectrum of m/z 141 in Fig. 3(a) shows the loss of a neutral of 82 (to form m/z 59 back). This indicates that the cluster ions [(ACN)$_2$ + CH$_3$COO]$^-$ contribute to produce the background ions m/z 141, which is also confirmed by the elemental composition studies using accurate mass measurements (see Table S1, Supporting Information). Other possibilities are the formation of clusters [CH$_3$COONa + CH$_3$COO]$^-$ by the addition of one CH$_3$COONa molecule (M$_w$ 82) to [CH$_3$COO]$^-$ and some minor isobaric contribution from [CH$_3$COOH + HCOOH + 2H$_2$O$^+$$^-$]$^-$, respectively. This sodium contribution may depend highly on its availability in mobile phases or samples. The ions at m/z 141 further form clusters with the solvent components and contaminants (Fig. 3(b)). Furthermore, m/z 157 and m/z 217 (Fig. 2(a)) are mainly related to clusters between neutral molecule CH$_3$COOH and deprotonated phosphoric acid (H$_3$PO$_4$$^-$) (m/z 97), a well-known contaminant derived from the plasticizer phosphates, which will be discussed in detail later in this paper. A minor portion of [(CH$_3$COOH)$_2$ + 37Cl]$^-$ (m/z 157) was also confirmed with accurate mass measurement.

In negative electrospray ionization mode using the given MS parameters (curtain gas, de-clustering potential and focusing potential), neither CH$_3$COONH$_4$ (M$_w$ 77, a mobile phase additive) nor HCOONH$_4$ (M$_w$ 63 in the other one discussed later) clusters could be detected. Instead of these ions, CH$_3$COONa (M$_w$ 82) and HCOONa (M$_w$ 68) adducts were observed. The presence of sodium as contaminant can be explained due to its ubiquitous nature. The corresponding adducts between formic acid and sodium were also shown in Fig. 1(c) (m/z 113) and the further product and precursor ions indicating the involvement of HCOONa are given later in Figs 5 and 6, respectively. Therefore, most components of the mobile phase including CH$_3$COOH, HCOOH, H$_2$O, 44 (CO$_2$), CH$_3$CN and CH$_3$COONa are more or less involved in the cluster formation and the possible cluster-related noise ions are summarized in Table 1.

In Fig. 1(b), the ions at m/z 179 are assigned as [(CH$_3$COOH)$_2$$^-$H$^-$]$.^-$. This contribution appears to be the main species, and further tandem mass spectrometric experiments indicate that only minor isobaric [97 + CH$_3$COONa]$^-$ (or, [97 + (ACN)$_2$]$^-$, m/z 179) are present (data not shown). This is additionally confirmed by the precursor ion scan of m/z 97 as discussed later in the text.

The small background ions at m/z 45, 46 and 62 in Fig. 1 do not directly interfere much in most tandem LC–MS studies (only contribute to the full-scan chromatogram baseline); however,
Figure 2. Precursor ion scan mass spectrum of (a) $m/z$ 59 [CH$_3$COO]$^-$ at a collision energy of 15 eV and (b) $m/z$ 119 [(CH$_3$COOH)$_2$-H]$^-$ at a collision energy of 5 eV under a relatively low curtain gas condition.

their further clusters with other mobile phase components make significant contribution to the background noise. The presence of the ion [HCOO]$^-$ at $m/z$ 45 in the full-scan mass spectrum when CH$_3$COONa and CH$_3$COOH as the additives (Fig. 1(a and b)) is most probably not only a contamination of the eluent with formic acid or formate but also likely due to the formation of [HCOO]$^-$ during the ESI process (heat, discharge and high voltage). The ions at $m/z$ 46 may result from only a very small amount (ca 1%) of the $^{13}$C-isotope of the [HCOO]$^-$ anion but the major contribution might be related to an electron attached anion [HCOOH]$^-$ formed during discharge processes. The other discharge-related species is [HCOOH + O]$^-$ ($m/z$ 62), which underwent neutral loss of 16 a.m.u. in its product ion scan spectrum.

The ions at $m/z$ 89 comprise mainly deprotonated lactic acid [CH$_3$-CH(OH)-COO]$^-$, which can result from the contamination of lactic acid-related plastic (polylactic and polyactic acid) materials or detergents. This is in accordance with the two fragment ions at $m/z$ 43 (likely an enolate anion [CH$_2$≡CH-O]$^-$ by the loss of a neutral formic acid molecule HCOOH) and 45 (deprotonated formic acid [HCOO]$^-$), respectively (see Fig. S1(a) in the Supporting Information). This assignment is also supported by its accurate mass as also shown in Table S1 in the Supporting Information. The other possible structures for $m/z$ 89 such as the cluster [HCOO + CO$_2$]$^-$ (formed during ionization) and the contribution from ethylene glycol derivatives (contamination from detergents) cannot be excluded either. All the above-mentioned small ions are the original nuclei for further clustering with the eluent components as summarized in Table 1. As another example for cluster formation, the precursor ion scan mass spectrum of $m/z$ 89 is also given in the Supporting Information (Fig. S1(b)), where further clustering with CH$_3$COOH, HCOOH and CH$_3$COONa were observed and confirmed by elemental composition.

A comparison of the background noise ions in the range of $m/z$ 220 – 350 in the full-scan mass spectra and that in the precursor ion scans of $m/z$ 59 and $m/z$ 119 is given in Fig. 4. It is obvious that many
other background ions, which are not related to the clusters of m/z 59 [CH3COO]– or m/z 119 [(CH3COO)2-H]–, are also present in the full-scan spectra. Some of them are included in the classification of other clusters. Anyway, there are still many minor contributions, which are not studied in detail due to their low abundances. However, all these noise ions with low abundances will also surely contribute to the chemical background and interfere LC–MS studies.

Cluster ions involving the mobile phase components while ammonium formate and formic acid are used as the additives

Generally, the mobile phase eluents investigated in this study are very alike with respect to the types of background ions. In Fig. 1(c) two dominant peaks of [HCOO]– (m/z 45) and [(HCOOH)2-H]– (m/z 91) are similar to m/z 59 and 119 in Fig. 1(a and b) as the typical cluster-related species from mobile phase components. The precursor ion scan mass spectrum of m/z 45 showing the background ions derived from [HCOO]– is given in Fig. 5. Beside the pure cluster ions from the mobile phase components HCOOH and water, what also worth-mentioned is the formation of the significant cluster ions [HCOONa + HCOO]– at m/z 113, which has also been reported as a possible artifact peak in the negative ESI in the literature.14 Their analogous clusters [CH3COO Na + CH3COO]– at m/z 141 derived from acetic acid have already been discussed above in Fig. 3. In addition, the further clustering of the ions at m/z 45, 113 and 137 with a neutral species of 44 a.m.u. was only detected when HCOOH was used as an additive, which indicates that the addition of 44 might relate to CO2 or ethylene glycol oxide C2H4O from contamination. Both the elemental composition (as shown in Table S1 in the Supporting Information) and the product ion scan mass spectrum of m/z 113 in Fig. 6(a) confirm this assignment, where the product ions at m/z 69 and 45 by the loss of the neutrals 44 and 68 (HCOONa) are present. And the precursor ion scan mass spectrum of m/z 113 in Fig. 6(b) shows its further clustering with the molecules HCOOH and 44. Moreover, the involvement of sodium in clustering can also be observed in another form as in m/z 129 in Fig. 5. The separate accurate mass FT-ICR experiment confirms that at least the ions with a composition equivalent to [NaHCO3 + HCOO]– were found. Although here the ions at m/z 129 can also due to potassium salt [HCOOK + HCOO]– similar to [HCOONa + HCOO]– (m/z 113), no direct evidence was obtained from its product ion spectrum as shown in Fig. S2 in the Supporting Information, where the formation of the fragment ions at m/z 89 and 74 can only be explained better by [NaHCO3 + HCOO]–.

As a summary, the background ions resulting from solvation/clustering reactions of mobile phase components and additives are given in Table 1. The contribution of the cluster ions purely derived from the mobile phase components to chemical background noise can be relatively easily reduced by improving de-clustering conditions such as higher curtain gas flow and temperature, high de-clustering potential and high temperature of the heated capillary. An alternative is the choice of a different mobile phase with lower declustering conditions such as higher curtain gas flow and temperature, high de-clustering potential and high temperature of the heated capillary.
Clustering between contaminants and mobile phase components

Beside the common negative background ions derived from solvent/additives exemplarily discussed above, abundant chemical background ions, which are not or not only originated from the mobile phase components, are also often observed in ESI LC–MS. These ions are presumably originated from the mobile phase or ion source contaminants including plasticizers and silicones.

As demonstrated in the full-scan mass spectrum in Fig. 1(a) and some precursor and product ion spectra while CH₃COONH₄ and CH₃COOH were used as the additives, e.g. the involvement of an anion with \( m/z \) 97 (or a neutral mass of 98) was often observed. The product ion mass spectrum of \( m/z \) 97 generated under a relatively high curtain gas condition and collision energy (25 eV) shows predominately the loss of water as a detectable fragment (mass spectrum given as Fig. S3 in the Supporting Information), which at least excludes a possible contribution of \([\text{HSO}_4^-](m/z \ 97)\) as, however, suggested by Tong et al.[14] Increasing the collision energy did not induce any further dissociation. This indicates that the majority of \( m/z \) 97 is likely from deprotonated phosphoric acid \([\text{H}_2\text{PO}_4^-](m/z \ 97)\) derived from a typical plasticizer triphenyl phosphate, which is also supported by our previous studies [12] in positive mode where the cations at \( m/z \) 99 are assigned as \([\text{H}_4\text{PO}_4]^+\).

However, in this study, the product ion spectrum of \( m/z \) 97 formed under a relatively low curtain gas condition and low collision energy (5 eV) particularly shows the typical trace contribution of clusters between the mobile phase components and contaminants (Fig. 7(a)) beside \([\text{H}_2\text{PO}_4^-]\). For instance, one small portion of \( m/z \) 97 consists probably of an adduct of an anion (\( m/z \) 37) with CH₃COOH, which is likely the \(^{37}\text{Cl}\) isotope of \([\text{CH}_3\text{COOH} + \text{Cl}^-](m/z \ 95)\) according to the high-resolution measurement. This is further confirmed by the loss of 38 \(^{37}\text{Cl}\) from \( m/z \) 97 to form anions at \( m/z \) 59, which is apparently \([\text{CH}_3\text{COO}^-]\). Accordingly, the loss of 36 (which could be interpreted as \( \text{H}^{35}\text{Cl}\) from \( m/z \) 97 to form an anion product at \( m/z \) 61 may be explained by the \(^{13}\text{C}\) isotope contribution from \( m/z \) 97 due to its overwhelmingly high abundance.

Based on the above-mentioned mixed contributions at \( m/z \) 97, further clustering with the mobile phase components CH₃COOH, H₂O and CH₃COONa was observed both under the low and high de-clustering conditions as shown in its precursor ion scan mass spectrum in Fig. 7(b).

Figure 4. ESI-MS partial full-scan mass spectra of the eluent with 1 mM CH₃COONH₄ and 0.1% CH₃COOH in ACN/H₂O (1:1, v/v) under (a) the standard curtain gas and (b) the low curtain gas conditions. For comparison, the precursor ion scan mass spectra of the ions at (c) \( m/z \) 59 and (d) \( m/z \) 119 obtained using the standard curtain gas are given. The result indicates that although most ions in the whole spectra can be found in the precursors of \( m/z \) 59 and 119, there are always ions which are not related to either of them.
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Figure 5. Precursor ion scan mass spectrum of m/z 45 at a collision energy of 15 eV.

Figure 6. (a) Product ion scan and (b) precursor ion scan mass spectra of m/z 113 at a collision energy of 10 eV.

Figure 7. (a) Product ion scan mass spectrum of m/z 97 at a collision energy of 5 eV and (b) precursor ion scan mass spectrum of m/z 97 at a collision energy of 8 eV under a relatively low curtain gas condition when CH3COONH4 and CH3COOH were used as the additives.

[CH3COOK + H2PO4]− cannot be supported by the accurate mass measurement. Although the ions at m/z 179 in Fig. 2(a) are no doubt as [(CH3COOH)2 + CH3COO]−, that as the precursors of m/z 97 in Fig. 7(b) certainly have a different structure (likely [CH3COONa + H2PO4]−) as suggested by its product ion spectrum. A summary of possible background ions between the contaminants and mobile phase components is given in Table 1.

In addition, other contaminants and their clusters with mobile phase additives are also identified in the investigated eluents. For example, the product ion scan mass spectrum of m/z 133 represents the contribution of the typical silicon rubber-related contaminant Si(CH3)2O (Mw 74), where both the anion [Si(CH3)2O−H]− at m/z 73 and its cluster with acetic acid molecules were observed. Similar evidence is also shown in the product ion scan mass spectrum of m/z 171 (tandem MS data of m/z 133 and 171 not shown). This anion at m/z 73 has also been reported in a previous investigation.[14]

CONCLUSIONS

In this study the characteristics as well as their structure relationship of the most common chemical background ions under the negative ESI LC–MS mode have been investigated systematically. The result provides a general overview about
the interfering background ions in negative ESI with regard to their classification and characteristics depending on their origins. The knowledge about the background ions of the investigated typical ESI-MS eluents can partially be transferred accordingly to other mobile phases due to the similarity of clustering. The main negative interference in the negative mode results from the cluster ions of solvents and additives. Additionally, there are also the clusters arising from the solvation of some typical contaminants (e.g. additives and degradation products from tubing, impurities in mobile phase, etc.), and accompanied by some minor contribution from individual contaminants. This differs somehow from the typical chemical background interferences in the positive ESI mode, where much significant contribution of chemical background noise derived from contaminants including plasticizers (phthalates, phenyl phosphates, adipates, etc.) and silicones are concluded.[12]

Since the aim of this study is to give a general overview about the correlation of background ions in negative ESI mode, not all ions discussed here were structurally identified. However, based on the current tandem mass spectrometric experiments, elemental compositions and literature, most structurally related ions are successfully classified into groups. Furthermore, it should be mentioned that, in general, the negative background cluster ions have significantly different structures compared with deprotonated analytes. This distinction may possibly contribute to developing a novel noise reduction technique using selective chemical reactions in LC–MS as already discussed in the positive mode.[10]

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Supporting information

Supporting information may be found in the online version of this article.

REFERENCES