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## Review article

# Interferences and contaminants encountered in modern mass spectrometry

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## ABSTRACT

With the invention of electrospray ionization and matrix-assisted laser desorption/ionization, scientists employing modern mass spectrometry naturally face new challenges with respect to background interferences and contaminants that might not play a significant role in traditional or other analytical techniques. Efforts to continuously minimize sample volumes and measurable concentrations increase the need to understand where these interferences come from, how they can be identified, and if they can be eliminated. Knowledge of identity enables their use as internal calibrants for accurate mass measurements. This review/tutorial summarizes current literature on reported contaminants and introduces a number of novel interferences that have been observed and identified in our laboratories over the past decade. These include both compounds of proteinaceous and non-proteinaceous nature. In the supplemental data a spreadsheet is provided that contains a searchable ion list of all compounds identified to date.

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## 1. Introduction

“Scheidekunst”—the ‘art’ to separate materials into their individual components is an old German word for alchemy and analytical chemistry related sciences [1] and its basic concepts are still valid today. The last hundred years have brought enormous advances in chromatographic and other separation methods in combination with a large variety of analyte detection technologies. Any separation and detection technique has the potential to inadvertently introduce new components or contaminants into the analytical system that must be evaluated and carefully considered. It is therefore no coincidence that Modern Analytical Chemistry takes advantage of ultra-pure chemicals and reagents and ultraclean sample handling containers whenever possible to minimize any potential and unwanted background interference. In addition, all routine modern analytical methods following good laboratory practices (GLP) will include blank tests such as system-, solvent-, method-, matrix- and equipment blanks [2].

With the introduction of novel ionization methods for modern mass spectrometric (MS) analysis, such as electrospray ionization (ESI) by Fenn et al. [3] and matrix-assisted laser desorption/ionization (MALDI) by Tanaka et al. [4] and independently by Karas and Hillenkamp [5], in the late eighties, scientists employing modern mass spectrometric tools face new challenges with respect to background ions that might not have played significant roles in traditional or other well-established routine analytical methodologies. The ongoing efforts to further miniaturize liquid chromatography (LC) methods [6], combinations of LC/MS [7–12], the development of capillary electrophoresis combined with MS (CE/MS) [13] including microfluidic chip-based mass spectrometry [14–17], make it easy to predict that knowledge of potential interferences and background ions will become increasingly important for successful future development of GLP-adhering methods in routine and research analytical methodologies. Miniaturization of sample transfer procedures and handling tools increases exponentially the surface to sample volume ratio and thus any interferences resulting from contaminated or background leaching surfaces will consequently also multiply.

The main focus of this review/tutorial is the introduction and description of known and identified interfering compounds that have either been reported in the scientific literature or have been observed in our own laboratories over the past decade or so. Where deemed appropriate and possible, general technical advice is included on how to minimize impacts of the described interferences.

This report does not include or further discuss specific techniques or instrumentation that allow minimization or elimination of certain background interferences, such as ion mobility MS [18,19], high-field asymmetric waveform ion mobility spectrometry (FAIMS) [20], matrix-free laser desorption/ionization techniques [21] including desorption/ionization on silicon (DIOS) [22], desorption electrospray ionization (DESI) [23,24], or direct analysis in real time (DART) [25]. Sources and handling of random background noise, either of electrical [26–28] or chemical nature [29], noise reduction through special software application [30], interferences introduced through degradation or metabolism of analytes/drugs during analysis [31,32], co- or post-translational protein modifications [33,34], background ion scrubbing via specific reactions with dimethylsulfide [35,36], or any analyte-specific interferences are also beyond the scope of this work. We would like to refer interested readers in the above-mentioned topics to the respective cited literature and the references therein.

The supplemental data contains a spreadsheet (Microsoft Excel) with a searchable compilation of all identified compounds to date. The list contains accurate mass-to-charge ratios for singly charged species and these can be exploited for calibration purposes in applications that require accurate mass measurements.

## 2. Proteinaceous interferences or contaminants

One of modern mass spectrometry's great impacts is on protein analysis and characterization. MS related techniques are now the preferred and well-established methods for protein identification [37] and quantification [38]. The complexity of biological samples requires extensive purification and separation methodologies, not only to remove non-proteinaceous components but also to address present proteins that are not of interest [39,40]. Here we will focus on potentially interfering proteins that are not indigenous to the original samples and are either involuntarily introduced into the sample (e.g. keratins) or deliberately added as enzymatic reagents for example in bottom up sample preparation for subsequent mass spectrometric analysis.

### 2.1. Enzymes used in sample preparations for bioanalytical mass spectrometry

A typical bottom up approach of protein sample preparation for subsequent mass spectrometric analysis involves enzymatic digestion of the protein of interest followed by

**Table 1 – List of some enzymes<sup>a</sup> commonly used in bottom up sample preparations for MS analysis**

| Common enzyme name (type)  | Cleavage sites<br>$\backslash X = N\text{-terminal}; X \backslash = C\text{-terminal}$ | Enzyme commission# | SwissProt accession# <sup>b</sup> |
|--|--|--------------------|-----------------------------------|
| Bovine trypsin (endopeptidase)                                   | K\; R\   | 3.4.21.4           | P00760                            |
| Porcine trypsin (endopeptidase)                                  | K\; R\   | 3.4.21.4           | P00761                            |
| Bovine chymotrypsin (endopeptidase)                              | F\; Y\; W\; L\ M\; E\; D\; N\  | 3.4.21.1           | P00766                            |
| Porcine pepsin (endopeptidase)                                   | Low or broad cleavage specificity, preferred hydrophobic residues F\; L\; E\           | 3.4.23.1           | P00791                            |
| Endoproteinase Arg-C   | R\   | 3.4.21.35          | n/a                               |
| Endoproteinase Asp-N   | \D\; \E  | 3.4.24.33          | n/a                               |
| Endoproteinase Glu-C (V8), <i>Staphylococcus aureus</i>          | D\; E\   | 3.4.21.19          | Q2FZL2                            |
| Endoproteinase Lys-C, <i>Lysobacter enzymogenes</i>              | K\   | 3.4.21.50          | Q7M135                            |
| Porcine leucine aminopeptidase, microsomal (exopeptidase)        | Cleaves N-terminal amino acids   | 3.4.11.2           | P15145                            |
| Thermolysin, <i>Bacillus thermoproteolyticus</i> (endopeptidase) | \L\; \F\; \I\; \V\; \M\; \A  | 3.4.24.27          | P00800                            |

<sup>a</sup> Adopted from the Proteolytic Enzymes Index at [www.sigmaaldrich.com](http://www.sigmaaldrich.com).  
<sup>b</sup> Accessible at [www.expasy.org](http://www.expasy.org) (Expert Protein Analysis System, ExPASy).

peptide mapping and/or MS/MS fragmentation of gas-phase separated peptide ions [37]. In an ideal case the sample protein is in large excess so that potential interferences from the added enzyme are minimal. However, one of modern mass spectrometry's great successes is the analysis of often low nanogram amounts of proteins separated and purified from complex mixtures with chromatographic or gel electrophoresis techniques. In these cases enzyme-to-protein ratios are often reversed so that potential interferences from enzyme autolysis products become significant and need to be addressed. Bovine and porcine trypsin are the most commonly employed enzymes in MS protein analysis protocols but "difficult" proteins, such as membrane proteins with few or non-accessible tryptic cleavage sites, might require other enzymes for sufficient digestion. Although, autolysis products of trypsin have been described in the literature [41,42], due to variability of digestion results under different experimental conditions, blank testing of enzyme autolysis remains advisable. To minimize interferences from peptide artifacts, either from enzymes or other sources, it is necessary to optimize digestion conditions and employ proteolytic enzymes of high purity [43]. The latter can be challenging; purification of enzymes requires techniques that do not affect their activity [44]. Table 1 summarizes the most commonly employed enzymes, including their specific cleavage sites and their online database accession number (SwissProt database). In cases where sequential multienzymatic digestions are necessary, for example in experiments where maximum protein sequence coverage is required [45], the complexity of potential interference will naturally increase and sophisticated and adequate blank test experiments are required.

New promising methods and technologies have recently evolved that avoid the use of enzymes in sample preparations for mass spectrometric characterization of proteins. These include microwave-assisted acid hydrolysis for controlled protein digestion [46], or top-down MS analysis of whole proteins [47]. However due to the wide acceptance of the robust enzymatic digestion methods and initial challenges with the new approaches, it is unlikely that the use of enzymes in mass spectrometric sample preparation protocols will be significantly replaced in the near future [48].

## 2.2. Keratins and other abundant, involuntarily introduced proteins

Keratins are ubiquitous proteins stemming predominantly from skin cells and are commonly found in house-hold and laboratory dust [49,50] and have the potential to contaminate biological samples if appropriate care and precautions are not taken [51]. Due to their proteinaceous nature, contaminating keratins will inevitably also undergo enzymatic digestion in respective bottom up sample preparations for mass spectrometry, and the resulting peptides will interfere in the analysis of the proteins of interest. The less abundant the proteins of interest, the greater the possibility that keratins will interfere with analysis. Necessary measures to avoid keratin interference will thus depend on the investigated samples and can range from simply wearing gloves, to working in laminar flow hoods or to working in special clean rooms. An excellent guideline on how and when to avoid keratin interferences has been developed by Biringer [51]. In 1999, Mann and co-workers presented a comprehensive list with respective MS/MS data of common peptide contaminants [41] including keratins, and these contaminants are contained in our supplemental spreadsheet. One could expect that most keratins found in dust are of human origin and this is most likely true for the majority of laboratories, however if lab animal facilities are included or nearby or rodent infestation has occurred, respective keratins from the species in question should also be expected. Another prerogative in prudent lab practices is to be prepared and vigilant; during a major project investigating the mouse endoplasmic reticulum proteome [52], we started identifying a large number of highly abundant sheep keratins at one point. As it turned out, a lab member had started wearing a wool sweater in the collaborator's sample preparation laboratory, initiated by a sudden outside temperature drop [53].

A number of other proteins can be involuntarily introduced into biological samples. Among these are bovine serum albumin (BSA) which is commonly employed in immunoassays and other techniques as a reagent to block non-specific binding sites on surfaces [54], or Protein A and G which are used as specific binding partners for immunoglobulins (IgG) in

**Table 2 – Selection of common contaminant proteins in sample preparation for MS analysis**

| Protein name and origin                    | SwissProt accession number(s) <sup>a</sup>     |
|--|--|
| Bovine serum albumin (BSA)                 | P02769   |
| Protein A ( <i>Staphylococcus aureus</i> ) | P99134; P0A015                                 |
| Protein G ( <i>Streptococcus</i> sp.)      | P06654   |
| Keratin, type I cytoskeletal, human, skin  | P08727; P08779; P13645; P13646; P35527; Q7Z3Z0 |
| Keratin, type II cytoskeletal, human, skin | P02538; P04259; P04264; P08729; P35908         |
| Keratin, type II cuticular, human, hair    | O43790; Q9NSB4                                 |

<sup>a</sup> Accessible at [www.expasy.org](http://www.expasy.org) (Expert Protein Analysis System, ExPASy), only primary accession numbers are listed for keratins and only species that have been reported in the literature or have been observed in our laboratories are included.

affinity purification methods [55]. Generally, all proteinaceous components of affinity columns, including immobilized antibodies, can potentially leak into sample fractions. Antibodies employed in solution-based immunoprecipitations are often in such excess that a mass spectrometric characterization of the proteins of interest is impossible, unless the interfering antibody chains are efficiently removed (e.g. by HPLC or gel electrophoresis) from the proteins of interest [56]. More complex protein mixtures can be introduced into samples when non-fat dry milk, fish gelatine or whole serum is used as reagents to block non-specific binding sites on surfaces of equipment used in bioanalytical methods [54].

Table 2 summarizes the most commonly observed contaminating keratins and other proteins with their respective online-database accession number (SwissProt database). Occurrence of interfering peptides from these proteins strongly depends on the specific applied experimental conditions and can vary greatly. In our supplemental database we have only included some of the most prominent examples that we have observed in our own laboratories or that have been reported in the literature. Nevertheless, it is a simple task to create 'in silico' peptide maps of these proteins adjusted to the employed experimental conditions. These theoretical peptide maps can then be compared with the experimental results and potential peptide artifacts originating from these proteins be identified.

### 2.3. Instrument-induced peptide fragment interferences

Non-specific in-source peptide fragmentation during analyte ionization can lead to unexpected signals from enzymatically created peptides. This phenomenon has been exploited for pseudo MS<sup>3</sup> experiments both in ESI [57] and MALDI [58]. The intensity of non-specific fragmentation in the ionization source is related to the applied skimmer-nozzle voltage in ESI as well as to the employed matrix and to the applied laser intensity in MALDI. Modification and optimization of above-mentioned factors can be employed to minimize these interferences. In a recent study on low-energy collision induced peptide fragmentation chemistry, Huang et al. [59] demonstrated that C-terminal cleavage to acidic residues (D, E) dominates when the proton on the peptide is localized, whereas cleavage N-terminal to proline dominates when the proton is mobile or partially mobile. This is consistent with our observations that above-described in-source fragmentation often yields specific peptide fragments (C-terminal cleavage after D, E or N-terminal cleave before P) and we have included

the most prominent and persistent examples resulting from keratin or enzyme origin in our supplemental database and labeled as ion-source fragments.

## 3. Non-proteinaceous interferences or contaminants

The long list of potential non-proteinaceous interferences contains both contaminants and compounds that have been recognized in traditional and established analytical methodologies, such as for example plasticizers and anti-oxidative additives, but also includes a large variety of interferences that are specific to modern mass spectrometric analysis, like matrix clusters in MALDI MS, and metal ion or solvent adducts and other solvent effects in ESI. Several instrument and sample preparation tool manufacturers have published their own lists of specific interferences [60–63] and with the ongoing development of novel materials, introduction of new matrix substances for MALDI MS, and increasing awareness of interferences from the ambient environment in atmospheric pressure ionization sources, the list of potential background ions is poised to keep growing steadily.

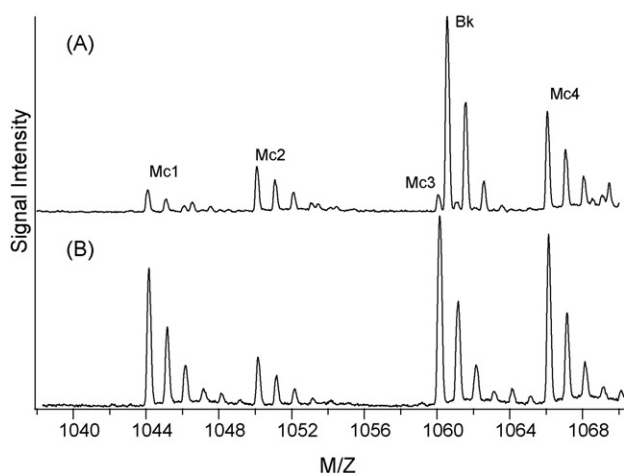
### 3.1. Matrix clusters in MALDI MS

The observation of matrix clusters was first described in detail by Dubois et al. [64]. Since then a number of reports have dealt with this subject; mainly for the common matrix compounds  $\alpha$ -cyano-4-hydroxy cinnamic acid (4-HCCA) and 2,5-dihydroxybenzoic acid (DHB) [29,34,42,65–68]. Keller and Li established the following algorithm on the composition of matrix clusters from 4-HCCA and DHB as agglomerates of matrix molecules and alkali ions, typically sodium and potassium [69]:

$$M_{\text{cluster}} = nM - xH + yK + zNa,$$

$$x = y + z - 1 \text{ and } y + z = n + 1 \text{ or } x = n$$

This algorithm allows the prediction of  $m/z$  values at which matrix clusters can potentially appear. For example, in the case of the commonly employed matrix substance 4-HCCA, matrix clusters typically appear in bundles containing  $\sim 3$ – $7$  specific signals within a bundle. Bundles are separated by  $\sim 190$ – $227$  mass units, which represent a matrix molecule unit (i.e. either  $[M+H]$ ,  $[M+Na]$ , or  $[M+K]$ ). Within a bundle there can be several strong individual signals surrounded



**Fig. 1** – MALDI mass spectra excerpts of two-layer matrix preparations employing alpha-cyano-4-hydroxycinnamic acid ( $M = 4\text{-HCCA}$ ) according to Dai et al. [96] using a Voyager DE MALDI TOF MS (Applied Biosystems, instrument located at Queen's University) in positive reflectron mode. Matrix clusters are labeled Mc1–Mc4 (Mc1:  $[5M - 2H + 2K + Na]^+$ ,  $m/z$  1044.12; Mc2:  $[5M - 3H + K + 3Na]^+$ ,  $m/z$  1050.12; Mc3:  $[5M - 2H + 3K]^+$ ,  $m/z$  1060.09; Mc4:  $[5M - 3H + 2K + 2Na]^+$ ,  $m/z$  1066.10). (A) Sample containing Bradykinin (Bk, sequence: RPPGFSPFR,  $m/z$  for  $[M + H]^+ = 1060.57$ ) (B) Matrix preparation without Bradykinin. Matrix clusters in this mass range can easily be distinguished from peptide signals in well resolved mass spectra due to the larger mass defect effect for matrix clusters containing alkali ions compared to peptides consisting only of C, H, N, O and S (i.e. different “fractional” mass) [42]. Matrix cluster compositions were obtained from an online program available on Dr. Liang Li's group website [70].

by less intense cluster signals, and average bundle intensities decrease with increasing average mass of the bundle. With modern, high-resolution mass analyzers, matrix clusters can easily be discerned from potential peptide signals as is demonstrated in Fig. 1. An online program is available on Dr. Liang Li's group website that allows the prediction and confirmation of potential matrix cluster masses of 4-HCCA or DHB based on the above-described algorithm including potential neutral water losses [70] (see: [www.chem.ualberta.ca/~liweb/links/MaClust.htm](http://www.chem.ualberta.ca/~liweb/links/MaClust.htm)). Harris et al. reported the use of accurate masses of matrix clusters of 4-HCCA and trypsin autolysis signals in calibration of peptide mapping experiments [42]. Interestingly, they found that the above-described algorithm fails, when 4-HCCA matrix clusters contained only potassium as the alkali ion and the number of potassium ions matched the number of matrix molecules. In this case the observed cluster mass was one mass unit less than predicted and the authors explained this with a hydrogen atom loss due to a potential photoionization mechanism [42].

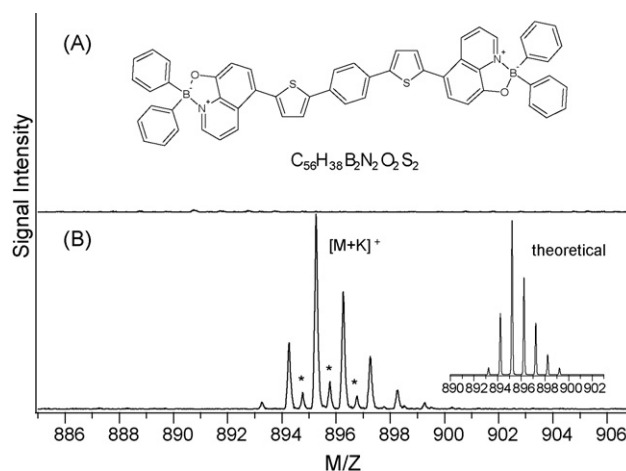
The obvious means to decrease occurrence and interference of matrix clusters is the avoidance or minimization of salt contaminations of the matrix and the sample which can be achieved by on-target washing of sample/matrix spots

[65,71,72], sample purification before sample/matrix deposition [73,74], or a combination of both approaches. Neubert et al. employed post source decay MS/MS analysis to distinguish matrix clusters from peptide signals [67], Smirnov et al. utilized washing steps with diammonium citrate buffer to eliminate or reduce 4-HCCA matrix clusters [66], and Kim et al. added nitrilotriacetic acid to sample/matrix preparation and achieved matrix cluster suppression and peptide signal enhancement [68].

Since matrix cluster formation is very dynamic and dependant on many factors including salt contamination, applied laser power, choice of matrix substance, sample type and analyte abundance [65], we included only the most prominent examples of 4-HCCA matrix cluster signals in our supplemental database, often observed during typical peptide mapping experiments in our laboratories.

### 3.2. Adducts, solvents and polymeric interferences

Adduct formation, either with solvent molecules, alkali or other metal ions, or with other contaminating components is frequently observed both in ESI and MALDI analysis. In fact, the majority of observed ions in modern mass spectrometry are adduct ions or pseudomolecular ions (e.g.  $[M + H]^+$ ,  $[M + NH_4]^+$ ,  $[M + Na]^+$ , or  $[M + K]^+$ ). The preferred ion type is the protonated molecular ion, especially in peptide analysis, since the partially mobile proton charge enables more meaningful fragmentation analysis, as compared to a sodiated

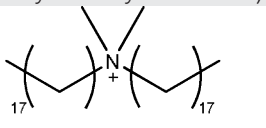
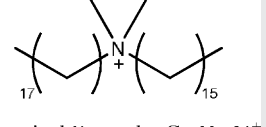
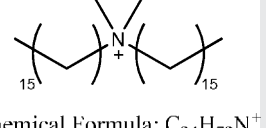


**Fig. 2** – Electrospray MS spectrum excerpt of a diboron compound (Cui and Wang, 2006 [78]) acquired on a QSTAR XL QTOF MS (Applied Biosystems, instrument located at Queen's University) with a regular electrospray source in positive mode with a flow rate of  $6 \mu\text{l}/\text{min}$ . (A) No signal was obtained when using combinations of different solvents such as methanol, acetonitrile, water and added  $K^+$  ions (as  $KNO_3$ ). (B) An intense potassiumated signal for this compound was obtained when dry nitromethane (Sigma–Aldrich, Oakville, ON) was used. Potassium was not deliberately added but is inherent in the solvent, most likely originating from the molecular sieves. The obtained singly charged isotope pattern agrees well with the theoretical model (see insert). A weak interference from the doubly charged dimer ( $[M_2 + 2K]^{2+}$ ) can be observed (labeled signals).

**Table 3 – Repeating units commonly observed in background interferences**

| Mass difference (accurate mass) | Origin   | Reference(s) |
|---------------------------------|--|--------------|
| 14.01565                        | -[CH <sub>2</sub> ]-, alkane chains, waxes, fatty acids, methylation   | [79]         |
| 15.99492                        | O, oxidation   | [79]         |
| 18.01057                        | H <sub>2</sub> O, water clusters   | [79]         |
| 28.03130                        | -[C <sub>2</sub> H <sub>4</sub> ]-, natural alkane chains such as fatty acids  | [79]         |
| 32.02622                        | CH <sub>3</sub> OH, methanol clusters  | [79]         |
| 41.02655                        | CH <sub>3</sub> CN, acetonitrile clusters  | [79]         |
| 42.04695                        | -[C <sub>3</sub> H <sub>6</sub> ]-, propyl repeating units, propylation  | [79]         |
| 44.02622                        | -[C <sub>2</sub> H <sub>4</sub> O]-, polyethylene glycol, PEG, Triton, Tween   | [79]         |
| 49.99681                        | -[CF <sub>2</sub> ]-, from perfluoro compounds   | [79]         |
| 53.00323                        | NH <sub>4</sub> Cl salt adducts/clusters   | [79]         |
| 56.06260                        | -[C <sub>4</sub> H <sub>8</sub> ]-, butyl repeating units, butylation  | [79]         |
| 57.95862                        | NaCl, sodium chloride clusters   | [79], [60]   |
| 58.04187                        | -[C <sub>3</sub> H <sub>6</sub> O]-, polypropylene glycol and related compounds, PPG                                 | [79]         |
| 63.03203                        | CHOONH <sub>4</sub> , ammonium formate adducts/clusters  | [79]         |
| 67.98742                        | NaHCO <sub>2</sub> , sodium formate clusters   | [79]         |
| 67.98742                        | CHOONa, sodium formate adducts/clusters  | [79]         |
| 72.03953                        | -OH replacement with -OSi(CH <sub>3</sub> ) <sub>3</sub> , (= [C <sub>3</sub> H <sub>8</sub> Si]), trimethylsiloxane | [79]         |
| 73.93256                        | KCl adducts/clusters   | [79]         |
| 74.01879                        | -[O-Si(CH <sub>3</sub> ) <sub>2</sub> ]-, polysiloxane, silicone rubber polymer                                      | [79]         |
| 78.01394                        | C <sub>2</sub> H <sub>6</sub> OS, DMSO adducts/clusters, dimethylsulfoxide solvent                                   | [79]         |
| 82.00307                        | NaCH <sub>3</sub> CO <sub>2</sub> , sodium acetate clusters  | [79]         |
| 84.05159                        | C <sub>2</sub> D <sub>6</sub> OS, deuterated DMSO adducts/clusters, NMR solvent                                      | [79]         |
| 106.90509                       | <sup>107</sup> Ag, silver clusters in MALDI analysis of non-polar polymers   | [76]         |
| 108.90476                       | <sup>109</sup> Ag, silver clusters in MALDI analysis of non-polar polymers   | [76]         |
| 113.99286                       | CF <sub>3</sub> COOH, trifluoroacetic acid, TFA adducts/clusters (negative mode)                                     | [79]         |
| 121.93828                       | NaClO <sub>4</sub> , sodium perchlorate adducts/clusters (negative mode)   | [79]         |
| 135.97481                       | NaCF <sub>3</sub> CO <sub>2</sub> , sodium trifluoroacetate clusters (NaTFA)   | [79]         |
| 162.05283                       | -[C <sub>6</sub> H <sub>10</sub> O <sub>5</sub> ]-, polysaccharides residues   | [79]         |
| 226.16813                       | -[C <sub>12</sub> H <sub>22</sub> N <sub>2</sub> O <sub>2</sub> ]-, cyclic oligomers from polyamide 66               | [86]         |
| 259.80992                       | CsI, cesium iodide clusters, used for calibration  | [97]         |
| 288.13713                       | C <sub>12</sub> H <sub>25</sub> SO <sub>4</sub> Na, sodium dodecylsulfate (SDS), clusters (negative mode)            | [79]         |

**Table 4 – Identified quaternary ammonium compounds by MALDI-FTICR-MS**

| Name and structure of compound  | Calculated theoretical mass | Observed mass |
|---|-----------------------------|---------------|
| (I) Dimethyl-dioctadecyl-ammonium (or distearyl-dimethyl-ammonium)<br><br>Chemical Formula: C <sub>38</sub> H <sub>80</sub> N <sup>+</sup><br>Exact Mass: 550.63 | 550.62853                   | 550.62849     |
| (II) Dimethyl-hexadecyl-octadecyl-ammonium<br><br>Chemical Formula: C <sub>36</sub> H <sub>76</sub> N <sup>+</sup><br>Exact Mass: 522.60                         | 522.59723                   | 522.59711     |
| (III) Dimethyl-dihexadecyl-ammonium<br><br>Chemical Formula: C <sub>34</sub> H <sub>72</sub> N <sup>+</sup><br>Exact Mass: 494.57                                | 494.56593                   | 494.56623     |

peptide ion. In addition, the occurrence of protonated analyte signals indicates that the employed instrument is clean and does not contribute any contaminating cationic components to the analytical process. However, there are also many examples where adduct formations, such as alkali or other metal ions, are unavoidable or indeed desirable since they enhance ionization efficiency of specific compounds or help stabilize the gas phase form of the analyte. In such cases it might be even necessary to deliberately add salt components to the solvent system in ESI or to the sample/matrix mixture in MALDI. Examples for deliberate adduct additions are the adding of silver or copper ions in the MALDI MS of non-polar polymers [75], which also can lead to cluster interferences in the case of silver [76], or the deliberate addition of halide anions in negative mode and ammonium, alkylammonium buffers and ammonium salts in positive mode for quantitative LC/MS studies of specific analyte groups [77].

It is not always possible to predict what solvent system, matrix compound and potential additives will work best for specific analytes and more often than not, individual method development is required for each new analyte class. A particular problem, frequently encountered in academic service- and research laboratories, is the analysis of metal-organic or other compounds that are not compatible with typical electrospray solvents such as methanol, acetonitrile, water or other alcohols. For these cases we find that dry nitromethane is often a good alternative that enables or enhances the mass spectrometric analysis, such as in the case of metal-organic compounds without forming solvent adducts or inducing analyte degradation. Fig. 2 shows an example of a diboron compound [78] that could not be detected in any other solvent system but dry nitromethane.

A comprehensive account of typical adduct formations in electrospray mass spectrometry was published by Tong et al. in 1999, including a description on how this information is used in the automatic “data massaging” of mass spectra interpretation in high-throughput applications [79]. We have included Tong et al.’s results on adduct formation in our supplemental spreadsheet and complemented it with available information of several instrument or accessory vendors [60–63] and our own lab results.

Two components of silicon oils employed in diffusion pumps of a home-built ESI-ion trap-TOF mass spectrometer, namely tetraphenyl-tetramethyl-trisiloxane and pentaphenyl-trimethyl-trisiloxane, were found to form adducts in protein analysis and have been described by Purves et al. in 1997 [80]. Change to higher grade diffusion pump oil resolved the issue.

In the MALDI analysis of proteins employing sinapic acid as the matrix compound, the protein signal is often accompanied by a second signal about 206 mass units apart, which has been proposed to be a photochemical adduct of sinapic acid [81].

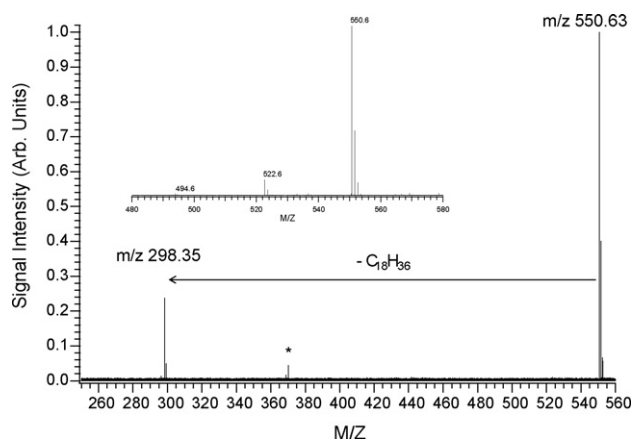
Polymeric interferences have been a problem in established and traditional analytical technologies for decades; among these are the ubiquitously employed polyethylene glycol (PEG) and polypropylene glycol (PPG). With the ability to analyze biological samples, modern mass spectrometry encounters additional polymeric interferences that are often intentionally added to biological sample preparations. Among these are the non-ionic detergents polyoxyethylene sorbitan monolaurate

(Tween®), used for removal of peripheral membrane proteins and benzyl-polyethylene glycol tert-octylphenyl ether (Triton®), for aiding the gel electrophoretic separation of proteins [82].

Polymeric interference sources can potentially come from all types of plasticware in the laboratory, including sample bottles, vials, pipette tips, pipette bulbs and filter membranes. Depending on the nature of the samples and sample preparation procedures, careful choices have to be made in terms of plasticware versus glassware, or types of plasticware used (e.g. Teflon versus nylon, etc. . .). A good starting point is consultation with the manufacturers of respective plastic products on compatibility issues [83], but in most cases this approach will not fully eliminate the need for lab-internal tests and development of good laboratory practice protocols that minimize leaching of polymeric interferences from plasticware. In this respect it needs to be mentioned that the use of aggressive solvents, acids or bases for cleaning of plasticware should be avoided.

Interfering polydimethylcyclosiloxanes in ambient air were reported by Schlosser and Volkmer-Engert in 2003 [84] and cyclic polyamide oligomers leaching from nylon 66 filters into the LC solvent stream were described by Önerfjord in 2004 [85] and identified by Tran and Doucette in 2006 [86].

It should be noted that both adduct formation and polymeric interferences can often be identified by their distinct repeating units. Table 3 summarizes the most commonly



**Fig. 3 – MALDI MS/MS spectrum of background ion (distearyldimethyl quaternary ammonium) acquired on an Apex-Qe 9.4 Tesla FT-ICR-MS (Bruker Daltonics, Billerica, MA, instrument located at the University of Alberta) equipped with an Apollo II Dual source using *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene]malononitrile as matrix. Fragmentation was induced using infrared multi-photon dissociation (IRMPD). Accurate mass measurement of the fragment ion at *m/z* 298 confirmed its identity (Theoretical value for  $[C_{20}H_{44}N]^+$ : 298.34683, found: 298.34678). The signals observed at *m/z* ~370 (\*) are instrument induced artefacts. The insert shows a MALDI MS spectrum of all three identified quaternary ammonium compounds as listed with structures in Table 4, showing the typically observed intensity ratios. It should be noted that sample preparations involving chromatography will separate these components.**

|    | Monoisotopic ion mass (singly charged) | Ion type   | Formula for M or subunit or sequence                                | Compound ID or species   | Possible origin and other comments              | ESI | MALDI |
|----|--|--|---|--------------------------|---|-----|-------|
| 8  | 33.03349                               | [M+H] <sup>+</sup>                               | CH <sub>3</sub> OH  | Methanol                 | Acetonitrile, solvent                           | X   |       |
| 9  | 42.03383                               | [M+H] <sup>+</sup>                               | CH <sub>2</sub> CN  | ACN                      | Acetonitrile, solvent                           | X   |       |
| 10 | 59.06037                               | [M+NH <sub>4</sub> ] <sup>+</sup>                | CH <sub>2</sub> CN  | ACN                      | Acetonitrile, solvent                           | X   |       |
| 11 | 83.04406                               | [A <sub>1</sub> B+H] <sup>+</sup>                | [C <sub>2</sub> H <sub>4</sub> O] <sub>2</sub> H <sub>2</sub> O     | PEG                      | Polyethylene glycol, ubiquitous polyether       | X   |       |
| 12 | 84.01577                               | [M+H] <sup>+</sup>                               | CH <sub>2</sub> CN  | ACN                      | Acetonitrile, solvent                           | X   |       |
| 13 | 85.05971                               | [M <sub>2</sub> +H] <sup>+</sup>                 | CH <sub>3</sub> OH  | Methanol                 | Acetonitrile, solvent                           | X   |       |
| 14 | 74.06004                               | [A <sub>1</sub> B <sub>2</sub> +H] <sup>+</sup>  | (CH <sub>2</sub> CN) <sub>2</sub> (CH <sub>2</sub> OH) <sub>2</sub> | Acetonitrile/Methanol    | ESI solvents                                    | X   |       |
| 15 | 74.06004                               | [M+H] <sup>+</sup>                               | C <sub>2</sub> H <sub>5</sub> NO                                    | Dimethyl formamide       | solvent   | X   |       |
| 16 | 77.05971                               | [A <sub>1</sub> B+H] <sup>+</sup>                | [C <sub>2</sub> H <sub>4</sub> O] <sub>2</sub> H <sub>2</sub> O     | PPG                      | Polypropylene glycol, ubiquitous polyether      | X   |       |
| 17 | 79.02121                               | [M+H] <sup>+</sup>                               | C <sub>2</sub> H <sub>6</sub> OS                                    | DMSO                     | Dimethylsulfoxide, solvent                      | X   |       |
| 18 | 83.06037                               | [M <sub>2</sub> +H] <sup>+</sup>                 | CH <sub>2</sub> CN  | Acetonitrile             | ESI solvents                                    | X   |       |
| 19 | 85.02600                               | [A <sub>1</sub> B+Na] <sup>+</sup>               | [C <sub>2</sub> H <sub>4</sub> O] <sub>2</sub> H <sub>2</sub> O     | PEG                      | Polyethylene glycol, ubiquitous polyether       | X   |       |
| 20 | 85.05887                               | [M+H] <sup>+</sup>                               | C <sub>2</sub> H <sub>6</sub> OS                                    | d6-DMSO                  | d6-Dimethylsulfoxide, solvent                   | X   |       |
| 21 | 88.03931                               | [A <sub>1</sub> B <sub>2</sub> +H] <sup>+</sup>  | (CH <sub>2</sub> CN) <sub>2</sub> (HCOOH) <sub>2</sub>              | Acetonitrile/Formic Acid | ESI solvents                                    | X   |       |
| 22 | 95.04198                               | [A <sub>1</sub> B <sub>2</sub> +Na] <sup>+</sup> | (CH <sub>2</sub> CN) <sub>2</sub> (CH <sub>2</sub> OH) <sub>2</sub> | Acetonitrile/Methanol    | ESI solvents                                    | X   |       |
| 23 | 99.04165                               | [A <sub>1</sub> B <sub>2</sub> +Na] <sup>+</sup> | [C <sub>2</sub> H <sub>4</sub> O] <sub>2</sub> H <sub>2</sub> O     | PPG                      | Polypropylene glycol, ubiquitous polyether      | X   |       |
| 24 | 100.07569                              | [M+H] <sup>+</sup>                               | C <sub>2</sub> H <sub>5</sub> NO                                    | NMP                      | N-methyl 2-pyrrolidone, solvent, floor stripper | X   |       |

**Fig. 4** – Screen snap shot of the spreadsheet in the supplemental data. The Excel worksheet contains tabs for positive (+ve) and negative (–ve) ions, repeating units, adducts, losses and replacements, a list of atomic masses used in the molecular weight calculations, a list of common solvents, references and a glossary of used abbreviations.

observed repeating units and their origins. This list is also included in the supplemental data spreadsheet. Repeating units are typically not charge specific, however a number of repeating units, such as clusters of trifluoroacetic acid (TFA, acid form), sodium perchlorate (NaClO<sub>4</sub>) and sodium dodecyl-sulfate (SDS) have only been reported in negative mode [79].

### 3.3. Plasticizers, additives and other interferences

Plasticizers such as phthalate esters and additives such as anti-oxidants have also long been known to interfere in analytical techniques, have been well characterized and are listed in technical notes of many instrument or accessory vendors [60–63]. Verge and Agnes reported the outgassing and interference of phthalates from vacuum o-rings employed in their electrospray mass spectrometry setup in 2002 [87]. Interference from diethylene glycol monobutyl ether (DGBE), a component in scintillation cocktails, was reported employing LC/MS coupled to online radioactive material detection (LC/RAM/MS/MS) by Gibson and Brown in 2003 [88]. Leaching of the sulfoxide oxidative products of didodecyl 3,3'-thiodipropionate (DDTDP), an anti-oxidant found in polypropylene vials, was reported by Xia et al. in 2005 [89]. In a comprehensive study on electrospray background interferences, Guo et al. classified common plasticizers and other contaminants into family trees [90].

Autoclaving is a common practice to sterilize scientific analytical equipment or storage containers for biological samples which unfortunately has the potential to introduce contaminants into the analytical stream. For example, leaching of xenoestrogens such as bisphenol A from lacquer-coated containers [91], leaching of plasticizers such as phthalates from storage bags or medical tubing [92,93], and formation

of acrylamide in rat food [94], during autoclaving has been reported.

Recently, we were able to identify three quaternary ammonium compounds, which are frequently observed as strong background ions both in MALDI and ESI and have previously found entry into a number of background ion lists as unknown contaminants [61,79]. The identities of these compounds was confirmed with accurate mass measurements both of the precursor ions and the MS/MS fragments. Table 4 lists the results for the three compounds and Fig. 3 shows an MS/MS spectrum of the distearyldimethyl quaternary ammonium ion; all measured masses are within 1 ppm mass error of the calculated theoretical molecular weight. Possible sources for these compounds are personal care products, wherein these compounds are often added as softeners [95].

## 4. Explanation of background ions spreadsheet

Fig. 4 shows a screen snapshot of the Excel spreadsheet available in the supplemental data. The spreadsheet contains a total of eight worksheets.

The first two worksheet tabs lead to singly charged background ion lists either in positive (+ve) or negative (–ve) mode. The positive ion list contains more than 650 species either reported in the literature or observed in our laboratories. The negative ion list contains only species that have been reported in the literature, however it should be noted that most background species (except quaternary ammonium species) listed in the positive ion list could potentially interfere in negative mode after de-protonation or anion adduct formation.



The widespread introduction of novel mass analyzers such as orthogonal TOFs or FT-ICR-MS with accurate mass measurement capabilities was responsible for our decision to calculate the accurate monoisotopic masses of the background ions; a feature that distinguishes this list from most other available interference lists. We also incorporated the mass of the electron in our calculations.

For both ion lists we decided not to include any multiply charged ions, since the formation of multiply charged ions depends on individual setups, solvents utilized, acids and other conditions and could potentially lead to high complexity if multiple charges are due to both various cations and possibly protons. Also, in many instances the appearance of multiply charged ions is often accompanied by the singly charged species and most software programs will have a software option to transform multiply charged ions into singly charged species.

The third tab contains the list of common repeating units of polymeric or adduct interferences which is also shown in Table 3. The fourth tab contains a list of common adduct formations, replacement reactions, and observed losses reported in the literature. The fifth tab includes a spreadsheet with a list of the atomic masses used in our calculations. The spreadsheet is set up to be used for determination of the accurate theoretical mass of any compound which then can easily be compared to an observed mass. The sixth tab contains a list of commonly used solvents including deuterated solvents, often employed in NMR studies. The seventh tab contains a list of the references reporting these background interferences. The letter label for each reference is included in the background ions, repeating units, or adducts list in a separate column. The eighth and final tab contains a glossary with a brief explanation of some of the abbreviations used in the database.

## 5. Conclusions

We have compiled a comprehensive database of currently known potential interferences and background-ions in modern mass spectrometry reported in the literature and observed in our own laboratories. It is clear that such a list can never be complete as modern mass spectrometry is still a young discipline enjoying rapid and dynamic growth, and new chemicals and materials are continually introduced into sample preparation assemblies and mass spectrometers themselves. Increasing emissions of emerging daily-life and other synthetic products into the ambient environment will also add to this growing list of potential interferences. However, we anticipate that this database provides a solid foundation for this evolving field and serves as a valuable and expandable quality control tool that contributes to sound method development in all types of research and service laboratories employing modern mass spectrometry.

## Note added in proof

Recently, Manier et al. published a more detailed identification and fragmentation study of the three quarternary ammonium compounds (listed in Table 4), employing ESI-FT-ICR-MS [98].

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2008.04.043.

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