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Review

# Ion suppression; A critical review on causes, evaluation, prevention and applications

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ABSTRACT

The consequences of matrix effects in mass spectrometry analysis are a major issue of concern to analytical chemists. The identification of any ion suppressing (or enhancing) agents caused by sample matrix, solvent or LC–MS system components should be quantified and measures should be taken to eliminate or reduce the problem. Taking account of ion suppression should form part of the optimisation and validation of any quantitative LC–MS method. For example the US Food and Drug Administration has included the evaluation of matrix effects in its "Guidance for Industry on Bioanalytical Method Validation" (F.D.A. Department of Health and Human Services, Guidance for industry on bioanalytical method validation, Fed. Regist. 66 (100) 2001). If ion suppression is not assessed and corrected in an analytical method, the sensitivity of the LC–MS method can be seriously undermined, and it is possible that the target analyte may be undetected even when using very sensitive instrumentation. Sample analysis may be further complicated in cases where there are large sample-to-sample matrix variations (e.g. blood samples from different people can sometimes vary in certain matrix components, shellfish tissue samples sourced from different regions where different phytoplankton food sources are present, etc) and therefore exhibit varying ion-suppression effects. Although it is widely agreed that there is no generic method to overcome ion suppression, the purpose of this review is to:

- provide an overview of how ion suppression occurs,
- outline the methodologies used to assess and quantify the impact of ion suppression,
- discuss the various corrective actions that have been used to eliminate ion suppression in sample analysis, that is to say the deployment of techniques that eliminate or reduce the components in the sample matrix that cause ion suppression.

This review article aims to collect together the latest information on the causes of ion suppression in LC-MS analysis and to consider the efficacy of common approaches to eliminate or reduce the problem using relevant examples published in the literature.



**Contents**



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## 1. Introduction

When liquid chromatography–mass spectrometry (LC–MS) techniques were first introduced, they solved a great deal of problems for analytical chemists. LC–MS had unprecedented capabilities, especially for compounds that were incompatible with gas chromatography–mass spectrometry (GC–MS) due to their high polarity and/or high mass. LC–MS systems allowed for the elimination of derivatisation steps prior to injection, saving on reagent costs and time. The technique was considered to require only minimal sample clean-up and preparation, as well as enabling high throughput analysis with minimum (or in some case no) chromatographic separation of constituent compounds. These beliefs resulted in the large uptake of LC–MS instruments in analytical laboratories. However, in recent years users have realised that LC–MS can be susceptible to interferences from the sample matrix that may affect analysis [2]. Antignac et al. highlighted in their study of ion suppression in LC–MS that difficulties can arise with reproducibility and accuracy when measuring trace analytes in complex matrices, such as biological fluids [3].

In the recent past, the strongest marketing point for LC–MS technology was its unrivalled selectivity. Electrospray ionisation (ESI) sources allowed for the facile ionisation of the LC effluent; ion focusing optics were capable of delivering ions of (more or less) specific mass-to-charge ($m/z$) ratios directly into the MS detector while eliminating all 'other' ions [4]. These features of LC–MS offered a significant advantage over techniques such as LC-UV/DAD and LC-FLD that were susceptible to interferences from all matrix constituents in possession of structural features that would enable them to absorb UV radiation (for example chromophores, structural rigidity, etc.) [5]. LC–MS was embraced by analysts as a robust and sensitive qualitative and quantitative tool capable of dealing with trace target analytes in a veritable sea of matrix. However, it is now accepted that the selectivity and sensitivity of the technique can be diminished by matrix components that can affect the ionisation efficiency of the analyte.

It is imperative to distinguish between matrix effects and interferences. An *interference* is a constant pre-determinate error that effects the response of the analyte, independent of its concentration [6]. *Matrix effects* interfere with the response of the analyte in proportion to analyte concentration. Matrix effects occur through reactions between the analyte(s) and some matrix constituent resulting in a species that can either suppress or enhance signal response in the MS [7]. In LC–MS techniques where single ion monitoring (SIM) or selected reaction monitoring (SRM) is employed, parameters are set so that only ions of desired $m/z$ ratio are evident in the spectra and matrix components (unless coincidently isobaric) do not appear in the spectra. However, though not apparent in the resultant mass spectra, these matrix constituents may alter the ionisation efficiency of the target analyte thereby compromising the efficiency and reproducibility of the quantitative method. In consequence this will affect the precision and sensitivity of the LC–MS method [8,9]. Therefore, during the initial stages of LC–MS method development, it would be prudent to operate the MS in full scan mode to identify potential interferences.

Validation is an integral part of all modern day analysis and is an important regulatory requirement in many areas of analytical chemistry [10]. The US Food and Drug Administration's (FDA) *Guidance for Industry on Bioanalytical Method Validation* requires matrix effect assessment "to ensure that precision, selectivity, and sensitivity will not be compromised" [1]. Although these guidelines require the evaluation of matrix effects in method validation, they do not state what procedures are required to carry out this assessment. Ion suppression or matrix effect studies are an important part of method validation due to the possible detrimental effects ion suppression may have on analyte response. By initially detecting, then measuring and/or removing any ion suppressing interferences from the matrix, together with other validation requirements, the quality of analysis is assured. It is important in method optimisation to also consider the fact that internal standards may possess even slightly different functionalities and polarities to the target analyte, so the ion suppression of these agents must also be examined [10].

There is a greater potential for ion suppression in the following cases:

- when the target analyte(s) are present at only trace amounts in complex matrices,
- when there is a number of compounds to be analysed in a single sample,
- when only minimal sample clean-up is preformed,
- when acid or alkaline buffers or ion pairing agents are present in the LC effluent,
- when using short non-resolving chromatographic runs [5].

## 2. Causes of ion suppression

The sources of ion suppression include endogenous compounds such as organic or inorganic molecules present in the sample, as well as exogenous substances not present in the original sample but which may have been introduced during sample preparation [3]. Large differences in the extent of matrix effects can be observed between different matrix types (e.g. urine, oral fluid and plasma) and between different sample preparation techniques, including direct injection, dilution, protein precipitation (PP), solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [11]. Besides ion suppression, it is also possible for analytes to undergo ion enhancement, which involves an increase in the analyte response due to the presence of matric effects. However, ion suppression is much more frequently encountered than ion enhancement and therefore ion enhancement will not be discussed in detail in this review. The objective of this section is to give a brief overview of the possible causes of ion suppression. A number of books and review papers have already been written on the topics of MS ioninsation and ion suppression and can be consulted for more in-depth detail.

Endogenous components that can act as ion suppressors include: ionic species (inorganic electrolytes, salts), highly polar compounds (phenols, pigments) and various organic molecules including carbohydrates, amines, urea, lipids, peptides, analogous compounds or metabolites with a chemical structure close to that of the target analyte. Some factors make a matrix component a prime candidate for inducing ion suppression, for example, high concentration, high mass, basicity and co-elution that appear in the same chromatographic retention window as the analyte of interest. The second source of ion suppression, which is less frequently encountered in the literature, is due to the presence of molecules being introduced to the sample from various external sources during the sample collection and preparation steps. Among this second group of ion suppressor agents are plastic and polymer residues, phthalates, detergent degradation products (alkylphenols), ion pairing reagents, proton exchange agents (e.g. organic acids), calibration products, buffers, and material released by the SPE, LC or GC stationary phases [3].

Ion suppression is compound dependent and occurs in the early stages of the ionisation process in the ion source. It can be caused by polar and unretained matrix components or by overloading of the LC column [11]. Different mechanisms have been proposed to explain the ion suppression phenomenon that have been to date recorded. These include:

(a) competition between matrix components and analyte ions that are co-eluting in the sprayed solution for access to the droplet surface for gas-phase emission [11,12].
(b) matrix interferences that compete for available charge.
(c) matrix that binds to analyte or causes the analyte to co-precipitate.
(d) analyte ions that may be neutralised through gas phase acid/base reactions.
(e) mobile phase additives, and
(f) equipment design.

All of the above have the potential to cause a decrease in the MS signal response [13,14].

(a) In ESI ionisation, evaporation of the LC eluent begins in the ion source where the electrical density at the surface of the droplets increases as the solvent evaporates from the droplets. This electrical charge on the droplet surface increases up to a critical point called the Rayleigh stability limit. At this point the droplet is divided into smaller droplets by electrostatic repulsion until analyte ions are emitted into the gas phase [15]. Any interfering compounds present in relatively high concentrations can increase the viscosity and surface tension of the droplets produced in the electrospray ionisation (ESI) and reduce the ability of the analytes to reach the gas phase by reducing access to the droplet surface [16].
(b) There can be competition between analyte and matrix components for access to available charge, causing different analyte response between standard solutions and samples [16]. Depending on the environment in which ionisation and ion-evaporation take place, this competition between matrix and analyte may effectively decrease (ion suppression) or increase (ion enhancement) the efficiency of formation of the desired analyte ion [11].
(c) Co-precipitation of the analytes with nonvolatile material such as macromolecules can also limit their transfer in the gas phase. It has been shown that molecules with higher mass will suppress the signal of smaller molecules and that moderate- to high-polarity analytes are especially susceptible to ion suppression [17].
(d) If during positive ESI mode ionisation a charged analyte is transferred to the gas phase, proton transfer reactions may cause neutralisation of the analyte if another neutral species is present in the gas phase with a higher proton affinity than the analyte [7,18]. In addition, other ionic species present in a sample with high ionisation efficiency or surface activity, such as salts, may compete with analytes during ion evaporation [7]. High levels of nonvolatile substances can also cause changes in the droplet solution properties and may affect the transfer of ionised analyte into the gas phase by preventing the radius and surface charge of the droplets from reaching the levels necessary for ionisation to occur [7,18].
(e) While addition of low levels of mobile phase additives, such as formic acid [20], can improve analyte response high levels of buffers and the use of known ion suppressants (e.g. TFA) can have a suprressing effect on ionisation [21]. See Section 5.5 for more information on the influence of mobile phase additives.
(f) ESI and APCI are two of the most commonly used ionisation techniques in LC-MS. They are particularly useful when coupled to liquid chromatography because of their effective desolvation of mobile phase. ESI is a sensitive ionisation technique, although it has been observed that it is highly susceptible to ion suppression [13]. It has been reported that ESI is more susceptible to ion suppression than APCI [13]. Poor sensitivity in ESI has been observed for compounds which lack an ionic functional group or which cannot efficiently transfer ions into the gas phase [7]. There are several possible mechanisms which occur during desolvation and ionisation which could be responsible for a loss of analyte response [7]. In ESI, the compounds are ionised in the liquid phase and ions are released from charged droplets. The applied electric field between the capillary tube and the counter electrode results in accumulation of charged compounds at the droplet surface. Supplementary Fig. 1 shows a schematic diagram of the ESI source. The upper limit of the total number of ions which can be formed during ESI is related to the total surface area of all droplets and this upper limit is usually reached at $10^{-5}$ M sample ion concentration [3]. Here, the response no longer increases but levels off and eventually decreases [3,18,22]. If many compounds are co-eluting, their relative liquid phase basicities and surface activities will determine the ionisation efficiency of the individual compounds. Since endogenous compounds can be present at high concentrations and their relative basicities and surface activities can be comparable or higher than those of the analyte, the limit of $10^{-5}$ M of ions is rapidly exceeded leading to ion suppression [3,22].

It has been suggested that APCI is associated with a smaller degree of ion suppression because analytes are already in the gas

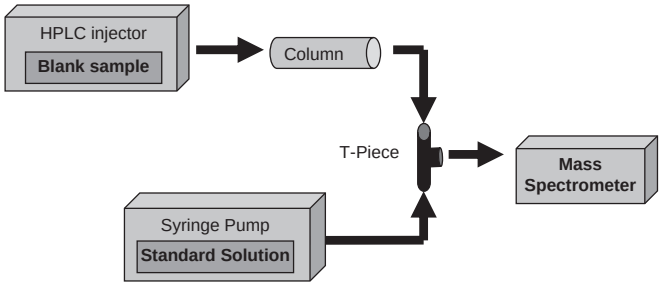


**Fig. 1.** Schematic of the set-up of a post-column infusion experiment as described by Antiganac et al. [3]. The line from the column where a blank sample has been injected is connected through a T-piece to a line from a syringe pump containing a standard solution, which mix together and travel to the mass spectrometer for detection.

phase when ionisation occurs [7,11,22]. The reagent ions generated from the vaporised mobile phase by corona discharge are formed in excess and therefore signal saturation or ion suppression is expected to occur at higher ion concentrations compared with ESI [22]. A schematic diagram of the APCI is shown in Supplementary Fig. 1. Unlike ESI, there is less competition between analytes to enter the gas phase, because neutral analytes are transferred into the gas phase by vaporising the liquid in a heated stream. APCI does experience some ion suppression, which has been explained by the effect of sample composition on the efficiency of charge transfer from the corona discharge needle [23]. In addition, because there is very little chance for analytes to pass through the vaporisation region and remain in solution, another mechanism of ion suppression in APCI is solid formation, either as pure analyte or as a solid co-precipitate with other nonvolatile sample components [24].

## 3. Effects of ion suppression

The consequences of ion suppression on the analytical result are numerous.

- The detection capability is reduced due to a decrease in the analyte signal, which also leads to a higher limit of detection (LOD), lower signal-to-noise (S/N) ratio and a smaller dynamic range of the method.
- Precision is affected as the degree of suppression may vary from one sample to another.
- Ion ratio, linearity and quantitation can be affected due to the variability of the matrix effects between samples.
- Severe ion suppression can lead to the non-detection of an analyte. This situation can result in an analysis not meeting the identification criteria set out in an SOP and/or result in false negative results for positive samples [3].
- The opposite is also true if ion suppression affects the internal standard rather than the analyte. This can result in an overestimation of analyte concentration and an increased risk of false positive results [3].

In summary, ion suppression affects the reliability of the analytical result and ultimately the integrity of the analytical process and analysts must take precautions to avoid or reduce its effects.

## 4. Detecting and evaluating of ion suppression

(i) The simplest way of detecting the presence of any matrix effects in a sample is to compare the signal of the analyte in a blank sample extract which has been spiked with standard post-extraction to the analyte response of a standard solution in neat mobile phase or solvent. If the spiked sample response is lower than the standard solution this will indicate the presence of interfering agents causing ion suppression (or enhancement). Similarly, calibration curves prepared in solvent and in matrix extract (matrix-matched standards) can be compared to each other. Any ion suppression present can be seen by the differences in the slopes of the two curves, which is caused by the different sensitivities of the two sets of standards. A matrix that causes no ion suppression should have a calibration curve which superimposes on that obtained for the standard in solvent [25]. While these methods provide information on the presence and extent of matrix effects they do not provide information on the chromatographic profile of ion suppressors in the matrix or on their exact elution region in the chromatogram.

An investigation of the effectiveness of this approach was carried out by Ito et al. in the analysis of diarrheic shellfish (DSP) toxins (okadaic acid, dinophysistoxin-1, pectenotoxin-6 and yessotoxin) in scallops [26]. They compared spiked extracts of shellfish with standard solutions in methanol. They found that the spiked extracts gave a signal ranging from 19 to 42% lower than that of the standard solutions. Their results demonstrated that there were some co-eluting substances present in the shellfish extract that induced signal suppression of the analytes (Table 1).

(ii) Another method for detecting ion suppression is through a post-column infusion experiment first described by Bonfiglio et al. [24,27]. This is carried out by the continuous introduction of a standard solution containing the analyte under investigation (together with the internal standard if required) to the column effluent through a T-piece (see Fig. 1). A blank matrix sample is then injected into the system (while the analyte is infused continuously) and the blank sample is separated in the column using the developed analytical method. A drop in the baseline signal (suppression of signal) indicates the presence of interfering matrix components [3,28,29]. Note that when a blank biological extract is injected onto the LC system, the resulting total ion current increases due to the sample extract arriving in the interface to undergo ionisation. Therefore, to detect ion suppression a SIM or SRM scan of the analyte is required, which allows the matrix effects of the extract to be viewed in relation to the target analyte. Continuous post-column infusion of a standard solution ensures that all matrix components in a sample that elute from the column are ionised along with the analyte [20].

Fig. 2 shows the result of a post-column infusion experiment for serotonin and dopamine in serum (authors own work). The shaded areas correspond to the elution time of the analytes. In the serotonin TIC graph (A) it can be seen that there is little ion suppression affecting the serotonin signal response as it elutes away from the chromatographic region

**Table 1**
Comparison of standard addition method to theoretical recovery and external standard methods [26]. Reprinted with permission.

| Calibration method | Amount ± SD (ng/g) [cd] | | | |
|---|---|---|---|---|
| | PTX6 | OA | YTX | DTX1 |
| Model sample (theoretical) | 200 | 200 | 200 | 200 |
| External standard method[a] | 170 ± 8 | 134 ± 14 | 135 ± 8 | 138 ± 6 |
| Standard addition method[b] | 197 ± 9 | 213 ± 20 | 215 ± 12 | 214 ± 10 |

[a] The calibration curves were prepared using DSPs standard solutions in methanol.
[b] The calculation was carried out by adding the DSPs standard solution to the model sample.
[c] $n=6$.
[d] ng/g hepatopancrea extract.

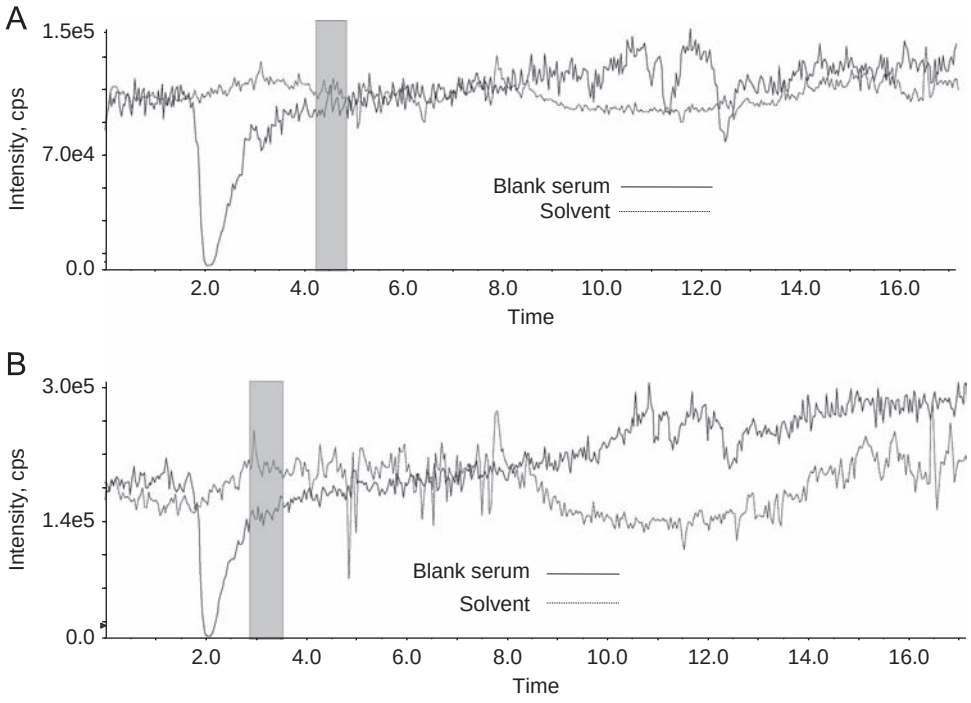


**Fig. 2.** Post-column infusion experiment for evaluation of ion suppression in serum matrix for serotonin (A) and dopamine (B). Shaded areas correspond to elution times of analytes (authors' own work).

where ion suppression occurs. In contrast, the dopamine peak (B) is eluting in an area of ion suppression, with the signal intensity being lower (≈20–25%) for the sample in matrix compared with that of the solvent-dissolved standard.

Post-column infusion can also be exploited in preventing ion suppression in an LC–MS method. This can be done by infusing a buffer to either adjust the pH of the column eluent or improve ionisation efficiency by promoting the formation of alternative adduct ions [30]. This technique has the effect of minimising ion suppression without having to change the optimised chromatographic conditions in order to elute the analyte away from precarious problematic regions where ion suppression may take place.

(iii) A third method for detecting ion suppression is a more complicated version of the first method. It involves comparing peak areas of an analyte that is spiked into the matrix before extraction, into the sample extract after extraction (matrix-matched standard) and into neat solvent. Using this method the recovery and matrix effects associated with the sample can be assessed. In a study by Matuszewski et al. [10], the 'absolute' matrix effect (ME) was calculated using the formula:

$$\mathrm{ME}(\%) = X/Y \times 100$$

where $X$ is the peak area for analyte in solvent and $Y$ is the peak area of standards spiked into matched matrix after extraction. Recovery (RE) can be calculated using:

$$\mathrm{RE}(\%) = Z/Y \times 100$$

where $Z$ is the peak area of standards spiked before extraction. Overall process efficiency (PE) can then be calculated by

$$\mathrm{PE}\ (\%) = Z/X \times 100 = (\mathrm{ME} \times \mathrm{RE})/100$$

Any difference in the analyte response of sample extracts that were spiked with standard after extraction but before evaporation and reconstitution in the mobile phase will be due to the sample matrix. Samples that were spiked with standard before extraction may have differences corresponding to possible variability in the recovery and ion suppression [31]. Fig. 3 shows three chromatograms with peak areas for (a) an extracted sample, (b) a post-extraction spiked sample and (c) solvent standard (authors own work). The difference in response between (a) and (c) is due to reduced recovery and ion suppression. The difference between (b) and (c) is ion suppression and the difference between (a) and (b) is recovery alone. Due to the more complex and time-consuming nature of this approach, the post-column infusion and post-extraction addition methods are the most commonly used approaches for matrix effect evaluation [8].

## 5. Strategies for removing or reducing ion suppression

Once ion suppression is observed to be present in a sample matrix, the next step is to decide the most appropriate method for reducing or removing the interfering matrix components that cause ion suppression. There are several strategies available to remove or reduce matrix effects, Table 2 including:

a) selective extraction [8,11–15,59,]
b) effective sample clean-up after extraction,
c) re-optimisation of the chromatographic method to attain full separation of sample components from analytes of interest [8,16–18,22].

Sometimes, these approaches are not completely effective as they could lead to analyte losses (a and b) or long analysis times (c). Other methods for reducing the effects of ion suppression include the use of suitable calibration approaches, such as

- external calibration using matrix-matched samples (see Sections 4 and 5.4)
- standard addition [9,12,23] or

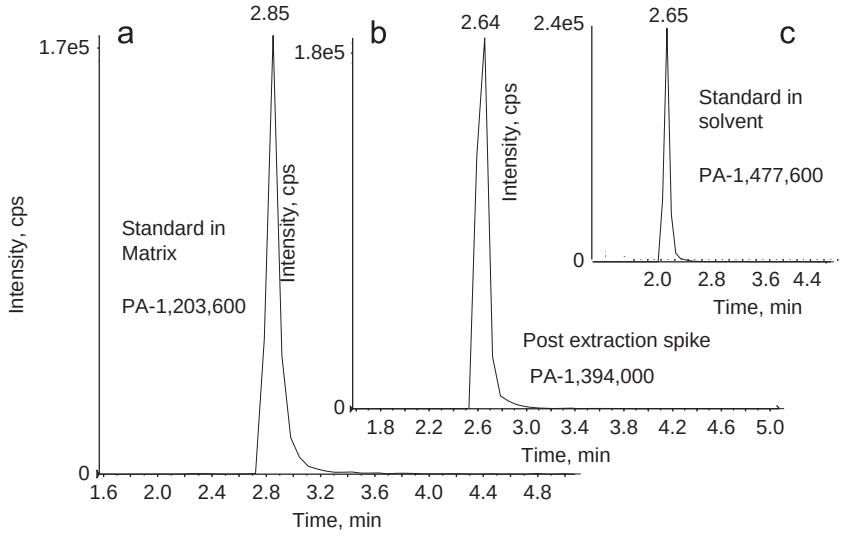


**Fig. 3.** Chromatographs showing differences in response between standards in matrix (a), standard spiked post extraction (b) and standard in solvent (c). The difference in response between (a) and (c) is due to reduced recovery and ion suppression, difference between (b) and (c) is ion suppression and difference between (a) and (b) is recovery. PA=peak area (authors' own work).

- internal standard [12,24–27], as well as
- dilution of sample extracts to reduce the matrix effects [2,8–9,20,25,28-30,42,48,55,67,77,84].

Choosing the appropriate approach will depend on a number of factors, including the complexity of the sample matrix, the availability of a suitable internal standard and the MS technology employed, and will be discussed in more detail in the next section.

### 5.1. Mass spectrometer source

The first option to consider changing (and often the easiest) is the ionisation mechanism (polarity) from positive ion mode (majority of compounds are ionised this way) to negative ion mode. The extent of ion suppression may be reduced but this will only be effective if the analytes being investigated give a good response in negative mode and the matrix interferences have little or no response [10]. As there are fewer compounds that give a good signal response in negative mode, it may be possible that interferences will not cause any matrix effects.

When a given ionisation mode is not effective the next step is to change the ionisation source. Both ionisation mode (e.g. APCI vs. ESI) and source design (which varies with different manufacturers and includes three main spray orientations: linear, orthogonal or Z-spray) can affect the extent of ion suppression [2]. APCI is less prone to ion suppression than ESI, although a compromise is required since there may be a loss of sensitivity and less applicability than with ESI [2]. It has been found that on studying the effects of ion pairing reagents on signal suppression of sulphonated dyes that different source designs influence ion suppression according to the general rule: Z-spray < orthogonal spray < linear spray (ranked in order of reducing ion suppression effects) [32]. The opposite was found by Ghosh et al. who found that the Z-spray caused nearly complete ion suppression and orthogonal spray caused almost zero ion suppression (with some minor ion enhancement) when looking at acamprosate (*N*-acetyl homotaurine, brand name Campral, a drug used for treating alcohol dependence) in biofluids. The ion suppression was seen to be caused by the presence of phospholipids [33]. Stahnke et al. carried out a study comparing five contemporary ESI sources to investigate to what extent their design influences the susceptibility of LC–MS to matrix effects [19]. The sources tested include Turbo Ion Spray (Applied Biosystems), Turbo VTM Source (Applied Biosystems), Jet Stream ESI (Agilent Technologies Inc.), Standard ESI and Standard Z-Spray Source. The authors concluded that the Jet Stream ESI obtained higher sensitivity through the additional superheated sheath gas which increases desolvation of ions. However, it also suffered from significantly increased signal suppression compared to the Standard ESI without sheath gas. The Turbo Ion Spray, Turbo VTM Source and Standard Z-Spray Source did not differ much in their susceptibility to matrix effects.

The Jet Stream source uses thermal gradient focusing technology to enhance sensitivity in ESI-MS by improving the desolvation and spatial focusing of ions. This is accomplished using superheated nitrogen sheath gas to confine the nebuliser spray to more effectively dry ions and concentrate them in a thermal confinement zone.

An alternative MS source is atmospheric pressure photoionisation (APPI) which may offer improved ionisation efficency and ought to be tried if it is available and when ESI and APCI have been already been evaluated. Unlike ESI and APCI, APPI can be used to analyse nonpolar molecules and is less susceptible to ion-suppression and salt-buffer effects [33]. It is clear therefore that a trial and error approach is required to determine the source/interface that produces the least ion suppression.

Over the years advances in MS technology have resulted in instruments capable of high mass accuracy and mass resolution, including time-of-flight (ToF) and Orbitrap and ion mobility spectrometry hyphenated with mass spectrometry (IMS–MS). The incorporation of these advances in MS technology can lead to increases in *S*/*N* ratio and consequently improved sensitivity (LOD/LOQ). However, while the advent of new technologies can undoubtedly help to overcome some of the problems associated with ion suppression, low resolution instruments such as triple quadrupoles and ion traps remain the most widely used MS instruments. Therefore, it remains important in quantitative LC–MS, and in particular with low resolution instruments that are more prone to matrix effect, to identify regions in the chromatography where ion suppression of analytes does occur and try to overcome or reduce it

### 5.2. Sample clean-up

An effective way to reduce ion suppression is to remove the matrix components that can introduce interference during

**Table 2**
Collection of methods that deal with ion suppression, their method parameters and evaluation and solutions to ion suppression.

| Author | Compound investigated | Matrix | MS | LC conditions | Evaluation of matrix effect | | | Matrix effect | Amount of matrix effect | Solution to matrix effect | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pre- and post-extraction | Post-column infusion | Matrix/solvent study | | | Dilution | Std Add | Extract-ion | Internal Standard | Column Switching |
| Fong et al. [72] | Acylglycines | Urine | ESI-Qtrap MS | – | ✓ (SPE) | ✓ | | Ion suppression | Reduction in ion suppression with derivatisation and use of Agilent SAX SPE | | | ✓ | ✓ | |
| Berg et al. [73] | Amphetamine and metamphetamine | Biological samples | UPLC–ESI-Quattro Premier Xe MS/MS | ACQUITY UPLC BEH C18 (50 × 2.1 mm, 1.7 μm) MP A-5 mM ammonium formate MP B-methanol Gradient elution | | | ✓ | Ion suppression and enhancement | Deuterated internal standands showed higher ion suppression than $C^{13}$ labelled internal standards. 20% compared to 80% | | | ✓ | ✓ | |
| Hasselstrom et al. [74] | Antidepressants and antipsychotics | Serum | UPLC-ESI-triple quadrupole | Zorbax SB-C8 (50 × 2 mm, 1.8 μm) MP-water/Methanol with 0.1% formic acid Gradient elution | ✓ | ✓ | | Ion suppression | Suppression ranged from −1% to 22% | | | ✓ | ✓ | |
| Clark et al. [75] | Metanephrines | Urine | LC-turbolonspray-triple quadrupole MS | Ultra II PFP propyl (50 × 2.1 mm, 1.7 μm) MP- 0.2% Formic acid in 5% Methanol. Isocratic gradient. | ✓ (SPE) | ✓ | | Ion suppression | 10-20% suppression just after solvent front, internal standard compensated for most of this. | | | ✓ | ✓ | |
| Kuhn et al. [76] | Amiodarone and desethylamiodarone | Plasma and serum | LC-Z-spray-ESI-Quattro Tandem MS | Phenomen × hydro RP (20 × 2 mm, 2.5 μm) MP-water/methanol with 0.1% formic acid and 2 mM ammonium acetate Gradient elution | | ✓ | | Ion suppression | Higher flow of NaCl solution resulted in higher suppression but internal standard was affected proportionally | | | ✓ | ✓ | |
| Moragues et al. [61] | β-agonists | Urine and Bovine liver | LC-ESI-Ion TrapMS | Hypersil Gold C18 (50 × 3 mm, 3 μm) 10 mM acetic acid/ACN gradient elution | ✓ (SPE and LLE) | | | Ion suppression | Reduction of ion suppression from 25-52% to 7-14% | | | ✓ | | |
| Ismaiel et al. [61] | Chlorheniramine | Plasma | LC-ESI-Micromass Quattro API Micro | ESI mode-Betasil Diol-100 (50 × 2.1 mm, 5 μm) 20/80 MeOH with 2mMammonium formate and ACN APCI mode-Zorbax-SB C18 (50 × 4.6 mm, 3.5 μm) 70/30/1 ACN/$H_2O$/formic acid | ✓ (LLE) | ✓ | | Ion suppression and enhancement | Reduced from 65% using ESI to 15% using APCI | | | ✓ | ✓ | |
| Kasprzyk-Hordern et al. [77] | Pharmaceutical and personal care products | Surface water | LC–ESI-Quattro Mirco triple stage quadrupole MS | ACQUITY UPLC BEH C18 (100 × 1 mm, 1.7 μm) MP A-79.5/20/0.5H2O/ MeOH/acetic acid and 10 mM TrBA MP B-5/94.5/0.5H2O/ MeOH/acetic acid and 10 mM TrBA | ✓ (SPE) | | ✓ | Ion suppression | Ion suppression ranged between ~10–80% | ✓ | | ✓ | ✓ | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chen et al. [55] | Estrogenic Steroids | Water | LC-ESI- Triple quadrupole | BetaBasic C18 (150 × 2.1 mm, 3 μm), A=ACN B=10 mM N-methylmorpholine, gradient elution | ✓ (SPE) | | | Ion suppression | Increase in recoveries by 1.5 - 4 fold after elimination | ✓ | | | ✓ | |
| Klawitter et al. [64] | Nucleotides | Tissue | LC/LC-ESI-MS (Agilent mass selective detector) | Zorbax C18 (50 × 4.6, 5 μm) +synergy hydro C18 (250 × 4.6, 3 μm) 95% 4 mmol/L dibuthylammonium formate/ 5% MeOH | | ✓ | | Ion suppression (also by nucleotides themselves) | Chromatographic separation eliminated nucleotide matrix effect | | | ✓ | ✓ | ✓ |
| Yang et al. [78] | Daunorubicin | Plasma | LC-Turbo ionspray-API 4000Triple quadrupole | BetaBasic Phenyl (50x2.1 mm, 3μm) A=25%ACN/75%water/ 0.1% formic acid B= 90% ACN/10% water/0.1% formic acid, gradient elution | ✓ (PP) | | | Ion suppression | Suppression of around 20% | | | ✓ | ✓ | |
| Wang et al. [8] | Matrix effect study on internal standards | Plasma | LC-Turbo ionspray-API 4000Triple quadrupole | – | ✓ (LLE) | ✓ | | Ion Suppression | Decrease in peak area ratios by 18.9% | ✓ | | | ✓ | |
| Zhang et al. [79] | Vancomycin | Serum | LC-ESI LCQ-Orbitrap Hybrid MS | ACE-3-C8 (50 × 3 mm, 3 μm) 90/10 0.1% formic acid and ACN | ✓ (SPE) | | | Slight Ion Enhancement | + 9-14% in response | | | ✓ | ✓ | |
| Johanson et al. [65] | Phos-phoinositide | Brain lipid | MALDI - TOF | - | ✓ (SCX column) | | | Ion Suppression | S/N x 2 after elimination | | | ✓ | ✓ | |
| Villagrasa et al. [9] | Benzoazinoid derivatives | Plant material | LC-ESI-MS (mass selective detector) | Synergi Max-RP C-12 (250 × 4.6 mm) Acidified $H_2O$ and MeOH, gradient elution | ✓ | | | Ion suppression | 1-17% lower without dilution (best method) | ✓ | ✓ | | ✓ | |
| Ismaiel et al. [59] | Hydrocodone and pseudoephedrine | Plasma | LC-ESI-Micromass Quattro API Micro | Betasil Diol-100 (50 × 2.1 mm, 5 μm) 2 mM $NH_3$ formate in MeOH and ACN, isocratic elution 20/80 | ✓ (LLE) | ✓ | | Ion suppression | Chromatographic separation eliminated phospholipid matrix effects | | | ✓ | ✓ | |
| Gros et al. [25] | Pharmaceuticals | Surface and waste waters | LC-ESI-Micromass Quattri triple quadrupole MS | Purospher Star RP-18 (125 × 2 mm, 5 μm) MeOH and water, gradient elution | ✓ (SPE) | | ✓ | Ion Suppression | Ranging from signal suppression of 15-60% for effluent waters and 40- > 60% for influent waters | ✓ | | ✓ | ✓ | |
| Naldi et al. [80] | Hisone proteins | Cancer cells | LC-ESI- ion trap MS | C4 jupiter column (150 × 2 mm, 5 μm) Water/HFBA/FA 100/ 0.04/0.4 and ACN/ HFBA/FA 100/0.04/0.4 isocratic elution, 30/70 | | | | Ion suppression | Ion suppression reduced by the combination of HFBA and FA added to the mobile phase | | | | | |
| Josefsson et al. [81] | Beta-agonists and beta-antagonists | Whole blood | LC-ESI-API 2000 triple quadrupole MS | Hypersil Polar-RP 150 × 3 mm) ACN/20 mM ammonium formate 10/ 90 for step 1 and 80/20 for step two, gradient elution | ✓ (PP and SPE) | | | Ion suppression | Matrix effects reduced by optimising chromatography and gradient program | | | ✓ | | |
| Carducci et al. [43] | Guanidinoacetate and creatinine | Blood | ESI-triple quadrupole MS | - | ✓ (LLE) | | ✓ | Ion suppression | Ion suppression decreased from 66 and 69% to -1.2 to 6.8% with I. S. for compounds | | | ✓ | ✓ | |

**Table 2** (*continued*)

| Author | Compound investigated | Matrix | MS | LC conditions | Evaluation of matrix effect | | | Matrix effect | Amount of matrix effect | Solution to matrix effect | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pre- and post-extraction | Post-column infusion | Matrix/solvent study | | | Dilution | Std Add | Extract-ion | Internal Standard | Column Switching |
| Li et al. [42] | Perchlorate | Water | LC-ESI-Quattro micro API triple quadrupole MS | Gemini C18 (150 × 2 mm, 5 μm) MeOH and 0.1% formic acid, Isocratic elution 10:90 | | | ✓ | Some suppression/ enhancement at low conc. | +/−20% matrix effect at concentration > 0.1 μg/L | ✓ | | | ✓ | |
| Lanckmans et al. [41] | Small molecules In microdialysis | Dialysis matrix | LC-ESI- Quattro triple quadrupole MS | Pepap C18 (15 × 75(300) mm, 5 μm) ACN/water A=2/98 B=80/20, gradient elution | | | ✓ | Ion suppression | 20–30% lower peak areas with ringers soln compared to water | | | | ✓ | ✓ |
| Saudan et al. [62] | Testosterone and epitestosterone conjugates | Urine | LC-ESI-Linear ion trap MS | Uptisphere ODB (10 × 4 mm, 5 μm) MeOH and 0.5% acetic acid in water, gradient elution | ✓ (SPE) | | | Ion suppression and enhancement | Percentage of Ion suppression/ enhance-ment ranged from 32% to −6% | | | ✓ | ✓ | |
| Vieno et al. [70] | Neutral and basic pharmaceuticals | Sewage and river water | LC-ESI- Micro triple Quadrupole MS | Zorbax XDB-C18 (2.1 × 12.5 mm, 5 μm) ACN/1% acetic acid, gradient elution | ✓ (SPE) | | | Ion suppression, significant at high ACN proportions and for sewage water | Less than 8% for surface and over 40% for sewage treatment plant effluents | | | ✓ | | |
| Mezcua et al. [45] | Pesticides | Water | UPLC-ESI-micromass Quattro Premier MS | Acquity BEH C18 (100 × 2.1, 1.7 μm) 0.1% Formic acid and ACN, gradient elution | | | ✓ | Ion Suppression and enhancement | Variations in calibration slopes in the range of 80-120% compared to pure solvent | | | ✓ | | |
| Guan et al. [44] | Anabolic Steroids | Plasma | LC-ESI or APCI-Quantum triple stage quadrupole MS | Ace C8 (2.1 × 50 mm, 5 μm) Formate buffer and methanol, gradient elution | | | ✓ | Mostly ion enhancement | +/− 12% ion suppression/ enhancement | | | ✓ | | |
| Stoob et al. [82] | Sulfonimide antibiotics and neutral and acidic pesticides | water | LC-ESI-TSQ quantum triple quadrupole MS | Nucleodur C18 gravity (125 × 2 mm, 3 μm) Water, formic acid/ MeOH, formic acid/ MeOH/water, ammonium acetate, Gradient Elution | ✓ (on-line SPE) | | | Mostly ion suppression | Generally ion suppression of less than 25% | | | ✓ | ✓ | |
| Kintz et al. [83] | Benzodiazepines and hypnotics | Oral fluid | LC-ESI-micromass Quattro Micro tandem MS | XTerra MS C18 (100 × 2.1 mm, 3.5 μm) ACN/0.1% formic acid, gradient elution | | | ✓ | Ion suppression | Less than 10% | | | ✓ | ✓ | |
| Wood et al. [37] | Illicit drugs | Oral fluid | LC-ESI-Quattro Ultima tandem MS | XTerra MS C18 (2.1 × 150 mm, 3.5 μm) 10 mM ammonium bicarbonate and MeOH, gradient elution | ✓ (SPE) | ✓ | ✓ | Ion suppression and enhancement | After SPE < 13% suppression and < 12% enhancement | | | ✓ | ✓ | |
| | | plasma | | | | | ✓ | | Ion suppression of about 14% | ✓ | | ✓ | | |

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| John et al. [84] | Antimicrobial human peptide | | LC-ESI-LCQ Ion trap MS | Jupiter C18 (150 × 1 mm, 5 μm) 0.06% TFA and 80%ACN/ 0.05TFA, gradient elution | | | Ion suppression | | | | | |
| Larger et al. [2] | Unnamed | Plasma | LC-ESI-API 3000 triple quadrupole MS | Zorbax SB-C8 (4.6 × 75, 4.6 × 150, 3.5 μm) and symmetry C8 (4.6 × 50 mm, 3.5 μm) 10 mM Ammonium formate and ACN, various Gradient elutions | ✓ | | Ion suppression | Study on ion suppression with isocratic and different elution gradients and also testing for suppression with dilution test | ✓ | | | |
| Salm et al. [34] | Cyclosporin | Whole blood | LC-ESI-API III triple quadrupole MS | Zorbax Bonus C18 (50 × 2.1 mm, 5 μm) MeOH/40 mM ammonium acetate, isocratic elution 80/20 | | ✓ | Ion suppression | Less than 8% of total signal | | ✓ | ✓ | |
| Fiori et al. [46] | Beta agonists | Urine | LC-MSD TRAP SL | MS C8 × Terra Waters (2.1 × 150 mm, 5 μm) Acetic Acid and Methanol, Gradient elution | | ✓ | Total matrix interferences | Matrix interferences higher for SPE ~0.5% than with molecular imprinted polymers (MIPS) ~0.2% | | ✓ | | |
| Lindberg et al. [50] | Antibiotic substances | Sewage water | LC-ESI-Ion Trap MS | YMC hydrosphere C18 (150 × 4.6 mm, 5 μm) Water and ACN with 0.1% formic acid, gradient elution | ✓ | | Ion suppression | Improvement of RSDs ( < 10%) using I.S. to overcome suppression | | ✓ | ✓ | |
| Hendrickson et al. [47] | Methamphetamine and amphetamine | Rat serum | LC-Zspray interface-Quattro LC triple quadrupole MS | Hypersil BDS C18 (100 × 2.1 mm, 3 μm) 10mM Ammonium acetate buffer pH 3.7 with 25% ACN and 2.5% MeOH | | ✓ | Ion suppression | Suppression for Methamphetamine: 44–63% Suppression for smphetamine: 39–52% | | ✓ | ✓ | |
| Mortier et al. [13] | Paclitaxel | Plasma | LC-APCI-API 2000 triple quadrupole MS | Phenomenex synergi Max RP (2 × 75 mm, 4 μm) MeOH and water with 0.5 mM acetic acid *A* = 70/30 (standard) *B* = 85/15 (fast), 2 isocratic methods | | ✓ | Ion suppression | Reduction of ion suppression by reducing the chromatographic analysis time and allowing simultaneous elution of std. and internal std. | | ✓ | ✓ | |
| Timperio et al. [66] | Proteins | Grana and stroma thylakoid membrane | LC-ESI-Ion trap MS | Vydac protein C4 (250 mM × 1 mM, 5 μm) ACN/water with 0.05% TFA, gradient elution | | | No ion suppression | All phospholipids removed with this method and therefore removal of all matrix effects | | ✓ | | |
| Freitas et al. [12] | Herbicides and metabolites | Natural Waters | LC-ESI-TSQ Quantum 2000 triple quadrupole MS | Nucleodur C18 gravity (125 × 2 mm, 3 μm) and gromsil polymer coated C18 (150 × 2 mm, 3 μm) Water and MeOH with 0.1% formic acid | | ✓ | Ion Suppression | Up to 71%, 59%, 42% suppression for waste, lake, and creek water samples | ✓ | ✓ | ✓ | |
| Chen et al. [49] | A M2 muscarinic receptor antagonist | Human plasma | 1.API 3000 triple quadrupole system with NanoMate 100 Nano-electrospray system | Betasil C18 (2 × 100 mm) Water and 50/50 MeOH/ACN with 0.2% formic acid, gradient elution | | ✓ | Ion suppression | Ion suppression was found to be 15% | | ✓ | ✓ | |

**Table 2** (*continued*)

| Author | Compound investigated | Matrix | MS | LC conditions | Evaluation of matrix effect | | | Matrix effect | Amount of matrix effect | Solution to matrix effect | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pre- and post-extraction | Post-column infusion | Matrix/solvent study | | | Dilution | Std Add | Extract-ion | Internal Standard | Column Switching |
| | | | 2.LC-Turbo ionspray API triple quadrupole | | | | | | | | | | | |
| Chin et al. [7] | Olanzapine and desmethyl olanzapine | Human serum | LC-turbo ionsource- PE Sciex API 3000 MS | Phenomenex LUNA phenyl hexyl (2 × 150 mm, 5 μm) ACN/ammonium acetate and 2% formic acid/ACN, gradient elution | | ✓ | ✓ | Ion suppression | Ion suppression accounted for 20–90% of the reduction of response of the analytes of interest | | | ✓ | ✓ | |
| Petrovic et al. [68] | Alkylphenolic compounds and sex hormones | Sediment samples | LC-LC-ESI-LC-MSD HP 1100 Mass Selective detector | LiChrospher ADS (25 × 4 mm, 25 μm) and LiChrospher 100 RP-18 (250 × 4 mm, 5 μm) Water/ACN, gradient elution | ✓ (On-line RAM (restricted access material)) | | | Ion suppression | Ion suppression: 8.3% Uses dual columns and RAM (online clean-up) to reduce ion suppression | | | ✓ | ✓ | ✓ |
| Muller et al. [58] | Codeine and glafenine | serum | LC-ESI- API 365 triple quadrupole MS | Phenomenex synergy polar-RP phenyl-propyl (150 × 2 mm, 4 μm) A=1mM ammonium formate-0.1%formic acid B=ACN, gradient elution | | ✓ | | Ion suppression | Compares different extraction methods on removal of ion suppression with LLE and SPE proving the favourable | | | ✓ | | |
| Qu et al. [67] | Scutellarin | Erigeron Bre-viscapus extract | LC-turbo ionspray-API 3000 triple quadrupole MS | Megachem C18 (50 × 2.1 mm, 5 μm) Water/ACN (20/80), isocratic elution | | | ✓ | Ion suppression | Uses a 1000-fold dilution and increase in temp of ion source to improve ion suppression. Improvement from 20–30% to 1–5% in signal suppression | ✓ | | ✓ | | |
| Ternes et al. [71] | Neutral pharmaceuticals | Waste water | LC-ESI-API 365 triple quadrupole MS | Merck LiChrospher 100 RP-18 (125 × 3 mm, 5 μm) 20 mM ammonium acetate in water/ACN | | | | Ion suppression | Matrix effects were found to reduce the sorption efficiencies of extraction materials and/or cause ion suppression Spiked stds before column to elucidate between the two. | | | ✓ | ✓ | |
| Majumdar et al. [85] | Cholesterol lowering reagent | Human Plasma | LC-ESI-TSQ 700 triple quadrupole MS | Asahipak ODP microbore (50 × 1 mm, 5 μm) MeOH/water/5 M ammonium hydroxide (60/55/5), isocratic elution | | | | Ion suppression | Adding a hydrolysis step followed by automated SPE during sample preparation was critical for cleanness of the extract and minimized the matrix mediated ion suppression | | | ✓ | ✓ | |
| Kitamura et al. [48] | 3-C-ethynylcytidine | Rat plasma | LC-ESI-Finnigan TSQ 7000 triple quadrupole MS | Inertsil ODS-2 (150 × 2.1 mm, 5 μm) MeOH/Acetic Acid (25/75), isocratic elution | | | ✓ | Ion suppression | Ion suppression of 78% std. dev. improved by manipulating chromatography to allow I.S. to elute simultaneously with analyte | ✓ | | ✓ | ✓ | |
| Bruins et al. [22] | Clenbuterol | Urine | APiI-Nermag R 3010 triple quadrupole MS | – | | | ✓ | Ion suppression | Compared APCI to ESI for ion suppression, results were 10 and 40% respectively | | | ✓ | | |

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|---|---|---|---|---|---|---|---|---|---|---|
| Buhrman et al. [18] | Unknown compound SR 27417 | Human Plasma | LC-ESI-Sciex API III Plus MS | Keystone BDS Hypersil C18 (100 × 2 mm, 5 μm) 2 mM ammonium acetate with 0.2% formic acid in water and 2 mM ammonium acetate with 0.2% formic acid in ACN, gradient elution | | ✓ | Ion suppression | Compared 3 extraction methods to see which gave the least ion suppression, Back extraction gave the best with 0% suppression, followed by LLE with 26% and SPE with 41% | ✓ | ✓ |

analysis. To achieve this, good sample preparation methods are required which involve more effective extraction and clean-up to prevent the co-extraction of interfering components from the matrix. The most common extraction procedures are solid-phase extraction (SPE), liquid–liquid extraction (LLE) and protein precipitation (PP). These techniques can be done individually or combined to allow for more efficient extraction and sample purification. A compromise between interference removal and good recoveries can often be an issue but sample extraction and clean-up may be joined with other methods to effectively reduce ion suppression. These techniques can be performed individually or in tandem depending on how 'dirty' the sample is. The disadvantage with this solution is that a lot of time is required to develop and optimise clean-up methods and there is an increased risk of poor analyte recoveries and also a risk of *adding* interferents to the sample during the extraction/clean-up process. Salm et al. reported very low levels of ion suppression ($<8\%$) when effective sample clean-up was combined with the use of a labelled internal standard in the analysis of cyclosporine in whole blood samples [35].

#### 5.2.1. Liquid–liquid extraction

LLE can be effective in obtaining high extractability of analytes and removal of matrix components. However, it comes with many disadvantages including large solvent consumption, time consuming, the need for an evaporation step to remove excess solvent, and highly polar and ionic compounds can be difficult to extract with this technique. It also lacks the specificity that is associated with SPE and there is a risk that some samples may form emulsions which can be difficult to separate.

#### 5.2.2. Solid-phase extraction

SPE can provide clean extracts, high recoveries and can be automated using on-line methods to speed up sample preparation time. However, method development using SPE can often be long and complicated with many steps to optimise. The wash step in SPE should remove most of the matrix interferences. A major drawback with using SPE as a sample purification method is that SPE depends on large differences in chromatographic behaviour for matrix removal, i.e. matrix components that remain after SPE clean-up typically have similar chromatographic behaviour to that of the analyte.

As a result, these interferences are likely to co-elute with the analyte during LC–MS analysis and continue to cause ionisation suppression [35]. Benijits et al. found that using a stepwise wash, whereby the organic content was incrementally adjusted, rather than a one-step low organic wash, proved to significantly reduce matrix effects (reduced ion suppression from approximately 30% to 11%) for the analysis of endocrine disrupting chemicals [28]. An additional benefit of SPE is the ability to concentrate a sample during the elution step. However, there is a danger that the matrix interferences will also be concentrated, and it is therefore important to include sufficient wash steps in a SPE method or optimise the elution solvent to reduce the amount of co-eluting matrix components. While conducting a study on the presence of illicit drugs in oral fluid, Wood et al. investigated ion suppression caused by the sample collection system (the system in which the sample was collected, stored and/or preserved, e.g. a sample tube containing EDTA), which contained stabilizing salts, non-ionic surfactants and antibacterial agents [37]. The study found that these agents caused extensive ion suppression in samples although the samples did not go through a clean-up procedure. Protein precipitation resulted in ion suppression of 50–70%, while SPE provided the most effective clean-up of samples resulting in low levels of ion suppression at 10–15% [37].

#### 5.2.3. Protein precipitation

PP is used as a technique in biological analysis and can eliminate up to 98% of protein contained in blood samples [38], which is a major source of ion suppression. The disadvantages with this technique include incomplete precipitation, low recoveries of target analyte due to entrapment in the protein precipitate and adsorption of the analyte onto proteins [38]. PP is often used as a pre-treatment step before SPE, which PP might not be effective alone at removing all sample protein. The SPE step then allows for further extraction and concentration of the analyte(s) of interest.

### 5.3. Modifying liquid chromatography conditions

Changing the chromatographic conditions can have a significant effect on ion suppression by eliminating endogenous sample compounds that compromise the ionisation efficiency of analytes under investigation [31]. Varying column chemistry, length, diameter and particle size, flow rate, mobile phase composition and temperature can improve retention and/or separation of analytes from matrix components. Endogenous compounds that cause matrix effects are often not detected with mass spectrometry (unknown and/or different *m/z*) but may be retained on the column. Post-column infusion experiments will aid in determining where these components elute and the chromatography can be adjusted to resolve these interferences from the analyte(s). The two areas of the chromatographic run that are most commonly affected by interferences, and therefore ion suppression, are (i) the solvent front, where highly polar and unretained compounds elute and (ii) the end of the elution gradient, where the strongly retained compounds are eluted [3]. This can be very significant, causing inaccurate quantiation in circumstances where an internal standard is being used but does not have the same retention time as the corresponding analyte. If the chromatographic profile shows that ion suppression occurs near an analytes retention time but does not affect its response, it may still affect an internal standard that does not co-elute and thus affect the standardised ratio for the analyte. The best way to avoid this is to adjust the mobile phase or gradient so that the analyte and internal standard co-elute [13]. While this may be easy to accomplish in a single compound analysis, it becomes more difficult in multi-analyte analysis and sometimes it may be impossible to remove all analytes from ion suppression regions of the chromatographic run. As a method for reducing ion suppression this option has the advantage that there is no need to change the rest of the analytical procedure (sample preparation and mass spectrometry parameters). However, disadvantages may include longer analysis time, wider peaks and lower sensitivity.

Nano-LC has been found to reduce matrix effects. In a study by Gangl et al. [36], reducing the LC flow rate entering the ESI source to the nano-litre per minute range led to increased desolvation, ionisation and ion transfer efficiency compared with conventional LC that is carried out at much higher flow rates. Using the nL/min flow rate, it is believed that the smaller droplet size of the nanospray forces the target molecules more effectively to the droplet surface leading to enhanced ionisation. The higher surface-to-volume ratios of the smaller nano-ESI droplets results in higher sensitivity and resistance to ion suppression effects with a significant improvement observed when the flow was reduced from 200 μL/min to sub-microliter/min rates. Schneider et al. showed that 'total solvent consumption' can reduce matrix effects by using a low flow rate and heat to remove all solvent from the spray to improve ionisation [39]. However, this can be unsuitable for routine analysis as sufficient reproducibility may be difficult to achieve with such low volumes [40].

### 5.4. Calibration

#### 5.4.1. Matrix-matched calibration

When ion suppression cannot be eliminated, different calibration approaches can be applied to compensate for matrix effects. Matrix-matched calibration is the most commonly used technique to counter ion suppression as well as allowing the measurement of matrix effects by comparing the calibration set prepared in pure solvent [22,25,35,37,40–47]. In fact, this has become a basic validation requirement for analytical methods. Different concentrations of analyte are spiked into a blank matrix and linear calibrations are performed for each analyte. The biggest obstacle with this approach is that it can be difficult to obtain a blank matrix that is completely free from the analytes under investigation. The blank matrix must also be of similar composition to the samples or it will fail to sufficiently compensate for the ion suppression. In addition, the blank matrix must remain constant throughout the set of In addition the blank matrix must be constant throughout the set of spiked standards, controls and samples so as to ensure accurate quantiation for all samples and controls[16]. This can be difficult to achieve as the type and concentration of interfering components can vary between samples [2]. Matrix-match calibrations are often used in combination with an internal standard to better compensate for ion suppression [7,18,41,42,48,49]. Table 2 outlines methods that used a matrix/solvent study for evaluating ion suppression.

#### 5.4.2. Standard addition

This is the most suitable method for compensating for ion suppression effects as it may be applied to samples with variable matrices. In the standard addition calibration model, a sample is spiked with known concentrations of standard and a graph is prepared from the standard enriched sample that is extrapolated back to the *x*-axis to give the actual concentration in the sample (concentration obtained from the negative *x*-axis). The downside of this method is that it is time consuming, laborious and multiple spiked standards have to be run for each sample.

Ito et al. [26] carried out a study using standard addition to compensate for ion suppression in shellfish matrices. In their study, the sample extract was analysed and a known quantity of standard solution was added. The concentration of the sample was calculated using the formula:

$$X = SI_x/(I_s - I_x)$$

where $X$ is the amount of analyte in the extract solution, $S$ is the amount of analyte spiked into the sample, $I_x$ is the signal intensity of analyte in the extract solution, and $I_s$ is the signal intensity of the analyte in spiked extract. In this case, there are two analyses of the one extract, which is a minimum requirement for standard addition [26]. A larger number of runs would be more accurate for quantifying analyte concentration. Despite this, the method was found to be able to correct for the ion suppression effect caused by interfering

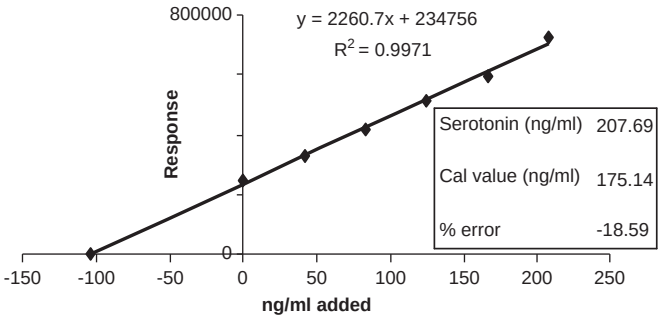


**Fig. 4.** Standard addition experiment for serotonin comparing result to value obtained from calibration curve (authors' own work).

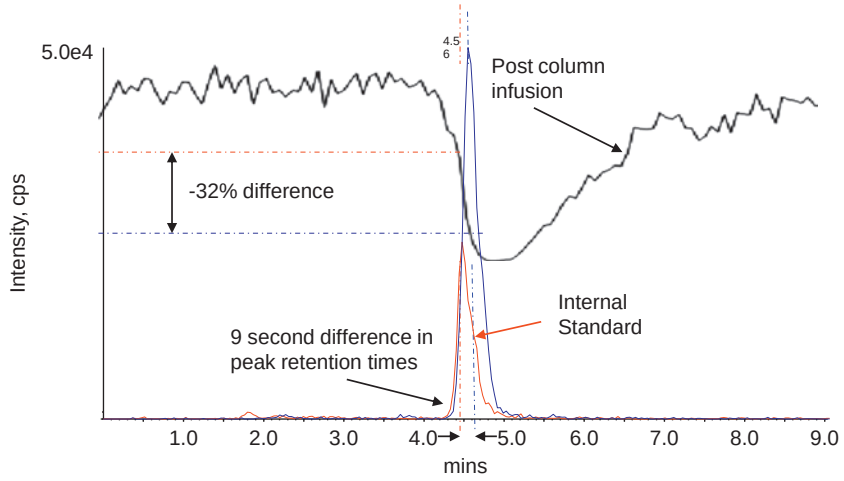


**Fig. 5.** Illustration of when a standard and internal standard are effected by different levels of ion suppression. This shows a 32% difference in signal response over a 9 second period of analysis (authors' own work).

matrix components and ensure accurate quantitation. Table 1 shows data from the experiment carried out by Ito et al.

Fig. 4 shows an example of a standard addition experiment with a serum sample. The results demonstrate that the standard addition method gave a higher concentration value for serotonin than for the matrix-matched calibration curve. In addition, a significant error (18.6%) was observed between the two values, which is most likely caused by ion suppression from the serum sample. When using the standard addition approach, the matrix is identical for each injection and therefore the ion suppression effect is also identical. As a result it is an extremely effective way of determining the true concentration of an analyte.

### 5.4.3. *Internal standard*

Internal standards are widely used in analytical methods and are used to normalise the response of the analyte signal to that of the internal standard response. The internal standard can be deployed during sample preparation or prior to, sample injection into the LC–MS. Internal standards reduce the effect of ion suppression during analysis because any matrix components co-eluting with the analyte will be identical for the internal standard, allowing the analyte-to-internal standard response ratio to compensate for any ion suppression that may be present. This provides for a more accurate, rugged and precise method.

In its most widely applied form, a known concentration and volume of an internal standard is added to each standard and sample preparation. The internal standard can be an isotopically labelled compound, a structural analogue or another compound that is similar to the analyte under investigation. The ratio of the sample response to the internal standard response is independent of the sample volume injected and instrument sensitivity and thus can provide reliable quantification. When using an internal standard to compensate for matrix effects it must have ionisation properties and retention time similar to the analyte. The best internal standard for LC–MS are stable isotope-labelled analogues of the analyte which are structurally very similar to the analyte. Such internal standards will behave almost identically to the analyte during sample preparation, chromatographic separation and MS ionisation. A major drawback of isotopically labelled internal standards is their high cost, which can escalate in the case of multi-analyte methods where many internal standards are required. In addition, isotopically labelled internal standards can often be difficult to obtain and/or synthesise, there can be a lack of confidence in the isotopic purity, and the isotopic integrity may be compromised due to exchange of the isotopic label during sample preparation [10,14]. It is also possible to encounter "cross-contamination" between the MS/MS transitions used for monitoring the analytes and internal standards. This is most often observed when there is only a 1 Da difference between the internal standard and the analyte, and they fragment to form the same product ion.

Wang et al. [8] found that deuterated internal standards may sometimes show unexpected behaviour which can affect the accurate measurement of analyte concentration. A suggested explanation for these undesired results was the partial resolution of the internal standard from the analyte during HPLC separation, and this is thought to be caused by the presence of deuterium on the molecule. The displacement alters the lipophilicity of the compound to a small degree which causes the retention time of the internal standard to be slightly different to that of the analyte. However, this is only of concern if ion suppression occurs near the elution time of either the analyte or internal standard [50].

Fig. 5 illustrates how the use of an internal standard does not always improve analysis by reducing ion suppression. Here the region of ion suppression affects the standard more than the internal standard, and there is a 32% drop in signal response in a 9 s period. To overcome this problem, the mobile phase gradient should be optimised so that the analyte and internal standard co-elute outside the ion suppression region.

In this approach an internal standard is required for every analyte because during chromatographic separation each analyte will have a different and unknown response [9]. The extent of compensation of ion suppression when using an internal standard can be seen by comparing the calibration curves of solvent with that of the spiked matrix. If the slopes overlap then ion suppression effect is negligible. If not, then re-evaluation of the method is required [25].

Stuber et al. described a method for using the analyte itself as an internal standard—the echo-peak technique [16]. This is an internal standard technique that is applied when no isotopically labelled standard is available [51]. In the echo-peak technique, the standard (labelled as reference) and sample solutions are injected sequentially during one chromatographic run with short time lags between their injections (≈30 s). The aim is to have the two peaks (standard and sample) completely resolved but eluting close to each other so that they undergo similar matrix effects. Fig. 6 shows chromatograms by Zrostlikova et al. showing the use of this technique.

The echo-peak technique will only be effective if the retention times of the standard and sample are close enough to be similarly affected by the matrix components. Since the two peaks need to be

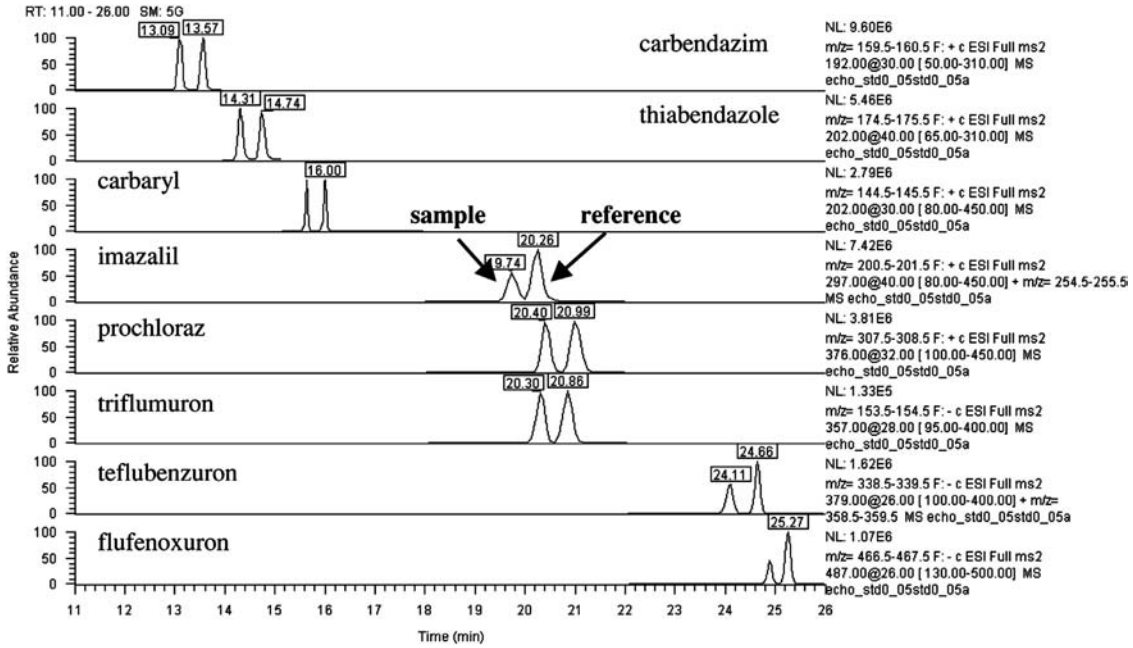


**Fig. 6.** LC–MS chromatogram of pesticide mixture—application of echo-peak technique. First peak, sample: standard of pesticides (0.5 mg/mL) in methanol–water (1:1, v/v). Second peak, reference: standard of pesticides (0.5 mg/mL) in methanol–water (1:1, v/v) [51].

well separated from each other and from other analytes in the run this method can prove difficult to develop in a multi-analyte analysis [51].

### 5.5. *Influence of mobile phase additives*

Using low concentrations of additives in mobile phase has been shown to reduce the effect of ion suppression by improving the ionisation efficiency. This however is based on the concentration of the additive as well as the chromatographic separation of the matrix components [20]. Common mobile phase additives include trifluoroacetic acid (TFA), formic acid, acetic acid, ammonium hydroxide, ammonium acetate and ammonium formate. The concentration of additives is an important parameter to optimise to improve ionisation efficiency while too high a concentration may cause suppression of analyte signal. It is recommended to use the lowest possible concentration of additive to achieve efficient ionisation of the analyte [20].

Gustavsson et al. carried out a study on the effect of mobile phase additives on the signal response of verapamil (a pharmaceutical compound used for the treatment of conditions such as hypertension and cardiacarrhythmia). The addition of formic acid to the mobile phase produced a large increase in signal response compared to the use of TFA ( > 60% decrease), heptafluorobutanoic acid and perfluoroheptanoic acid (between 15 and 30% for both). This shows that by using formic acid the ionisation efficiency of the analytes in the sample matrix increased [52]. TFA has been chosen in the past as a mobile phase additive due to its good ion-pairing and solvating characteristics but is being used less with mass spectrometric methods at present as it often results in spray instability and analyte signal reduction [21]. Temesi et al. [21] found that TFA was the least effective additive when used with ESI, while formic acid was found to be the best. In addition, TFA and other ion-pairing agents can cause ion suppression for both basic and acidic compounds, while formic acid, ammonium hydroxide and acetic acid have been shown to increase the response for the majority of compounds at low concentrations [30]. Marwah et al. [53] investigated the effect of TFA and other mobile phase additives on the sensitivity of steroids. They believed that the oxygen atom present in the ester functional group acts as a substrate for the proton. They determined that the analyte response increased several fold in the following order: formic acid (50–200 ppm, v/v) > acetic acid (100–500 ppm) > TFA (5–20 ppm). A phosphate buffer can also be used to improve chromatographic performance but is not amenable to MS analysis as it is prone to causing ion suppression and is not volatile enough to evaporate. In addition, it frequently results in salt precipitation that may block the ion source, resulting in the necessity of frequent dismantling and cleaning, and it may hinder the instrument's ability to perform long uninterrupted sequences of sample analysis.

Yamaguchi et al. [15] suggested that the ineffectiveness of conventional modifiers in improving ion suppression could be due to the high volatility of organic solvents. These solvents are more volatile than water and will evaporate from the droplet prior to reaching the critical point (Rayleigh stability limit), resulting in the formation of droplets containing water and buffer, which can lead to ion suppression. Addition of surface tension lowering modifiers can be added post-column to the mobile phase to allow buffer and water to evaporate prior to reaching the Rayleigh stability limit, which could also reduce or eliminate ion suppression [15]. Yamaguchi et al. [15] found that post-column infusion of 2-(2-methoxyethoxy) ethanol, in addition to using a buffer in the mobile phase, increased signal response up to 100-fold for urinary metabolites by lowering the surface tension of the mobile phase to encourage fine droplet formation and reduce ion suppression.

A study carried out by Benjits et al. [28] looked at two acids, formic and acetic acid at two concentrations (0.01 and 0.1%, v/v), and two bases, ammonium formate and ammonium acetate at two concentrations (1 and 5 mM for bases), as additives for analysing endocrine disrupting chemicals in water in positive and negative ESI mode. They found that, for the most part, both acids showed significant analyte signal suppression at both concentrations and in both positive and negative ion modes with recoveries of < 65% being observed. They reported that increasing concentrations of mobile phase additives showed a suppressive effect on analyte signal intensity in standard and matrix analysis. The analysis of estriol, using 1 mM ammonium acetate gave a signal enhancement of 172% but suppression occurred when the concentration was increased to 5 mM. The study found that ammonium formate at 1 mM was the most suitable additive for both positive and

negative mode as it gave the highest analyte recovery and induced the least ion suppression of all the buffers studied.

### 5.6. Dilution

Sample dilution is a very simple and straightforward method to reduce the amount of coexisting matrix components in a sample [55]. Diluting the sample increases the ionisation efficiency of the analytes by reducing the concentration of any components that may have signal suppressing properties and thereby reducing competition for ionisation. Its main disadvantage is that it will increase the limit of detection [2,9,42,55,56]. However, it can be an effective solution if there is a lack of matrix-matched materials or isotopically labelled standards available [25]. The amount by which a sample is diluted should be optimised to find the point where suppression is reduced to an acceptable level while still maintaining a good signal response, although this will depend on the type of matrix and the concentration of analyte present in the sample. Villagrasa et al. [9] found the optimum dilution of plant extract where ion suppression had been sufficiently reduced, by comparing the signal of a standard solution to that of a spiked matrix. Where the response of the spiked matrix matched the response of the standard solution, the optimum dilution was found. Fig. 7 shows an example where ion suppression is present during analysis. After a 10-fold dilution of the sample a peak is clearly observed. In this example the matrix effects are almost eliminated though sample dilution. Separatedly, Stahnke et al. carried out a dilution study where a reduction in matrix effects were measured at 10 levels of dilution up to 1000 fold [57]. They found that when analysing pesticides present in QuEChERS extracts, dilution by a factor of 25–40 reduces ion suppression to less than 20% if the initial ion suppression is $<80\%$. Higher dilutions (100-fold) were required for stronger ion suppression samples matrix effects but this is seen by the authors as a simpler than the alternative approach which would require the removal of 99% of extracted matrix sample constituents.

Mulder et al. [56] found that when analysing nitrofurazone and azodicarbonamide in bread, very little ion suppression was observed using a large dilution ($>200$ fold). However, using reduced sample

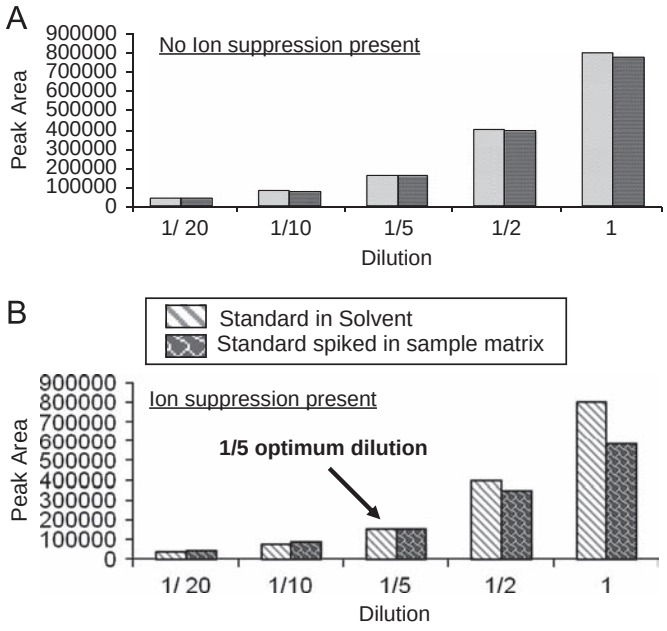


**Fig. 7.** Bar chart showing the effect of dilution on the analysis of a sample (A) without ion suppression and (B) with ion suppression. A 1/5 dilution was found to be the optimum dilution to remove ion suppression effects (authors' own work).

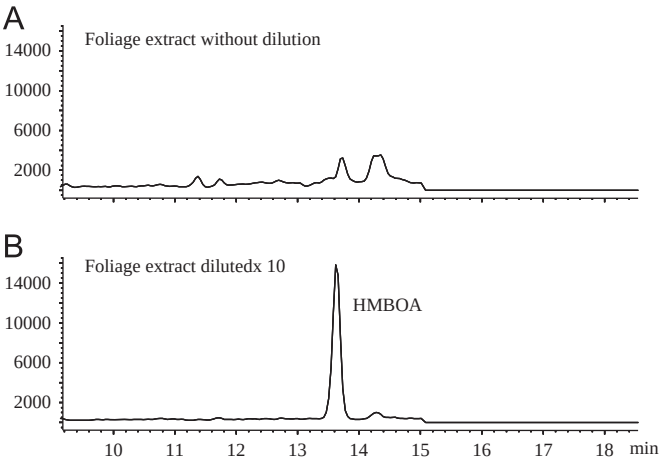


**Fig. 8.** LC–ESI–MS ($m/z$ 194) chromatogram obtained for: (A) a foliage extract without dilution and (B) the same extract diluted 10 times [9].

dilution (100–200 fold) resulted in strong ion suppression effect and recoveries dropped from 80 to 8%. For more concentrated extracts a 50-fold dilution could be used for fresh meat and baby food as these matrices were found to be less susceptible to ion suppression. Similarly, Gros et al. found that a change in dilution from 1:2 to 1:8 for surface and waste water samples, respectively, was sufficient to reduce or eliminate ion suppression [25]. Larger et al. also described a dilution study for formulation agents in plasma samples which corroborates the previous findings: dilution often reduces ion suppression effects [2]. This study resulted in the dilution method being used routinely as acceptance criteria for identifying the presence of ion suppression for new methods with minimum additional work required and no delay in analysis time Fig. 8

## 6. Types of matrices and applications

As stated previously, the amount of ion suppression observed when analysing a sample will be dependent on the type of matrix. Different components in different matrices will effect ionisation in various ways. Even when dealing with one type of matrix, for example blood, different levels of matrix effects can be seen from sample to sample. In fact, the matrix of a blood samples from the *same* subject can change over time as concentration of blood components change e.g. salt//mineral levels [31,11]. Table 2(A–G) shows a collection of different methods that deal with ion suppression. It also describes the parameters, evaluation and solutions applied to overcome the effects of ion suppression.

### 6.1. Blood

Whole blood consists mostly of water but also contains dissolved proteins, mineral ions, glucose, hormones, platelets and cells. Plasma or serum will also contain dissolved proteins and other materials which can be involved in charge transfer processes in the gas phase at the ionisation interface between the LC and the MS [57]. In blood and serum samples it would be almost impossible to remove all potential ion suppressing compounds from the matrix. Therefore for such samples, it is very important to identify regions of the chromatogram where ion suppression is taking place and ensure that the target analyte is well resolved from these regions.

Phospholipids have been shown to cause high amounts of matrix effects [2,33,58]. Phospholipids are the main component of cell membranes, and the most significant types found in plasma are glycerophosphocholines and phosphatidylcholine. Ismaiel et al. [59] investigated the effect of several biological extracts on the elution time of glycerophosphocholines to determine the effect of repeated

injections on analytical performance. They commented that "monitoring phospholipids may provide a means to ensure the avoidance of matrix effects in each individual sample and may provide a more practical tool for avoiding matrix effects than commonly used post extraction addition and post column infusion". In another study Ismaiel et al. [59] confirmed that ion suppression and enhancement coincided with the elution profiles of phospholipids in the analysis of chlorpheniramine in plasma. They also showed in their study that matrix effects were reduced by 75% using an APCI source. Larger et al. [2] have commented that in some cases spiking blank plasma may not completely compensate for ion suppression effects observed in real samples. This can be seen in examples where ion suppression is observed from the sample taken from the animal being tested but not observed in the control. They have suggested a solution may be to obtain plasma from animals being tested and use these real samples with a "dose addition", however this approach would have limited practicality.

In 2012, Ghosh et al. reinforced common beliefs that different source geometries resulted in ion suppression from different types of phospholipids in biofluids. This was due to different phospholipids being ionised by the different source designs caused by variations in parameters such as capillary diameter, distance from capillary tip to the counter electrode and radius of the droplets between the two designs [33].

### 6.2. Urine

Urine contains highly concentrated endogenous compounds like creatinine and enterolactone which have been shown to cause ion suppression in the analysis of hormonal residues [3]. Moragues et al. [60] developed a method for the analysis of β-agonists in urine and animal liver using a two-step extraction to reduce ion suppression. They started with an SPE extraction including a hexane wash and followed with by LLE using *tert*-butyl methyl ether (TBME). The hexane wash removed key matrix components such as fatty acids and other lipophilic components related to ion suppression and the TBME LLE allowed for a more selective extraction of the analytes. This protocol was also applied successfully to liver samples with relatively similar drops in ion suppression. Saudan et al. [61] used SPE for the clean-up of urine samples for the analysis of testosterone and epitestosterone conjugate. They also used deuterated internal standards to correct for the presence of ion suppression and enhancement. The matrix effects varied from 32% of suppression to 6% enhancement but the use of an internal standard did improve the method significantly.

Johnson et al. [63] found that using an ultra-performanace liquid chromatography (UPLC) system (500 μL/min) with an ESI source for the analysis of a dilute sample of acetaminophen provided reduced ion suppression when compared with a HPLC system (2 mL/min) fitted with an ESI interface. The UPLC produced sharp peaks and increased the ionisation efficiency of the analytes. Fiori et al. [46] compared two clean-up methods for their ability to minimise ion suppression when using a LC-ion trap mass spectrometry for the analysis of β-agonists in urine. The method used unendcapped C18 SPE and molecular imprinted polymer (MIP) columns for the clean-up of the urine samples. The MIP columns were successful in reducing ion suppression to below 10% while maintaining recovery and sensitivity.

### 6.3. Animal tissue

There are many components in animal tissue that can have ion suppression properties, including salts, fats, proteins and cells. Animal tissue nucleotides, which are a precursor for RNA synthesis, may also be a major cause of ion suppression. Nucleotide peaks will have to be separated so as not to interfere with each other's ionisation capacity as carried out by Klawitter et al. [64]. This method also used column switching together with internal standards to reduce matrix effects on the analysis. Johanson et al. [65] developed a method for the analysis of phosphoinositide in brain lipids using MALDI-TOF MS. They found that the removal of phosphatidylcholine from lipid extracts by using a SCX column for sample clean-up provides reduced levels of ion suppression during analysis. This process resulted in a two-fold signal-to-noise improvement compared to that of the original value after using the post extraction method.

### 6.4. Microdialysis Samples

Microdialysis samples are often considered relatively clean as they are highly filtered and protein free, but they can contain a large amount of salts and complex mixtures of small molecules which can interfere with the ionisation process and cause ion suppression [41]. Lanckmans et al. overcame these issues with the use of internal standards for correcting variations in signal response of analytes and column switching for the desalting and pre-concentration of samples (this was not completely effective when injection volume exceeded 1 μL). They also commented that they observed 20–30% lower peak areas when using Ringers solution (a salt solution used as perfusion fluid) when compared with water. A matrix-matched calibration curve was required for accurate quantitation due to the ion suppression effects [41].

### 6.5. Plant tissue and sediment samples

Plant tissue contains many components that may cause ion suppression; these include proteins, cells and lipids. Villagrasa et al. [9] found that the analysis of benzoazinoid derivatives in plant material can be affected by ion suppression within the range of 3–72%. They compared standard addition, use of an internal standard and a dilution method to evaluate the most effective way to reduce ion suppression. They found that dilution gave the best results in counteracting ion suppression. Timpero et al. [66] reported that for the analysis of proteins in the grana and stroma of the thylakoid membranes of higher plants, matrix effects were completely eliminated on removal of all phospholipids from the sample matrix. Qu et al. [67] used a 1000-fold dilution for sample clean-up and a high temperature setting on the ion source (350 °C) to reduce the amount of ion suppression in the analysis of scutellarin in *Erigeron Breviscap* extract. Pettrovic et al. [68] used column switching and restricted access material (RAM) for online sample clean-up and analysis of endocrine disrupting compounds in sediment samples. RAMs are bifunctional sorbents that separate macromolecular components from low molecular target analytes. RAM sorbents achieve this by "restricting" access of macromolecular matrix species to adsorption sites on the support material. Using RAM technology together with internal standards enabled an ion suppression level of 8.3% to be achieved.

### 6.6. Water samples

Depending on the type of water sample the level of ion suppression can vary considerably between the highly complex matrix of a waste water sample compared with the relatively clean matrix of a drinking water sample. Kasprzyk et al. [69] developed a method for the analysis of pharmaceutical and personal care products in surface water. Their results showed a significant influence of matrix effects on the reduction of analyte signal response ranging from 10 to 80%. The authors comment on the importance of using an internal standard to correct for signal loss due to reduced ionisation efficiency caused by matrix components. They also state for compounds where an internal standard is not effective, dilution of the samples should

be undertaken. Vieno et al. [70] found that ion suppression caused reduced lower absolute recoveries of the β-blockers carbamazepine and afloxacin in sewage treatment plant effluent and influent samples with a 40% ion suppression effect. There was a reduced level of ion suppression in surface water (8%) due to less interfering matrix components in this relatively cleaner water sample compared to the sewage treatment plant effluent. Hospital sewage water was analysed for antibiotic substances by Lindberg et al. [50]. Ion suppression/enhancement was found for all investigated analytes. They also found that the use of internal standards corrected the variation in signal response although the amount of correction varied with the analyte. It was observed that internal standards that have different retention times than the analyte will only be effective for compensating ion suppression that is constant throughout the analysis and not ion suppression that is localised to particular areas of the chromatographic run which may only effect the region in which an analyte of interest elutes.

Freitas et al. [12] developed a method for the analysis of herbicides and metabolites at low levels in surface waters. They applied isotope-labelled internal standards for each analyte to overcome ion suppression. Ion suppression was found to be highest in waste water at 71%, followed by lake water at 59% and creek water at 42%. Ternes et al. [71] analysed neutral pharmaceuticals in waste and river samples and found that matrix effects either reduced the sorption efficiencies of the extraction material (Isolute C18) or led to ion suppression in the ESI source. To distinguish between these two issues the authors spiked water extracts with analytes prior to injection onto the column. Recoveries were not significantly improved by these measures thus the signal suppression caused by the ESI source plays an important part in the loss of analyte response.

## 7. Conclusions

This review was written in an attempt to highlight the problem of ion suppression in LC–MS quantitative methods and to give an overview of the techniques that have been used to detect and subsequently overcome or reduce its effect. Consideration has been given to a variety of techniques that have been used across different types of sample matrices to reduce ion suppression/enhancement effects in LC–MS analyses.

Ion suppression can be a major obstacle in LC–MS analysis. It is difficult to know if ion suppression is present until it is investigated. It is clear that the probability of having an ion suppression effect on analyte response is proportional to the complexity of the sample matrix. There is no generic method for detecting and eliminating ion suppression; therefore, the problem must be addressed on a case-by-case basis. Identifying the presence/absence of ion suppression in an analysis should be a minimum requirement of every validation process. Post-column infusion is the most frequently used technique for identifying the presence of ion suppression throughout a whole chromatographic run. Furthermore, quantifying the degree of ion suppression is essential during method development because it allows an analyst to determine whether ion suppression negatively effects analyte quantitation, precision and sensitivity. Comparison of matrix and solvent calibrations along with recovery studies is a simple yet effective technique for quantifying the effect of ion suppression in a LC–MS method.

While it is clear that ion-suppression can vary between different matrices due to their vastly different composition, it is less clear that it can also vary between similar sample types, e.g. blood samples from different test subjects. Therefore, it is essential to develop a method (starting from sample preparation right through to the optimisation of the LC–MS conditions) that is sufficiently robust to compensate for variability. Usually the more complex the matrix, the more clean-up steps that are required to remove any interferent(s), this in turn can result in reduced recovery and/or add a significant amount of time, labour and cost to a method.

If the central question is “should ion suppression be eliminated or controlled” the answer must be that it depends on how badly it affects the analytical result and how difficult it is to eliminate the source of the ion suppression: Is it even possible or practical to eliminate it by 100%? The first step in controlling ion suppression in LC–MS is to identify the region in the LC where ion suppression occurs. Then if it is a simple matter of tweaking parameters to change the retention time of the target compound to isolate it from the supressing agents then this is the most practical (and fastest) solution. However, if the suppressing agent persists throughout the chromatographic run then a more multifaceted approach is required such as those outlined in this review.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.03.048.

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