Signal suppression/enhancement in high-performance liquid chromatography tandem mass spectrometry

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1. Introduction

When the analysis of a complex real sample is performed, the matrix effect is one of the most relevant drawbacks that the analyst can expect. In analytical chemistry the matrix effect is defined by IUPAC as “the combined effect of all components of the sample other than the analyte on the measurement of the quantity. If a specific component can be identified as causing an effect then this is referred to as interference” [1]. The definition, even if expressed in general terms, suggests the different and complex aspects of the phenomenon.

Matrix interference represents a disadvantage of practically all the instrumental techniques and unfortunately also of the technique of high-performance liquid chromatography hyphenated with mass spectrometry detection (HPLC–MS) [2]. On the other hand this technique is too important and always more diffusely

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used since HPLC represents the method of choice when the determination of thermally unstable, polar or nonvolatile species is required and MS and tandem MS detections greatly help in a selective identification process.

In mass spectrometry detection interference effects more frequently lead to ion suppression phenomena, induced by the presence in the matrix of volatile compounds able to change the efficiency of analyte droplet formation (or evaporation) as well as the amount of the analyte ions formed in the gas phase that reaches the detector [3]. The different phenomena potentially induced by the matrix components dramatically affect the method performance in terms of detection capability, selectivity, repeatability, accuracy, linearity of response (signal vs. concentration) and limit of quantification. Furthermore, also due to the spectra modifications brought by ion suppression phenomena, it is hard to build a spectrum library. In addition, the lack of fragment ions in full-scan spectra significantly reduces the library information. For this reason, spectrum libraries are mainly built for MS/MS spectra, where ion suppression is less relevant.

Ion suppression affects both identification and determination of analytes. It can lead to false negative diagnostics, when it precludes detection of analytes present but also false positive one when, for instance, the signal of the internal standard (I.S.) undergoes a suppression greater than that of the analyte.

The evaluation of matrix effects in MS detection and possible methods for its overcoming should therefore be included in the validation process of new methods [4]. Matrix effects are so common that the current regulatory for bioanalytical methods requires their assessment and elimination, even if general experimental procedures to be followed are not yet available [5].

Different species can be able to give ion suppression. They can be endogenic species, already present as components of the sample and still present after its pre-treatment or extraction: potential ion suppressors are ionic species (inorganic electrolytes, salts), polar compounds (phenols, arylsulfonates) and organic molecules as carbohydrates, amines, urea, lipids, peptides and, in general, compounds or metabolites characterised by a chemical structure similar to the target analyte. Co-extracted substances present in the injected sample can cause relevant determination problems, especially when they are present at high concentration and co-elute with the analyte, so modifying its signal. Co-extracted species may also affect ion intensity of the analyte when form adducts or react with the analyte in the HPLC–MS interface.

Also the extraction process can bring into the extract interfering materials as polymer residues and phthalates released from plastic tubes or from packing materials used in solid phase extraction (SPE) or LC columns [6].

Also reagents added to the mobile phase to improve the chromatographic peak shape, as salts, ion-pairing agents, buffers and organic acids are potential responsible of ion suppression [3,5,7–12]. In this case, since the additives are continuously introduced into the interface with the mobile phase, the signal suppression can be observed throughout the entire chromatographic run.

The elution flow-rate can also play a role: low flow-rates and nanospray systems may reduce the effects. Being the initial droplet diameter lower for flow-rate in nanoliter range, a lower number of subsequent coulombic explosions occur, that results in a lower concentration of salts in the droplet and then a lower suppression [13,14].

The level of signal suppression also depends on the hydrophobicity of the analyte and its affinity for the stationary phase. When using RP stationary packings the effect is generally lower for the more hydrophobic compounds.

The degree of ion suppression varies not only from sample to sample, but also from compound to compound and depends on the sample preparation [15]. But also for the same analyte, ion suppression entity may depend, even if in non-linear fashion, on the analyte concentration as well as on the matrix to analyte concentration ratio [13]. So for instance in the analysis of clenbuterol in urine, the percentage of ion suppression was shown to range from 37% at the analyte concentration level of 93 μg·L⁻¹ to 68% for concentration of 45 μg·L⁻¹ [3]. These data highlight the benefits to decrease, when possible, the matrix to analyte concentration ratio through a more extensive clean-up of the sample or by the choice of more suitable chromatographic conditions. They also show the importance, when the validation for the matrix effect is performed, to choose a concentration of the analyte as close as possible to that expected in the real sample. Due to the importance of the concentration, very likely also when using an I.S., its concentration must be controlled and chosen similar to that of the analyte.

Also the mass and the charge of the analyte can affect the ionisation process and the ion suppression entity [3]. In general, molecules with higher mass have been shown to suppress the signal of the smaller ones. More polar analytes are more susceptible to undergo ion suppression, while organic solvents generally enhance electrospray ionisation (ESI) signal, especially in positive ion (PI) mode [15,16].

Also in ion spray (ISI) and sonic spray (SSI) ionisations, the increase of organic modifier concentration favours ionisation, while increasing amounts of volatile acids and buffers increase signal suppression [3]. Another possible effect brought by high concentrations of nonvolatile solutes is the increase of the solution boiling point that in turn might cause lower efficiency in solvent evaporation and lower droplet formation rates. Nonvolatile solutes can also lead to be increased surface tension of the liquid whose effect is a lower efficiency in droplet formation and production of anlyte ions.

In general, to achieve higher sensitivity and selectivity, tandem MS is often used both in tandem-in-time MS/MS (ion trap analysers) and tandem-in-space MS/MS (triple quadrupole analysers).

An innovative ultra-high-pressure liquid chromatography (UHPLC) technique has been recently developed, based on the use of stationary phase packings with particles size smaller than conventional HPLC and of technology systems allowing pressures higher than 10,000 psi. Higher resolution and sensitivity, shorter analysis time and higher signal-to-noise (S/N) ratio can be obtained. UHPLC in combination with tandem MS is an excellent analytical tool for multi-residual determinations of pharmaceuticals, drugs, toxins and pesticides in environmental waters. To reduce dwell time and to increase selectivity without sacrificing sensitivity, UHPLC is coupled with a faster-acquisition triple quadrupole analyser [17–19]. As concern the matrix effect, even if the smaller particles of the stationary phase, that provide more resolution and more speed to the chromatographic analysis, should suggest a low or null matrix effect, relevant effects were instead observed by many authors, at least when I.S. were not used [17,20].

Different behaviours are reported in literature when using ESI or atmospheric pressure chemical ionisation (APCI) sources [5]. Many authors observed signal suppression both in ESI and APCI sources and most of them found that the effect is lower in APCI [4,21–24]. A signal enhancement in APCI was observed, especially in the presence of high percentages of organic modifier in the mobile phase. Other authors indicate lower effects when using negative ionisation (NI) mode, likely due to the higher selectivity of this mode of ionisation [17,25].

The particular situation that suggested the authors of this review to deepen the aspects of ion suppression/enhancement is represented by the experimental results obtained when developing HPLC–MS/MS methods for the simultaneous determination of six biogenic amines (mono- and poly-amines characterised by both aromatic and aliphatic structures) in cheese. This analysis is
Fig. 1. Example of signal suppression: comparison of external and internal (standard addition method) calibration plots in the determination of tyramine in cheese. Chromatographic conditions: Luna C18 column (150 mm × 2 mm, 3 μm) equipped with a precolumn as the stationary phase and a mixture of methanol and 15.0 mM ammonium acetate (alternatively at pH 6.00 and 3.50 for glacial acetic acid) eluting in gradient conditions as the mobile phase. Ion source: ESI in PI mode; mass spectrometer operates in SRM mode [26].

worldwide collecting particular interest, due to the relevant information that the presence and the relative concentration of biogenic amines in fermented food offer in food quality control, associated with some technical difficulties encountered in their simultaneous determination, due to the so different chemical properties.

A HPLC–MS/MS method was firstly developed, making use for the chromatographic separation of a Luna C18 stationary RP packing and of a mobile phase constituted by a mixture of methanol and ammonium acetate aqueous solution, adjusted to different pH values with acetic acid and flowing under gradient conditions [26]. The pre-treatment was performed by SPE on C18 sorbent, the extract dried under nitrogen and collected in HCl solution. In MS detection working in ESI a relevant suppression of the signals for all the analytes was observed (Fig. 1). The matrix effect was so heavy and so different for each amine considered that it was necessary to employ the standard addition method for the determination of each amine in each sample of cheese.

Afterwards, the method was improved by using a hydrophilic interaction liquid chromatography (HILIC) stationary phase and a mobile phase, eluting in gradient conditions, represented by a mixture of acetonitrile and ammonium formate brought to pH 4.0 for formic acid. A simplified pre-treatment step was employed and mass spectrometer worked in APCI source. The matrix effect was present also in these conditions but gave for all the analytes signal enhancement. With respect to the previous method, two pre-treatment steps have been avoided since HILIC stationary phase packing is compatible with the injection of organic extract, so that the nitrogen drying step of the eluate and the following collection in HCl aqueous solution were no more necessary (the extraction yields were of the same order). For the determination of the biogenic amines in cheese the standard addition method was employed. The slopes obtained in the calibration plots built through the standard addition method were for all the analytes always much greater (100–1000 folds) than those obtained in the external calibration plots, to indicate that the matrix effect gave here rise to a signal enhancement (a typical example is given in Fig. 2 for tyramine) [27]. We remind that in the method previously described a signal suppression was observed (Fig. 1) [26]. The different behaviour observed for the analytes in the same matrix under different chromatographic and MS conditions was firstly ascribed, in agreement with literature data, to the different mechanism of ionisation that characterises ESI and APCI sources [5,23,28–30]. But it must be also considered that the use of the HILIC stationary phase allows using in the mobile phase higher concentrations of organic solvent that, in turn, favour MS ionisation. In addition, the APCI source permits the use of higher concentration of volatile buffer.

Fig. 2. Example of signal enhancement: comparison of external and internal (standard addition method) calibration plots in the determination of tyramine in cheese. Chromatographic conditions: HILIC column (150.0 mm × 2.1 mm, 3 μm) as the stationary phase and a mixture of acetonitrile and 50.0 mM ammonium formate at pH 4.00 for formic acid as the mobile phase. Ion source: APCI in PI mode; mass spectrometer operates in multiple reaction monitoring mode [27].
The different behaviour obtained for the same analytes in the same matrix, but under different extraction, chromatographic and ionisation conditions, suggests that many factors simultaneously intervene in causing ion suppression or enhancement. A detailed study of literature results has therefore been conducted, in order to possibly evaluate at which extent the different observed matrix effect (suppression [26] or enhancement [27]) can be correlated to the different ionisation sources (ESI and APCI) used.

The review presents general considerations on matrix effects in HPLC–MS and HPLC–MS/MS analysis, reports the main effects observed and proposes hypotheses to explain the different behaviours, as well methods and strategies to overcome the effects.

2. How to evaluate matrix effect

According to Annesley the presence of matrix effect in MS analysis can be envisaged by comparing the instrumental response obtained for: (a) a calibrator as for example an I.S. directly injected in the mobile phase, (b) the same amount of I.S. added to the already extracted sample, (c) the same amount of I.S. added to the sample before extraction. The data for the calibrator in the mobile phase gives the 100% response value. The data for the same amount added to the extracted sample show the matrix effect on the MS response while the data obtained in the sample added with I.S. before extraction highlights whether the loss of signal is due to the extraction process or to the matrix effect.

The percentages of matrix effect (ME), of the recovery of the extraction process (RE) and of the overall process efficiency (PE) are calculated as [5]:

\[
\text{ME} (\%) = \frac{B}{A} \times 100
\]

\[
\text{RE} (\%) = \frac{C}{B} \times 100
\]

\[
\text{PE} (\%) = \frac{C}{A} \times 100 = \frac{\text{ME} \times \text{RE}}{100}
\]

where A is the peak area of the standard solution, B the peak area of the standard spiked after the extraction and C is the peak area of the standard spiked before the extraction.

The entity of matrix effect is defined as 100 – ME (%). If ME (%) = 100 no matrix effect is present, if ME (%) > 100 there is a signal enhancement and if ME (%) < 100 a signal suppression.

The presence and entity of the matrix effect can also be evaluated, when possible, by a direct comparison of the standard line slopes when different HPLC–MS interfaces (APCI and ESI) are used for the assay of the same compounds, when using the same I.S., the same sample preparation and the same chromatographic conditions.

The taking place of matrix effect can be also denounced by the comparison of the slopes of the calibration plots built for standard analytes and for the standard additions performed on the sample. In the absence of matrix effect and in the absence in the matrix of the analyte of interest, the slope and the intercept in the two plots correspond each other within the experimental error deviation. In the presence of the analyte and in the absence of matrix effect the slope is always the same and the intercept in the standard addition plot gives the amount of the analyte. A lower slope in the plot of standard addition method indicates the suppression of the signal while a greater slope indicates signal enhancement (examples are given in Figs. 1 and 2). For determination purposes therefore, the generalised use of the external standard calibration plot often gives biased results, especially in the analysis of complex samples such as food or biological fluids.

To overcome matrix effect when different samples of a similar matrix must be analysed, some authors suggest to perform a standard calibration plot for a standard matrix similar to that of the samples to be investigated, but free from analytes. This could indeed save time but the problem is to find a matrix with the same characteristics of the sample to be analysed and, in addition, completely free from the analyte or the analytes of interest.

3. Proposed mechanisms of ion suppression/enhancement in ESI and APCI sources

Even if the mechanism of matrix effect is not yet fully understood, it originates from the competition between the analyte and co-eluting interfering species. Depending on the conditions of ionisation and ion evaporation, competition may lead to a decrease (ion suppression) or an increase (ion enhancement) of the efficiency in the formation of the analyte ions. The ionisation reaction extent depends on the ionisation energy and on the proton affinity of all the molecules present in the interface, so that the efficiency of formation of the desired ions also depends on the possible competition with the interfering species. The extent of matrix effect can therefore be different for ESI and APCI interfaces, since different are the ionisation mechanisms. The extent of formation of the desired ions can therefore be different even in the presence of the same co-eluting compounds, since it depends on the mutual positive or negative effects that the co-eluting species play on the efficiency of ion formation. While in ESI the analyte is ionised in the liquid phase inside the electrically charged droplets and the analyte ions pass from the liquid to the gas phase, in APCI the neutral analytes are transferred into the gas phase by vapourising the liquid in a heated gas stream and the ionisation occurs through the chemical ionisation of the gas phase analyte [22,24].

Ionisation suppression in ESI has been explained by considering that the reactions in gas phase give rise to loss of charge of the analyte ions [24]. But this explanation holds always for gas phase reactions and therefore also when using APCI source.

According to another hypothesis, high concentrations of interfering compounds increase viscosity and surface tension of the droplets produced both in ESI and APCI interfaces. This phenomenon reduces the ability of the analytes to reach the gas phase, while favours the formation of adduct ions or ion pairs [24,31,32].

Also reactions taking place between the analyte and nonvolatile species can limit the transfer of the analytes to the gas phase. In particular, unlikely the analytes can pass through the APCI vapourisation region remaining in solution, while it seems more probable that suppression in APCI is due to formation of solid matter: a precipitate could form as the concentrations of both analyte and nonvolatile components increase with solvent evaporation. On the other hand, precipitation reactions are expected to affect ESI response but do not explain the greater suppression level observed, that is more likely due to variations in colligative solution properties, caused by the presence of nonvolatile materials [24].

Besides these mechanisms that take place in liquid phase, other possible mechanisms are proposed to occur in gas phase. In particular processes of neutralization are considered, linked to the basic properties assumed in gas phase by both the analytes and the interfering species. Although in ESI the analyte is transferred to the gas phase by electrospray mechanism, under suppression conditions the species transferred to the gas phase could be neutral. The formation of neutral species of the analyte rather than charged ones could be due to the depletion of the available charge from the drop when the interfering sample components are ionised. To test this hypothesis a combined ESI-APCI source was designed and built: an APCI discharge needle was added to the ESI source to provide charge in excess with respect to that available from ESI [33].
If the analyte evaporates as a neutral species, APCI should ionise the neutral analyte and so reduce the degree of ionisation suppression observed with ESI alone. Infusion experiments carried out for the same species and conditions showed, in the presence of the discharge needle, no significant change in suppression to indicate that the possible release from solution of gas phase neutral analyte does not explain ion suppression [33].

As a possible cause of ion suppression, also the radius of the droplets from which gas phase ions form has been considered. The nonvolatile species present in the matrix could in fact prevent the droplets to reach their critical radius, with a consequent decrease of ionisation efficiency [34].

Also the choice of PI or NI modes can affect the signal. When the species can ionise both in PI and NI modes, the use of NI mode is suggested because more selective and associated to lower matrix effects. So for instance, when ESI (NI mode) was used in the determination in environment and wastewaters of 37 pesticides (herbicides, insecticides and fungicides), the signal suppression, with only few exceptions, was not significant, in contrast with that observed in PI mode [17].

This behaviour is anyway not always verified. So for instance, in the determination of naproxen and ibuprofen, notwithstanding both the analytes and their I.S. underwent signal enhancement, the result of the analysis was not satisfactory, because the enhancement effect was much lower for the analytes than for their I.S.

To collect more information on this aspect, the matrix effect was studied in the determination under the same chromatographic conditions, of nine drugs and their corresponding I.S. both in ESI and APCI, in selected ion monitoring (SIM) and selected reaction monitoring (SRM) modes. The results showed that in ESI all the drugs and their I.S. suppress each other the ionisation responses, while in APCI, where a signal enhancement was expected for all the drugs, two drugs (and relative I.S.) showed no enhancement [29].

4. Possible actions to overcome ion suppression/enhancement

Various factors both in ESI and APCI interfaces affect the ionisation of the analytes and of consequence the identification and quantification processes. Therefore the choice of the sample preparation procedure, as well as of the chromatographic conditions should be harmonized with the final objective to optimise the performances of both the chromatographic separation and the ionisation process. On the basis of literature results, some actions can be suggested to minimise or to correct ion suppression/enhancement. They include modifications of the MS conditions, of the clean-up process, of the chromatographic conditions and of the calibration techniques.

4.1. Modification of mass spectrometric conditions

To overcome ion suppression/enhancement problems, it can be advantageous to modify MS conditions, especially when this operation does not require any other change in the already developed and optimised analytical procedure, as it concerns both sample preparation and chromatographic separation.

From literature examples, it can be concluded that, with some exceptions [6,40–43], ESI is more subjected to ion suppression than APCI [5,21,22,24,29,35–39]. When matrix effects are encountered, it is suggested to try to switch the ionisation mode or to use, if available, a different mass spectrometer. So for instance the performances of different atmospheric pressure ionisation (AP1) source designs from three different instrument manufacturers including both ESI and APCI sources have been compared [6]. In this study, unexpectedly, the APCI interface of the Micromass Quattro tandem mass spectrometer resulted more susceptible to matrix effect than the ESI interface of the same instrument [6].

The occurrence and compensation of matrix effects using both APCI and ESI were tested in the analysis of 22 pesticides in three kinds of matrix (vegetables, fruits and wheat flour). The pesticides affected by significant matrix effects were the same, both in APCI and in ESI analyses, but signal enhancement was always observed in APCI and suppression in ESI [28].

As it regards the choice of ionisation polarity, it can be tried to work, when the species can be ionised both in PI and NI modes. As mentioned, NI mode is usually considered less subjected to ion suppression because more selective, being relatively low the number of compounds undergoing NI mode.

Atmospheric pressure photo ionisation (APPI) is a more recent technique, with a limited range of application and therefore ion suppression for this kind of ionisation is still less investigated [44]. Since in this technique the ionisation of the analyte depends on the interaction of the molecule with a proton of energy greater than the ionisation potential, photoionisation is able to ionise many species more efficiently than ESI and APCI [45,46]. In particular APPI, due to its greater sensitivity with respect to APCI, is advantagously used in normal-phase chiral analysis especially at low mobile phase elution flow-rates [41] and in quantitative trace bioanalysis based on the use of miniaturised LC column and when limited sample size is available [47]. Since photoionisation is not based on charge affinity, ion suppression phenomena are generally lower than in ESI and APCI [42,47–54]. For this reason APPI technology can be chosen to circumvent ion suppression and to lower matrix effect [49]. The occurrence of ion suppression can be anyway expected also in this technique, due to the role of the liquid phase in the ionisation process. Since the APPI is particularly suitable for non-polar compounds, it must be however underlined that the potential interfering species are likely different from those that more affect the signal in ESI and APCI, in which suppression is mainly due to polar compounds.

As it concerns the influence on ion suppression of source geometry, the linear transfer from the capillary to the sample cone is described as more disturbing than the orthogonal design, because this latter improves the transmission of the ions through the simultaneous discarding of the interfering species. But, considering that ion suppression generally takes place in the early stages of the ionisation process (i.e. before or immediately after the transfer of the analyte into the gas phase as ionic species) the influence of the stage in which the ions are transferred to the analyser has likely a lower influence. For the same reason in our opinion it appears not justified to consider that a triple quadrupole or an ion trap system is more subjected to ion suppression. Since the difference between the two instruments concerns the mass filters and not the interfaces, the potential problems occurring during the ionisation should be similar for both the instruments.

In order to overcome matrix effects, an innovative LC–MS interface based on electron ionisation (direct-EI) has been presented, particularly suitable for volatile, not thermolabel and characterised by small-medium molecular mass analytes [14]. Direct-EI interface is considered a technique of ionisation harder than API interfaces. Because of the higher energy involved, EI produces several neutral and ionic fragments and gives a peculiar combination of m/z values characteristic of each compound like a chemical fingerprint. The EI spectra can be easily obtained for almost any compound under 600 u and are readily interpretable since, due to their high reproducibility, have been recorded in commercial available libraries. The relatively limited success of the technique can be ascribed to the low sensitivity associated to the low ionisation efficiency and to the necessity to convert the analytes in vapour phase. On the other hand, the EI source, based on gas phase ionisation, is able to highly lower matrix effect, since
it is not influenced by the mobile phase and components of the matrix: therefore the signal response is proportional only to the analyte concentration [55–57]. In particular, good results were obtained when interfacing ESI source with micro- and nano-HPLC. In a recent study of Cappiello et al., concerning the determination of 12 organochlorine pesticides in waters by an off-line SPE followed by nano-HPLC-direct-ESI MS method, no matrix effect was observed [58].

4.2. Pre-treatment and extraction process

To lower in the final extract the presence of interfering species, it is suggested to systematically check the entity of the matrix effect after different sample pre-treatments. In other words, the usual tendency to consider the recovery of the target analyte as the main performance indicator should be moderated by the necessity to evaluate also the method efficiency as it concerns the removal of the interfering compounds. There is no universal strategy, but only solutions to be used case by case for each analyte/matrix combination.

The only way to definitively circumvent the problem is to improve the sample preparation and purification, taking any way into account that the chemistry of the analyte affects the ESI response more than the sample pre-treatment [15].

Anyway, large differences in the matrix effect were observed after different sample preparation techniques [22]. Since the pre-concentration process not only increases the concentration of the target analyte but often also that of the potential interfering substances, SPE pre-concentration can often magnify matrix effect. It is also true that the omission of a pre-concentration step generally lowers analysis sensitivity.

It is evident that one approach that only consists in the dilution of the sample or in the injection of a lower volume should be advantageous in term of work and time, but it is also inappropriate for trace analysis when low detection limits are required. A dilution could be critical for the analyte present at ultra-trace level and without significant effects for interfering substances present at high concentration. Sample purifications are therefore always preferable, like liquid–liquid extraction (LLE) [15,21,59,60], protein precipitation (PPT) [15,21,59], or SPE [15,21,59–63], the choice depending on each specific application.

4.3. Modification of chromatographic conditions

Improving HPLC separation efficiency is a good approach to overcome or at least to decrease matrix effect. Chromatographic conditions can be modified in order to shift the retention time of the analytes away from the time windows more affected by matrix effect [35,59,64]. A special attention should be paid towards the analytes eluting in the solvent front (highly polar and non-retained compounds) or during the end of an elution gradient (or in the washing step of the chromatographic column where the strongly retained compounds are eluted). Since generally the areas of these peaks are more affected by interferences, the retention of the analytes can be adjusted in order that they elute in the time window between these two regions.

Different stationary phase packings can be used with characteristics suitable to the analytes of interest, as for example C18 packings containing polar moieties or HILIC stationary phases [21,27]. When the change of the stationary phase does not provide the expected results, column switching technology (two-dimensional chromatography) may improve resolution [65–67].

Modifications of the mobile phase composition can also be useful. Low concentrations of weak acids (such as formic, acetic, hexafluorobutyric, trifluoroacetic acids), ammonium formate, ammonium acetate or ammonium hydroxide can be added to the mobile phase [3,12,66]. It must anyway be noted that mobile phase additives used to improve separation may also affect the ionisation process and suppress the signal. In the presence of the same additives, strong similarities were observed between the performance of ISI and SSI, while APCI displayed a different behaviour. This can be explained by considering that ionisation in both ISI and SSI is predominantly a liquid phase process, while in APCI the ionisation mechanism is mostly based on gas phase chemistry.

APCI, in turn, is positively influenced by the presence of volatile acid additives and in addition is compatible with higher flow-rates and 4.6 mm I.D. or larger bore stationary phase packings.

When an I.S. is used, the chromatographic conditions must be adjusted in order that analyte and I.S. co-elute and of consequence ion suppression for the two compounds can be expected to be very similar [4].

To control possible interferences, a post-column infusion system has been developed that enables the researcher to follow the time profile and the extent of variation in the analyte response, when interfering components are present [15]. Matrix effect profiles, generated by post-column infusion of 129 analytes in ESI MS and PI mode, provided information about the effect of the sample matrix on the individual analytes, independently on their retention time. For a given retention time, the matrix effects more differ among matrices than among analytes and suggest that the extent of matrix effect is less influenced by the physicochemical properties of the analytes than by the properties of the matrix components that co-elute. The similarity of the effects played by a given matrix on the different analytes can offer the opportunity to compensate for most of the matrix effects occurring in ESI, through the help of matrix effect profiles obtained in post-column infusion. An example of the instrumental device is shown in Fig. 3 [25,68].

Also the use of species with lower surface tension in ESI source as well as post-column addition of acids to displace trifluoroacetic acid (TFA) and aid ionisation with a process called “TFA fix” have been reported [3,9]. Other authors suggest the use of 2-(2-methoxyethoxy) ethanol as a signal enhancer to eliminate ion suppression effects of acetate anions: about 100 folds enhancement of the signal was obtained [69].
Another approach to overcome the matrix effect is based on the building of matrix effect maps, with the aim to evaluate the impact on the matrix effect of the parameters correlated to the LC–MS method employed [70]. The matrix effects are studied as a function of the amount of the co-injected matrix extract: no variation was observed varying the analyte concentration and keeping constant the matrix amount, while a significant variation was observed when the amount of matrix was varied, being constant the analyte concentration. The results suggested that the ruggedness for API LC–MS methods must be considered as a function of the amount of the co-injected matrix [70].

4.4. Selection of the optimal calibration strategy

If matrix suppression/enhancement phenomena cannot be eliminated or made negligible through the application of the strategies described, appropriate calibration techniques can be used to compensate, as possible, the matrix effects. The following options can be experimented.

(i) The addition of I.S. is largely used. In order to achieve an efficient compensation of the matrix effects I.S. must be characterised by chemical structure and chromatographic retention similar as possible to those of the analyte. The co-eluting matrix components are expected to affect at comparable extent both analyte and I.S. In particular, isotopically labelled internal standard are the most powerful strategy for diminishing suppression effects and improve quantisation accuracy [67,71–81]. Unfortunately, their use is rather expensive, especially in a multicomponent analysis [71–73,82]. Species of structure analogue to the target analyte can be added to the eluate coming from the LC separation column and entering the MS detector (post-column addition). But also this strategy is not of easy application for multiclass multicomponent analysis [83].

(ii) Echo-peak technique represents a interesting alternative to the I.S. concept. With this technique each analysis comprises two injections into the LC–MS system. The unknown sample and a standard solution are injected consecutively within a short period of time, under the same chromatographic conditions. As a result, the peak of the standard elutes close to the peak of the analyte and is called the “echo-peak”. Provided that the retention times of these two peaks are close enough to be affected in the same manner by the co-eluting undesired species, matrix effects are compensated [84,85]. An example is reported in Fig. 4 for the analysis of eight pesticides.

(iii) Calibration using external matrix-matched standards-supposing. If standards with the same or similar matrix composition of the sample to be analysed are used, a practically full compensation of matrix effects is achieved. But unfortunately, this approach is not easy, mainly because an appropriate blank (i.e. material free of residues of target analyte) is generally not available.

(iv) The standard addition method above described can be usefully employed [26,27,86].

5. Conclusions

On the basis of the results, up to now available in literature, it can be concluded that the occurring of a signal suppression or enhancement in MS detection cannot be attributed to only one cause, but it depends on a synergic effect of all the conditions involved. These are

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**Fig. 4.** LC–MS chromatograms of a mixture of eight pesticides in echo-peak technique injection. 10 μl injection. 1st peak, sample: standard of pesticides (0.5 μg/ml) in methanol–water (1:1, v/v); 2nd peak, reference: standard of pesticides (0.5 μg/ml) in methanol–water (1:1, v/v) (Ref. [85], Fig. 3, with permission).
represented by the analyte chemical properties, the matrix components, the clean-up procedure, the chromatographic conditions as concerns both stationary and mobile phase (including the additives), the kind and the features of the mass spectrometer. The occurrence of ion suppression was shown in fact to also depend on the different ionisation techniques, ionisation modes (PI, NI) and on different equipments with different source design [6,11,39,64-87].

All the hypotheses proposed from different authors to explain ion suppression/enhancement phenomena are reasonable and worth to be considered. Very likely each mechanism proposed takes place or can take place as a function of the experimental conditions. On the basis of the different reactions and mechanisms postulated, very likely the cause is never only one, but more effects simultaneously occur. In particular when comparing the matrix effect in the most used ionisation sources ESI and APCI, the examples reported indicate that it is not possible to generalise and expect to observe signal suppression in ESI and signal enhancement in APCI. It was in fact shown a different behaviour for analytes of similar structure, under the same conditions of ionisation, instrument, chromatography, clean-up, kind of matrix. In this case the chemical properties of the analyte seemed to be the predominant cause of the different behaviours. On the other hand, an example showed a different matrix effect behaviour (suppression or enhancement) when the same analytes in the same matrix are determined under different extraction, chromatographic and MS conditions. It can perhaps also happen that among all the potential causes that lead matrix effects, one of them prevails and becomes the only responsible, but this situation can be hardly predicted.

References