Sample treatments prior to capillary electrophoresis-mass spectrometry

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Summary

Sample preparation is a crucial part of chemical analysis and in most cases can become the bottleneck of the whole analytical process. Its adequacy is a key factor in determining the success of the analysis and, therefore, careful selection and optimization of the parameters controlling sample treatment should be carried out. This work revises the different strategies that have been developed for sample preparation prior to capillary electrophoresis-mass spectrometry (CE-MS). Namely, the present work presents an exhaustive and critical revision of the different samples treatments used together with on-line CE-MS including works published from January 2000 to July 2006.

Keywords: Capillary electrophoresis/ Mass spectrometry/ CE-MS/ Sample pretreatment/ Couplings/ Hyphenated techniques/ Review

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1 Introduction.

It is generally assumed that in order to provide an adequate chemical analysis any analytical method must include the following steps: sampling (sample must be representative of the object under investigation), sample preservation (sample should be kept stable until the analysis is completed), sample preparation, sample analysis *per se* and data treatment. Often, one of the bottlenecks of this analytical process is sample preparation since it is usually a time-consuming and laborious step. The purpose of any sample preparation is the clean-up of the sample and/or the extraction, enrichment or preconcentration of the analytes, improving in this way the quality of the analytical results obtained. However, it has to be considered that any sample treatment will depend on both the sample nature and the following analytical technique that is going to be employed, requiring an almost case-by-case development. Therefore, no universal sample preparation is available.

The choice and optimization of a suitable sample pretreatment is not easy, especially with highly complex sample matrices like biological fluids (plasma, serum, whole blood, urine, etc.) or other natural matrices including e.g., foods, plant extracts or environmental samples. Ideally, sample preparation should be as simple as possible, not only because it will reduce the time required, but also because the greater the number of steps, the higher the probability of introducing errors. If possible, sample preparation should be carried out without loss of the analytes (or with the minimum loss) while eliminating as many interferences as possible from the matrix. Finally, it should also include, when necessary, a suitable dilution or concentration of the analytes in order to obtain an adequate concentration for the subsequent analysis. Sometimes, it may also include the transformation of the analytes into different chemical forms that can make

1 easier e.g., their separation or detection.

2 At the present time, developments in sample pretreatment strategies involve the
3 use of new extraction materials, the use of automated protocols and/or its integration
4 into miniaturized formats such as microchips or micrototal analysis systems (μ -TAS) that
5 should allow a rapid and sensitive analysis of the target analytes, especially in complex
6 samples [1]. This research area has provided interesting and promising results and it
7 will surely be one of the working areas in the future Analytical Chemistry.

8 Nowadays, the inherent advantages of the use of capillary electrophoresis (CE)
9 as separation technique are well known and can be summarized in high separation
10 efficiency, low analysis time, high resolution power and low consume of samples and
11 reagents. It is at the moment one of the premier analytical separation techniques for the
12 analysis of biological compounds such as peptides, proteins and polynucleotides and
13 has been applied with success to a great variety of analytes [2-6]. Its different separation
14 modes (CZE, MEKC, ITP, etc.) have allowed facing the problem of the separation of
15 either neutral or charged analytes based on different physico-chemical properties
16 (charge/mass ratio, molecular weight, polarity or isoelectric point). Besides, the
17 different detectors available (UV-Vis, laser induced fluorescence (LIF), mass
18 spectrometry (MS), electrochemical, etc.) have also broadened its use and applications,
19 although UV is the most widely used detector in CE equipments so far.

20 Nevertheless, the small capillaries used in CE separations accommodate only
21 small volumes of sample, which require either the use of a suitable on-line or off-line
22 preconcentration procedure or the use of a more sensitive detector like LIF or MS. In
23 this regard, MS gives information on the molecular weight of the analytes and enables
24 the separation of co-migrating molecules increasing selectivity and specificity acting as
25 a second dimension. Furthermore, it also compensates the migration time variation that

1 normally takes place in CE and provides unequivocal structural information via
2 fragmentation patterns that can be obtained for instance via MSⁿ procedures. Therefore,
3 the on-line coupling of CE with MS gives rise to an impressive analytical tool that
4 combines the high resolution power and separation speed of CE with the high sensitivity
5 and selectivity of the mass spectrometer [4, 7, 8]. However, in order to take advantage
6 of the many possibilities derived from using CE-MS, it is of extreme importance a
7 suitable selection of CE separation parameters (buffer composition, pH,
8 preconcentration procedures), ionization technique (usually electrospray, ESI), and ESI
9 and MS working parameters.

10 Thus, when developing a suitable CE-MS procedure several aspects have to be
11 taken into account. Only highly volatile buffers can be used and they are typically an
12 aqueous or hydroorganic solution containing e.g., acetic acid, formic acid, ammonium
13 hydroxide at low concentrations. The use of non volatile components like cyclodextrins
14 (CDs), inorganic salts (e.g., containing sodium, phosphate, etc) or surfactants (as SDS)
15 are precluded since they are strong inhibitors of ESI efficiency, increase the noise and
16 reduce the sensitivity of the system. Different strategies have been proposed to
17 overcome this limitation including the partial filling technique [9].

18 An additional consideration prior to use CE-MS is the development of suitable
19 sample pretreatment procedures [10]. As an example, direct injection of samples with a
20 high protein content results in short capillary longevity (proteins precipitate and can
21 irreversibly adsorb onto the silanol groups of the internal capillary wall) [11].
22 Furthermore, despite the selectivity of the mass spectrometer, highly complex samples
23 may also induce some ionization suppression or even a complete loss of the MS signal.
24 The objective of this work is, therefore, to provide an overview of the various samples
25 preparation protocols that have recently been proposed prior to on-line CE-MS covering

relevant publications from January-2000 till July-2006.

2. Sample treatments.

In the time covered by the present review, different and interesting sample treatments prior to CE-MS have been proposed, which are summarized in Table 1. As will be next discussed, in some occasions a single or simple treatment procedure was not enough to ensure the correct analysis of the sample, requiring the use of several consecutive sample treatments. In other cases, a single extraction or preconcentration procedure was enough to reduce the sample complexity or to improve the LODs achieved by CE-MS.

2.1 Liquid-liquid extraction (LLE).

This classical sample treatment allows the extraction of both trace analytes or macrocomponents. The selectivity and efficiency of the extraction process in LLE depends mainly on the election of the immiscible solvents, but other factors may also affect the distribution of the solute into both phases like the pH, the addition of a complexation agent, the addition of salts (salting out effect), etc. Although the use of LLE alone provides good results in terms of extraction efficiency and clean-up of the samples, it is often carried out in combination with other preconcentration procedures as it will clearly be seen in the subsequent examples and sections.

Rudaz et al [12] developed a CE-MS stereoselective analysis of tramadol and its main phase I metabolite in plasma after LLE with hexane-ethyl acetate (80:20, v/v); samples were evaporated and redissolved in 0.01 M HCl. The best enantioseparation

1 was achieved using a coated polyvinyl alcohol (PVA) capillary and a 40 mM
2 ammonium acetate buffer at pH 4.0 with 2.5 mg/ml sulfobutyl ether β -CD as the chiral
3 selector. To avoid the entrance of the CDs in the MS and, as a result, the loss of the MS
4 signal, the partial filling technique was applied.

5 Strickmann et al [13] developed an on-line capillary electrochromatography
6 (CEC)-ESI-MS method for the determination of etodolac and metabolites in urine.
7 CEC, although difficult to perform, is together with CZE the preferred CE mode for on-
8 line coupling with MS, because of the highly volatile buffers frequently used. The drug
9 and metabolites in urine could be analyzed by CEC-ESI-MS after LLE extraction using
10 an equal volume of ethyl-acetate and then evaporated and redissolved into the
11 separation buffer.

12 Wey et al [14] have developed a CE-ESI-MS method for the analysis and
13 confirmation testing of morphine and related opioids in human urine by using a BGE
14 containing 25 mM ammonium acetate at pH 9. High analyte concentrations (2-5 μ g/ml)
15 could be monitored in plain and diluted urine samples without further treatment directly
16 by CE-MS. However, for the recognition of lower concentrations LLE at alkaline pH
17 and solid phase extraction (SPE) were used and compared. Concerning the LLE
18 procedure, a mixture of dichloromethane and dichloroethylene was used as extraction
19 solvent. Mean recovery values ranged between 76 and 86% except for the metabolites
20 nordihydromorphine and normorphine which were 14 and 25% respectively. However,
21 the SPE procedure using a mixed mode polymer phase (namely, Bond Elut and Vac-
22 Elut) provided higher recoveries, between 83 and 96% for all the compounds. For this
23 particular application, SPE was shown to be more time consuming than LLE since an
24 additional evaporation step was required to eliminate water from the eluate.

2.2 Solid-phase extraction (SPE).

Sample preparation using SPE was firstly introduced in the mid-1970s, replacing LLE due to its simplicity, selectivity and the better LODs that it provides. Since then, SPE has gained a wide acceptance due to the ease of automation, high analyte recovery, extraction reproducibility, ability to increase selectively analyte concentration and commercial availability of many SPE devices and sorbents, including the use of molecular imprinted polymers (MIPs) [15, 16].

Concerning the use of SPE it is probably the most widely used sample pretreatment procedure prior to CE-MS. Recently, Hernández-Borges et al [17] have determined five triazolopyrimidine sulfoanilide herbicides (cloransulam-methyl, diclosulam, florasulam, flumetsulam and metosulam) in soy milk by SPE-CZE-MS using C₁₈ cartridges. For this purpose, CE-UV and CE-MS instruments were used. To increase the sensitivity of the method, normal stacking mode (NSM) was also used for on-line preconcentration of the SPE extract, providing LODs down to 74 µg/L. Mean recovery percentages ranged between 40 and 94% with good separations when working with aqueous solutions and SPE-NSM-CE-UV as shown in Figure 1A. However, the use of SPE combined with NSM-CZE-UV for analysis of the mentioned pesticides in soy milk did not provide suitable results because of the high number of interferences from the sample matrix (see Figure 1B). In order to overcome this limitation CE-MS was used. Thus, the main ESI-MS parameters (nebulizer pressure, dry gas flow rate, dry gas temperature and sheath-liquid composition) were optimized by means of a central composite design. Optimum separation buffer was composed of 24 mM formic acid and 16 mM ammonium carbonate at pH 6.4, while the sheath-liquid was composed of acetonitrile:water 82.5:17.5 (v/v) with 2% of TEA at 0.35 mL/h flow rate. The

1 combined use of SPE-NSM-CE-MS allowed the detection of these pesticides in soy
2 milk as can be seen in Figure 1C.

3 Peterson et al [18] developed a specific CE-ESI-TOF-MS method for the
4 determination of serotonin (5HT) and its precursors tryptophan (Trp) and 5-
5 hydroxytryptophan (5HTP) in human platelet rich plasma. Analytes were removed from
6 the plasma and preconcentrated by SPE using Oasis MCX columns with mean
7 recoveries between 71.6 and 95.3%. Submicromolar LODs were obtained for standard
8 mixtures of all the compounds except for 5HTP which had LODs in the low micromolar
9 range. When the method was applied to the analysis of plasma extracts from healthy
10 volunteers as well as from pathological samples the levels of both 5HT and Trp were
11 determined while 5HTP was not found present in any of the samples. In a previous
12 work of the same group [19] also a CE-ESI-TOF-MS method was used for the
13 determination of catecholamines (dopamine, norepinephrine [and](#) epinephrine) and their
14 O-methoxylated metabolites (3-methoxytyramine, normetanephrine, metanephrine) in
15 urine. In this case the capillary was coated with polyvinyl alcohol and the injection of
16 the samples was carried out electrokinetically. Catecholamines and metanephrines were
17 removed from the urine samples and preconcentrated by SPE using cation-exchange
18 sorbents (Oasis MCX) with mean recovery values over 80% for all the analytes, except
19 for epinephrine (75%).

20 Vuorensola et al [20] have also analyzed eight catecholamines in aqueous and
21 alcoholic (ethanol, methanol and 1-propanol) non-aqueous solutions by CE-MS but in
22 this case using sheathless nanospray coupling. A comparison was made between
23 different separation electrolytes for the separation of these compounds. Although non-
24 aqueous media (in methanol) was more efficient than water, both methods were applied
25 to the analysis of urine samples extracted with Oasis HLB cartridges using a previously

1 developed protocol [21]. The sensitivity of the non-aqueous nanospray method (0.48-
2 1.30 μM) was only slightly better than that of a previous aqueous method using coaxial
3 sheath-liquid coupling [22].

4 SPE procedures are often used after the LLE or solid-liquid extraction of the
5 analytes assisted or not by ultrasounds, microwaves, etc. Rodríguez et al [23] have used
6 C_8 cartridges for the extraction of pesticides thiabendazole and procymidone from fruits
7 (apples, grapes, oranges, pears, strawberries) and vegetables (tomatoes) after a suitable
8 sonication of the homogenized samples with methanol:water 1:1 for 15 min. Separation
9 was achieved using a buffer of formic acid-ammonium formate at pH 3.5 with 2% of
10 methanol (the sheath-liquid was the same as the separation buffer). LOQs of the SPE-
11 CE-MS procedure (using also a stacking technique) ranged between 0.005 and 0.05
12 mg/kg, with mean recovery values of 64 and 75% for thiabendazole and procymidone,
13 respectively.

14 Recently, Juan-García et al [24] have also extracted six pesticides
15 (thiabendazole, pyrifenoxy, pirimicarb, pyrimethanil, procymidone and dinosol) from
16 peaches and nectarines with a mixture water:acetone 1:1 (v/v) prior to their SPE
17 extraction with C_{18} cartridges before their CE-MS or CE-MS/MS determination. In this
18 case, a buffer consisting of 0.3 M ammonium acetate-acetic acid pH 4 in 10% methanol
19 (the sheath-liquid had the same composition) was used. Recovery percentages ranged
20 between 58 and 99% with relative standard deviation values (RSD %) between 9 and
21 19%. Under optimized CE-MS/MS conditions the minimum detectable levels of the six
22 pesticides in spiked samples were between 0.01 and 0.05 mg/kg.

23 Sentellas et al [25] described the optimization of a clean-up and preconcentration
24 procedure for the determination of fifteen heterocyclic amines in human urine samples.
25 In this work, Oasis MCX and LiChrolut TSC cartridges were studied by using UV

detection. Peak intensities obtained after clean up for both sorbents were similar for most of the amines; however, Oasis MCX cartridges were selected because they provided slightly better recoveries for some of the amines. When urine samples were analyzed, interferences preventing the analytes identification were observed with both cartridges, that is why a LLE procedure using dichloromethane was used. The optimized clean-up procedure together with a previously published field-amplified sample injection (FASI)-CE-MS method [26] was used for the quantification of heterocyclic amines in hydrolyzed spiked human urine, obtaining LOD down to 0.3 ng/mL.

As it has been previously indicated, BGE solutions as well as sheath-liquid compositions should be volatile enough in CE-MS. In spite of this limitation, several works have appeared in which CDs are used as components of the separation electrolyte to analyze SPE extracts by CE-MS [27-29]. For instance, Servais et al [29] have used nonaqueous CE (NACE)-ESI-MS with heptakis(2,3-di-*O*-acetyl-6-*O*-sulfo)- β -cyclodextrin (HDAS- β -CD) in the BGE for the enantioselective determination of low concentrations of salbutamol in SPE extracts from human urine. The selected separation electrolyte consisted of 10 mM ammonium formate and 15 mM HDAS- β -CD in methanol acidified with 0.75 M formic acid. This approach was applied to the quantitative determination of salbutamol enantiomers in human urine after SPE using Isolute HXC-3 cartridges. The SPE-NACE-MS allowed the determination of both compounds at concentrations ranging from 8 to 14 ng/mL.

2.3 Solid-liquid extraction.

Extraction from solid matrices has to be carried out after an adequate

1 homogenization or trituration of the sample, which can be enhanced (as well as the
2 extraction efficiency) by lyophilization or by the use of liquid-nitrogen. The extraction
3 of organic compounds, for example, involve the desorption of the analytes from the
4 sample matrix and their later dissolution into the solvent, which is controlled by the
5 solubility, mass transfer and matrix effects. Extraction can be improved by either
6 assisting the process with ultrasounds, microwaves, etc. Sonication, for example, helps
7 in the homogenization of the sample and, consequently, it can be used for the rapid and
8 easy extraction of analytes from solid samples. Thus, Groom et al [27] have analyzed
9 nitroaromatic and cyclic nitramine contaminants originated from military explosives
10 and propellants (TNT, TNB, RDX, HMX, CL-20) in soil and marine sediments using
11 sonication with acetonitrile together with sulfobutylether- β -cyclodextrin (SB- β -CD)
12 assisted CE-ESI-MS. In this work, it was also stated that the presence of highly charged
13 SB- β -CDs may affect the identification of target explosive analytes. Optimum BGE
14 consisted of 10 mM SB- β -CD and 10 mM ammonium acetate at pH 6.9 using
15 acetonitrile alone as sheath-liquid.

16 Feng et al [30] have analyzed several alkaloids (aconitine, hypaconitine,
17 mesaconitine, brucine, strychnine, icajine, atropine, novacine) and their hydrolysis
18 products in Chinese medicine preparations (Maqianzi, the seed of *Strychnos pierrian*,
19 and Wutou, aconite root of *Radix aconiti praeparata*) by CE-ESI-MS. Pulverized
20 samples were immersed in methanol overnight and afterwards ultrasonicated for 30 min
21 prior to their CE-MS determination. Goodwin et al. [31] were able to separate and
22 determine herbicides glyphosate and glufosinate and their derivatives
23 (aminomethylphosphonic acid and methylphosphinicpropionic acid) in wheat samples
24 using CE-ESI-MS with a sheathless interface. In this case wheat samples were extracted
25 in a mixture of water-acetone 1:1 with magnetic stirring for 1 h. The separation buffer

1 was 1 mM ammonium acetate/acetic acid at pH 6.3 in a mixture methanol:water (50:50,
2 v/v). The best reproducibility in terms of migration times and peak areas was obtained
3 using a capillary coated with linear polyacrilamide. The extract was directly injected in
4 the CE system and the final LOD was 1 μ M in water and 2.5 μ M in the wheat water-
5 acetone extract.

6 Suomi et al [32] determined five neutral irioid glycosides (cyclopentanol
7 monoterpene derivatives) in plant samples by micellar electrokinetic chromatography
8 (MEKC) using SDS as surfactant. The separation system was coupled via a coaxial
9 sheath-liquid flow ESI interface to a MS using the partial filling (PF) technique to avoid
10 the entrance of the micelles in the MS. The separation, which was optimized by MEKC-
11 UV, was achieved by using a BGE consisting of 100 mM SDS in 20 mM ammonium
12 acetate at pH 9.5 (Figure 2). The compounds were detected as lithium adducts by the
13 addition of 1.0 mM lithium acetate to the sheath-liquid (water-methanol 50:50, v/v).
14 The extraction of the samples was carried out with boiling water for 60 min of the
15 crushed dry leave samples (after 40 min at room temperature to wet the leaves
16 thoroughly), and after evaporation to dryness, the extract was redissolved in Milli-Q
17 water and injected in the CE system. Catalpol, verbenalin, loganin and possibly 10-
18 cinnamoyl catalpol were found in the examination of seven plants species in the genera
19 *Plantago*, *Veronica*, *Melampyrum*, *Succisa* and *Valeriana*. LODs for the iridoid
20 glycosides ranged were 25 mg/L except for catalpol which was 50 mg/L. In a second
21 work by the same group, Suomi et al [33] separated a higher number of irioid
22 glycosides (eleven) in several plants belonging also to the genera *Plantago*, *Veronica*,
23 *Melampyrum*, *Succisa* and *Valeriana* by PF-MEKC-ESI-MS. In this case, extraction of
24 the dry leave samples was also carried out with boiling water for 60 min.

25 Recently, Arráez-Román et al [34] have tested different liquid-phase extraction

procedures to establish which could provide the highest content of polyphenols and bitter acids from hop characterized by CE-MS (optimum BGE was 80 mM ammonium acetate at pH 10.5). For this purpose hop pellets were powdered and extracted with different solvents like hexane, methanol, methanol:water, etc. by shaking. Among them, the extraction with hexane to remove lipids, carotenoids and chlorophylls and later with methanol (to extract sugars, organic acids and phenolic compounds) allowed the detection of the highest number of compounds.

Juan-García et al [35] determined five quinolone residues (danofloxacin, enrofloxacin, flumequine, ofloxacin and pipemidic acid) in chicken and fish by CE-MS by solvent extraction of the minced muscle tissues. A sodium phosphate buffer at pH 7.0 was added to the spiked samples which were later extracted with dichloromethane (rotary shaking). The organic layers were then extracted with 0.5 M NaOH. This aqueous phase was adjusted to pH 7 and extracted with hexane to eliminate the fat and it was then passed through a C₁₈ cartridge following a suitable SPE protocol. Mean recovery values of the whole procedure ranged between 45 and 99% for chicken samples and between 52 and 90% for fish samples. The proposed method is sufficiently sensitive to analyse these quinolone in both samples because the LOQs achieved (50 ng/g) were below the maximum residue limits (100-200 ng/g) established by the EU.

2.4 Solid-phase microextraction (SPME).

SPME was firstly developed by Pawliszyn and co-workers in 1989 and became commercially available in 1993 [36]. Since its development, SPME has been increasingly used since its setup is small and convenient, it can be used to extract analytes from very small samples, it provides a rapid extraction and transfer to analytical instrument and

1 can be easily combined with other extraction and/or analytical procedures improving in a
2 large extent the sensitivity and selectivity of the whole method.

3 The on-line coupling of SPME with CE has been described in several occasions
4 [37, 38], however, the use of such coupling is still a non-resolved topic because of the
5 very small injection volumes required in CE. As a result, SPME-CE analyses are
6 typically carried out in an off-line mode, by manually desorbing the analytes in an
7 appropriate organic solvent, and later introducing it into the CE system. Rodríguez et al.
8 [39] carried out the analysis of a group of pesticides (ioxynil, *o*-phenylphenol,
9 haloxyfop, acifluorfen, picloram) in fruit samples by using SPME prior to CE-MS. In
10 that work, the buffer used consisted of 32 mM HCOONH₄/HCOOH at pH 3.1 while the
11 sheath-flow was made of 32 mM separation buffer with a 20 % of methanol with 14
12 µL/min flow. After testing different SPME fibers, the use of CW-TPR allowed the
13 extraction of these pesticides from water and fruit samples down to 0.02-5 mg/kg
14 (LOQ).

15 Hernández-Borges et al. [40] tested different SPME fibers and CE-MS for the
16 extraction and quantitative determination of a group of pesticides (pyrimethanil,
17 pyrifenox, cyprodinil, cyromazine and pirimicarb) in orange and grape juices. The
18 buffer used consisted of a volatile aqueous solution containing 0.3 M ammonium
19 acetate/acetic acid at pH 4 while the sheath-liquid was made of a mixture
20 isopropanol:water (65:35, v/v) at 0.22 ml/h flow. In this case, SPME parameters (e.g.
21 extraction time, sodium chloride percentage, pH and desorption time) were optimized
22 by means of a chemometrical approach. The best results were achieved by direct
23 immersion of a PDMS-DVB fiber which allowed achieving LODs of these pesticides at
24 concentrations down to 15 ng/mL in water samples and down to 40 ng/mL in fruit
25 juices.

Other SPME modifications like stir-bar sorptive extraction (SBSE) [41] or fiber-in-tube SPME [42] have not yet been combined with CE-MS.

2.5 Pressurized liquid extraction (PLE).

Pressurized liquid extraction (PLE), also called accelerated solvent extraction, is a sample preparation technique in which a solvent at elevated temperature and pressure is used as extractant. By adequately choosing the solvent, its temperature and pressure it is possible to control, among other factors, the dielectric constant of the extractant and with that the polarity of the compounds that can be obtained. Moreover, PLE works in an automatic way, it requires small amounts of solvents and low extraction times. Therefore, PLE can provide fast extractions and purifications allowing testing a high number of extraction conditions under controlled conditions.

The possibilities of the combined use of PLE and CE-MS were recently demonstrated by Herrero et al [43-45]. PLE-CE-MS was applied to the extraction and characterization of the main antioxidants (i.e., polyphenols) from rosemary [43] and the extraction and characterization of phycobiliproteins from the microalga *Spirulina platensis* [44, 45]. In this latter case, a thorough optimization of both the PLE extraction conditions (including sonication of the sample prior to PLE) and CE-MS conditions had to be carried out, demonstrating that PLE-CE-MS can be a fast, automatic and highly informative method for natural products investigations [46].

2.6 Other procedures.

Apart from the previously described sample treatment procedures, Table 1 also

1 shows different and interesting alternatives for this purpose. Thus, introduced in the
2 mid-nineteenth century, soxhlet extraction has been one of the extraction methods more
3 used until the development of modern extraction techniques. The need of cooled,
4 condensed solvents for the extraction makes this technique a slow alternative (up to 24-
5 48 hours of extraction) with a very high consumption of organic solvents that have to be
6 evaporated, although with very high recoveries and also with multiple sample extraction
7 possibilities. Concerning its combination prior to CE-MS several approaches have
8 appeared [47-49]. Very recently, Edwards et al [48] have used soxhlet extraction in
9 combination with LLE and SPE for the characterization by CE-ESI-MS of secondary
10 metabolites (flavonoids) from the antihyperglycaemic plant *Genista tenera*. In this case,
11 air-dried and powdered plants were extracted in a soxhlet apparatus with ethanol. After
12 filtration and evaporation the residue was redissolved in water and extracted
13 successively with diethyl ether, ethyl acetate and butanol. After another evaporation and
14 redissolution of part of the extract a SPE procedure with C₁₈ was carried out. Optimum
15 buffer was composed of water:2-propanol (95:5, v/v) containing 10 mM ammonium
16 carbonate at pH 9.25. The CE-MS study of the extract allowed the identification of five
17 flavonoid aglycones, five flavonoid-monoglycosides, two flavonoid-diglycosides, one
18 flavonoid-triglycoside, three monoacetyl-flavonoids, one diacetyl-flavonoid and one
19 acetyl-flavonoid-glycoside. Wahby et al [49] have also used soxhlet extraction for the
20 extraction of atropine (tropane alkaloid) and choline (quaternary base) in hairy root
21 cultures of *Cannabis sativa* L. Hairy root cultures were rinsed with tap and distilled
22 water, frozen in liquid nitrogen and lyophilized. The dry material was ground to a fine
23 powder and extracted in a soxhlet apparatus with 70% of aqueous methanol for 16 h.
24 After cooling, the extracts were filtered and concentrated. Both compounds could be
25 determined in the samples with LODs of 18 mg/L for choline and 320 µg/L for atropine

1 using a BGE of 20 mM ammonium acetate at pH 8.5 and a sheath-liquid composed of
2 50:50, v/v 2-propanol:water with 0.5% (v/v) formic acid 0.18 mL/h.

3 The combination of automated sample preparation in CE is especially useful for
4 the analysis of complex samples [50] since it can improve the selectivity and sensitivity
5 of the determination as well as to decrease the time involved in the sample treatment
6 [50, 51]. One of the main lines of research in this area is the combination of flow
7 injection systems with CE and, in a less extent, with CE-MS. Thus, Santos et al [52]
8 reported a new method for the separation and detection of 9 biogenic amines by the
9 used of a flow manifold coupled to a CE-ESI-MS for the automatic filtration of the
10 samples and their insertion into the CE vials. The on-line filtration was carried out using
11 a flow injection system coupled to the CE instrument. The BGE was composed of 25
12 mM citric acid at pH 2.0. Two injection modes (hydrodynamic and electrokinetic) were
13 tested. Although electrokinetic injection provided better sensitivity, it was also found to
14 give worse precision and linear range and, therefore, hydrodynamic injection was
15 selected. The method allowed the detection of amines between 0.018 and 0.09 µg/mL.
16 The method was applied to the determination of biogenic amines in red and white wines
17 with mean recovery values around 100%.

18 The use of microwave radiation for sample pretreatment has attracted growing
19 interest in the past few years and has yield a numerous amount of publications [53-56].
20 Microwave radiation provides a homegeneous and instant heating of the sample
21 yielding into very quick and effective extraction/digestion and thus strongly decreasing
22 sample pretreatment times. Van Lierde et al [57] used microwave-assisted acid
23 digestion of porcine and human skin to extract chromium species from these samples.
24 The mechanism of chromium transport through the skin and the relationship between
25 chromium allergy and chromium species (in vitro permeation experiments) was studied.

1 For this purpose, CE-was used with inductively coupled plasma-mass spectrometry
2 (ICP-MS) using a BGE composed of 50 mM phosphate buffer (pH 2.5). For the
3 digestion of the samples, skin membranes were dried at 30°C for 24 hours, after that
4 HNO₃ and H₂O₂ were added. Digestion was carried out at different microwave
5 intensities for a total of 25 min. The LODs of the method ranged between 6 and 12 µg
6 of Cr per liter.

8 3 Microfluidic devices.

10 Clearly, development and/or use of microchip-CE are not objectives of this
11 paper, however, microchip-CE devices deserve a special attention because they can
12 automate sample preparation and, furthermore, they can integrate this step together with
13 the chemical analysis under a single format that may allow a ultrarapid and sensitive
14 analysis of the target analytes [58]. However, at the moment most of the applied
15 approaches suffer from several limitations regarding their fabrication, manipulation or
16 the LODs that can be achieved. This can explain the very low number of publications
17 found showing the on-line coupling of microchip-CE with MS.

18 A recent application of a microbead-packed polydimethylsiloxane (PDMS)
19 microchip with an integrated electrospray emitter for sample pretreatment prior to
20 sheathless ESI-TOF-MS was presented by Lindberg et al [59]. This system was applied
21 for the desalting and enrichment of six neuropeptides from a physiological solution.
22 Figure 3 shows a schematic picture of the PDMS microchip design used in that work.
23 Electrical contact for the sheathless ESI was achieved by coating the integrated emitter
24 with a conductive graphite powder after applying a thin layer of PDMS as glue. Both
25 the coating and the bond of the PDMS structures were found to have a very good

1 durability (a continuous spray was obtained over 800 h). Another PDMS microfluidic
2 system was previously developed by the same group [60] and applied to the analysis of
3 peptides but in this case only sample injection, separation and ESI emitter structures
4 were integrated in a single platform. As found in the literature, PDMS microchips with
5 an integrated ESI-emitter have been fabricated using different principles [61-66]. As an
6 example, Dahlin et al [62] presented a PDMS-based microchip for in-line SPE-CE with
7 an integrated electrospray emitter tip coupled to a TOF-MS. The chip was fabricated in
8 such a way that mixed PDMS was cast over steel wires in a mold. The removed wires
9 defined 50 µm cylindrical channels where fused silica capillaries were inserted. The
10 microchip was fabricated in a two-level cross design. In one of these channels
11 hypercross-linked polystyrene beads acted as SPE sorbent for desalting. In this work,
12 six-peptide mixtures at different concentrations were dissolved in physiological salt
13 solutions and injected, desalted, separated and sprayed into the MS for the analysis.
14 LODs were in the femtomole levels.

15 Microfluidic devices have also been used for in-line digestion of proteins [67,
16 68]. Wang et al [67] have proposed the use of a microfluidic device with a CE channel
17 connected to a MS (via an ESI interface) which contains a digestion bed on a monolithic
18 substrate to carry out the in-line protein digestion. The application of this device for the
19 rapid digestion, separation and identification of proteins was demonstrated for melittin,
20 cytochrome c and bovine serum albumin. The rate and efficiency of the digestion was
21 related to the flow rate of the substrate solutions through the reactor. For cytochrome c
22 and bovine serum albumin the digestion time was 3-6 min at room temperature, while
23 for melittin was 5 s. Microdevices provide a convenient platform for automated sample
24 processing in proteomic applications.

25

4 Use of stacking techniques in CE-MS.

Although strictly speaking the use of stacking techniques (except on-line SPE) cannot be considered part of the sample treatment, we would like to include a brief comment here about the use of these techniques together with CE-MS. On-line preconcentration strategies based on sample stacking [69-71], sweeping [72] and/or solid-phase extraction (SPE) [73-75] have shown their usefulness for improving the limits of detection (LOD) achieved by CE. Concerning the use of these techniques together with CE-MS they are not easy to apply and, in some cases, their use is limited. For instance, many of these preconcentration strategies involve the use of CDs, surfactants, and other non-volatile compounds that are precluded in CE-MS or can affect the stability of the electrical circuit in CE-ESI-MS. For example, the use of stacking with matrix removal (SWMR) is not possible in CE-MS since there is not outlet vial necessary in this case to reverse the polarity and to eliminate the sample matrix. In Table 1 it can be observed that these techniques are not widely applied in CE-MS.

The use of the electrokinetic injection in the mode called field-enhancement sample injection (FESI) also called field-amplified sample injection (FASI) or field-amplified sample stacking (FASS) is one of the most commonly stacking techniques in CE-MS, because, despite the presence of the siphoning effect that can take place, the sensitivity improvement can be high, although in this case only one type of charged analyte (cations or anions) can be introduced into the capillary [25, 26, 76-79].

Hernández-Borges et al [17] have used normal stacking mode (NSM) for the preconcentration of pesticides after their SPE extraction from soy milk samples. This technique is easy to apply because only a low conductivity matrix is required (which

1 can be achieved by the use of organic solvent) since focusing takes place due to the
2 abrupt change in the local electric field between the sample matrix and the BGE. In this
3 case, the stacking was achieved by injecting a high amount of the sample (up to 100 s at
4 20 psi) dissolved in pure acetonitrile. This specific type of stacking is often called
5 *acetonitrile stacking* because of the good sensitivity improvement that the use of
6 acetonitrile alone in the sample matrix has provided, which has also been observed by
7 different authors [\[80-84\]](#).

9 **5 Conclusions and future outlook.**

10
11 Some current trends in the today's sample pretreatment area are expected to
12 continue in the future as important research areas within this attractive field of
13 Analytical Chemistry. This is the case for the search of new extraction materials
14 including the development of molecular imprinted polymers (MIPs) to adsorb specific
15 analytes mimicking for instance immunorecognition. These new extraction materials
16 can play a definitive role in the development of completely automated analytical
17 processes able to provide information on analyte composition and concentration without
18 the intervention of the operator. In this regard, the integration of sample preparation
19 devices into miniaturized formats (e.g., microchips, μ -TAS) seem to be a very attractive
20 way to achieve this goal while increasing even more the throughput and analysis speed
21 of these methods. These future procedures combined with on-line stacking techniques
22 and CE-MS can give rise to an ever more impressive and powerful analytical system.

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Figure captions

Figure 1.- NSM-CE-UV electropherogram of: A) a standard solution containing ca. 1 mg/L of each pesticide and; B) a SPE extract from soy milk sample containing 200 µg/L of each pesticide. Injection: 60 s at 0.5 psi. Running electrolyte: 24 mM formic acid, 16 mM ammonium carbonate at pH 6.4; Total length: 57 cm (50 cm effective length); Voltage: +23 kV; Temperature: 22°C. (1) Metosulam; (2) Cloransulam-methyl; (3) Diclosulam; (4) Florasulam; (5) Flumetsulam. C) Extracted ion electropherograms of a soy milk sample containing 200 µg/L of each pesticide analyzed under SPE-NSM-CE-ESI-MS optimized conditions. Redrawn from [17] with permission.

Figure 2.- (a) An on-line PF-MEKC-ESI-MS electropherogram of the mass spectrometric data. The capillary was 80 cm long and applied voltage was +15 kV (current 10 µA). Sample was injected at 50 mbar pressure for 10 s. The electrolyte solution contained 20 mM ammonium acetate at pH 9.2 and a solution of 100 mM SDS was injected for 200 s at 50 mbar pressure. The sheath-liquid contained 1 mM lithium acetate dissolved in water-methanol (50:50 v/v) and it was pumped to the electrospray interface at 180 µL/min. Mass area of 100-600 m/z was scanned. (b) A PF-MEKC-UV electropherogram of the sample of (a). Compounds were detected at 20 cm. Peak assignments: (1) catalpol; (2) ketologanin; (3) verbenalin; (4) loganin; (7) 10-cinnamoyl catalpol. Reprinted from [32] with permission.

Figure 3.- Schematic picture of the PDMS microchip design. A) Shows the microchip as mounted on the holder in front of the MS. Graphite coated emitter was placed between two brass plates (B) to which the high voltage was applied. Holder (C) was

- 1 mounted on an *xyz*-adjustable table for easy alignment of the microchip in front of the
- 2 TOF-MS orifice. Reprinted from [\[59\]](#) with permission.
- 3

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Table 1.- Some examples of sample treatments, analytes and matrices studied by CE-MS.

Analyte	Matrix	Treatment	Interface	Analyzer	Buffer	Observations	References
Hops acids, oxidized derivatives and iso- α -acids	Beer, hops pellets	Extraction (acetone/water) for hops pellets; SPE for beer	ESI (sheath-liquid: 2-propanol:water 50:50 v/v, 0.1% TEA, 3 μ L/min)	IT	160 mM (NH ₄) ₂ CO ₃ -NH ₄ OH pH 9	-	[85]
Glycoproteins	Bovine proteins	Microcon filtration, enzymatic deglycosilation	ESI (sheath-liquid: 1% HOAc in 2-propanol:water 1:1, 4 μ L/min)	IT, TOF	Several buffers	Coated capillary	[86]
Peptides	Horse cytochrome c and myoglobin	Enzymatic digestion, SPE	ESI (sheath-liquid: 0.1% HCOOH in 50% MeOH, 3 μ L/min)	IT	20/40/40 ACN/100 mM HCOONH ₄ pH 3/water v/v/v	FASII. LOD: 10 ⁻⁹ M	[79]
Carbohydrates	Urine	-	ESI (sheathless)	QTOF	50 mM NH ₄ Ac-32% NH ₃ pH 11 and 12	-	[87]
Glycopeptides	Plasma	Lyophilization, digestion	ESI (sheath-liquid: 2-propanol-water 1:1 v/v, 0.4% HCOOH, 2.5 μ L/min)	QIT	50 mM HCOONH ₄ pH 2.7; 50 mM triethylammonium acetate pH 5.0; 50 mM HCOONH ₄ pH 8.0	MS ⁿ , off line MALDI-TOF-MS, coated capillaries	[88]
Tobacco-N-nitrosamines	Rabbits' serum	SPE	ESI (sheath-liquid: MeOH-water 50:50 v/v, 0.5% formic acid, 10 μ L/min)	IT	75 mM ammonium formate (pH 2.5) or citrate (pH 2.4)	-	[89]
Cytokinins	Coconut water	SPE	ESI (sheath-liquid: 0.3% formic acid in 50% v/v MeOH:water, 4 μ L/min)	IT	25 mM ammonium formate/formic acid (pH 3.4), 3% v/v ACN	MS ² , stacking, LOD: 0.05-0.18 μ M	[84]
Phenolic compounds	Virgin olive oil	SPE	ESI (sheath-liquid: 2-propanol:water 60:40 v/v, 0.1% v/v TEA)	IT	60 mM NH ₄ OAc pH 9.5 with 5% 2-propanol	Standards obtained by semipreparative HPLC	[90]
Neuropeptides	Physiological salt solution	Microbead-packed PDMS microchip	ESI (sheathless)	TOF	25:75 v/v ACN:10 mM acetic acid	Microchip; LOD: 20 fmol	[59]
Chromium species	Porcine and human skin	Microwave assisted digestion	ICP	SF	50 mM phosphate buffer pH 2.5	LOD: 6-12 μ g/L	[57]
¹⁰ B-BPA (boronophenylalanine)	Cell culture	Trypsin digestion, freeze-thawing cycles-ultrasonication, ultrafiltration	ESI (sheath-liquid: 5 mM NH ₄ Ac in 50 % v/v MeOH- water, 10 μ L/min)	IT	0.5 M HCOOH	LOD: 3 μ M; use of HR-ICP-MS	[91]
Flavonoids	Plant (<i>Genista tenera</i>)	Soxhlet, LLE, SPE	ESI (sheath-liquid:	IT	Water:2-propanol 95:5, v/v,	MS ²	[48]

			IPA: water 50:50 v/v, 0.5 μ L/min)		10 mM ammonium carbonate (pH 9.25)		
Choline, atropine	Hairy root cultures of <i>Cannabis sativa</i> L.	Soxhlet	ESI (sheath-liquid: 50:50 v/v 2-propanol:water, 0.5% v/v formic acid 0.18 mL/h)	IT	20 mM NH ₄ OAc, pH 8.5	LOD: 18 mg/L (choline), 320 μ g/L (atropine)	[49]
Polyphenols, bitter acids and oxidation products	Hops	Extraction with different solvents	ESI (sheath-liquid: 60:40 v/v, 2-propanol:water, 0.1% TEA, 0.28 mL/h)	IT	80 mM NH ₄ OAc/NH ₄ OH, pH 10.5	Characterization of the methanolic extract of hops	[34]
Quinolone residues	Chicken, fish	Solvent extraction, SPE	ESI (sheath-liquid: 60 mM (NH ₄) ₂ CO ₃ , pH 9.2, 10 μ L/min)	QIT	60 mM (NH ₄) ₂ CO ₃ , pH 9.2	MS ⁿ , LOD: 20 ng/g	[35]
Glycopeptides	Urine	Gel filtration chromatography, anion exchange chromatography	ESI (sheathless)	QTOF	0.1 M HCOOH in MeOH:water 6:4, v/v	LOD: 0.05-0.25 mg/mL	[92]
Isoquinoline alkaloids	Herb(<i>Fumaria officinalis</i>) and phytopharmaceuticals	Soxhlet, LLE, SPE, ultrasounds	ESI (sheath-liquid: isopropanol-water 1:1 v/v, 3 μ L/min.)	IT	ACN-MeOH 9:1 v/v, 60 mM NH ₄ OAc and 2.2 M HOAc	MS ²	[47]
Antioxidants	<i>Rosmarinus officinalis</i> L.	PLE	ESI (sheath-liquid: 2-propanol-water 60:40 v/v, 0.1% v/v TEA; 0.24 mL/h)	IT	40 mM NH ₄ OAc/NH ₄ OH, pH 9	-	[43]
Nitroaromatic and cyclic nitramines	Soil and marine sediment	ACN sonication	ESI (sheath-liquid: 100% ACN, 6 μ L/min)	QIT	10 mM SB- β -CD-10 mM NH ₄ OAc (pH 6.9)	LOD: 0.025-0.5 mg/L	[27]
Caffeine and metabolites	Urine	SPE	ESI (sheath-liquid: MeOH-water-HCOOH 79.7:19.8:0.5 v/v/v, 0.5 mL/min)	Q	50 mM (NH ₄) ₂ CO ₃ , pH 11.0	-	[93]
Pesticide residues	Peaches and nectarines	Solvent extraction, SPE	ESI (sheath-liquid: 0.3 M NH ₄ OAc-HOAc, pH 4, in 10% MeOH, 5 μ L/min)	QIT	0.3 M NH ₄ OAc-HOAc, pH 4, in 10% MeOH	MS ³ , LOQ: 0.001-0.2 mg/kg	[24]
Salbutamol enantiomers	Human urine	SPE	ESI (sheath-liquid: ACN-water 75/25 v/v, 0.1% HCOOH, 2.5 μ L/min)	IT	10 mM HCOONH ₄ and 15 mM HDAS- β -CD in MeOH, 0.75 M HCOOH	LOQ: 18-20 ng/mL	[29]
Methylenedioxy-derivates of amphetamine	Urine	SPE	ESI (sheath-liquid: ACN-water-HOAc 50:49.5:0.5)	IT	50 mM NH ₄ OAc/ HOAc, pH 4.5	LOD: 0.31-4.29 ng/mL	[94]
Pesticides	Soy milk	SPE	ESI (sheath-liquid: ACN/water 82.5:17.5 v/v, 2% TEA, 0.35 mL/h)	IT	24 mM HCOOH and 16 mM (NH ₄) ₂ CO ₃ , pH 6.4	LOD: 74-150 μ g/L	[17]
Phosphorylated and acidic	<i>Escherichia coli</i> DH5- α	Cell lisation	ESI (sheathless)	QIT	80% v/v 20 mM NH ₄ OAc	MS ²	[95]

metabolites in prokaryotes					pH 9.5 and 20% v/v 2-propanol		
Selenium	Nuts (<i>Bertholletia excelsa</i>)	Defatted nuts hydrolyzation	ICP	Q	Ammonium pH 9.25 with 2% v/v OFM anion-BT	Study of the association of selenium to proteins; electrokinetic injections	[96]
Polypeptides and proteins	Urine	SPE, lyophilization	ESI (sheath-liquid: 30% MeOH, 0.5% HCOOH)	TOF	30% MeOH, 0.5% HCOOH, 69.5% water	Identification of protein pattern in Type 1 diabetics	[97]
Neuropeptides	-	SPE	ESI (sheathless)	TOF	25:75 ACN:10 mM HOAc	PDMS microchip, LOD: 0.1 µg/ml	[62]
Polypeptides	Human urine	Ultrafiltration, SPE, lyophilization	ESI (sheath-liquid: 30% v/v isopropanol, 0.4% v/v HCOOH, 2 µL/min)	TOF	20 % ACN, 0.25 M HCOOH, 79.5% water	Identification of polypeptides and patterns of polypeptides specific for prostate cancer	[98]
Lipophilic peptaibol alamethicin	Culture broth of <i>Trichoderma viride</i>	Preparative HPLC	ESI (sheath-liquid: 2-propanol:water 1:1 v/v; 1% HCOOH; 4 µL/min)	IT, TOF	NACE: 12.5 mM HCOONH ₄ in MeOH (pH _{app} = 7.4) Aqueous: 25 mM borate pH 11.0	MS ⁿ	[99]
Peptide mixture	-	Digestion	ESI (sheath-liquid: MeOH-water 50:50 v/v; 0.05 v/v HCOOH, 4 µL/min)	IT	0.9 M HCOOH, pH 2	Peptide modeling; characterization of enzyme cleavage patterns. MS ²	[100]
Amino acids	Orange juice	Derivatization with FITC and DNS	ESI (sheath-liquid: MeOH-water 50:50 v/v with 25% 100 mM NH ₄ Ac pH 6.5 mM β-CD; 3.5 µL/min)	IT	100 mM NH ₄ Ac pH 6.5 mM β-CD	Capillary coating,	[101]
γ-glutamyl-S-ethenyl-cysteine (GEC)	<i>Vicia narbonneensis</i> L. seeds	Solvent extraction stirring or ultrasounds	ESI (sheath-liquid: MeOH-water 50:50 v/v 0.1% v/v HOAc, 3 µL/min)	IT	20 mM NH ₄ HCO ₃ pH 7	LOD: 0.021 mg/mL	[102]

Proteins	<i>Spirulina platensis</i> microalga	Sonication, PLE, ultrafiltration, precipitation-dialysis-freeze drying	ESI (sheath-liquid: water-2-propanol 75:25 v/v, 0.5 % v/v HOAc, 6 µL/min)	IT	40 mM ammonium hydrogen carbonate pH 7.8 in water-ACN-2-propanol 45:50:5 % v/v/v	PLE optimization	[45]
Proteins	<i>Spirulina platensis</i> microalga	Sonication, PLE, freeze drying	ESI (sheath-liquid: water-2-propanol 75:25 v/v, 0.5 % v/v HOAc, 6 µL/min)	IT, TOF	40 mM ammonium hydrogen carbonate pH 7.8 in water:ACN 2-propanol 45:50:5 % v/v/v	-	[44]
Anthocyanins	Wine and wine musts	SPE	ESI (sheath-liquid: MeOH:water 80:20, 0.25% v/v HOAc)	IT	200 mM monochloroacetate-ammonium, pH 2 or 200 mM borate-ammonium, pH 9	Acidic and basic BGE. LOD: 0.8-1.5 mg/L (acidic); 4-10 mg/L (basic)	[103]
Antidepressants	Water	SPE	ESI (sheath-liquid: 5 mM HCOONH ₄ in 8:2 isopropanol/water, 1 µL/min)	QTOF	1.5 M HCOOH, 50 mM HCOONH ₄ in ACN/water 85:15	LOD: 22-280 µg/L	[104]
Basic proteins	Chicken and turkey egg white, wine, minced meat	Lyophilization (white egg); meat (homogenization and buffer extraction)	ESI (sheath-liquid: MeOH:water 50:50 v/v, 0.05 % v/v HOAc 4 µL/min)	IT	75 mM NHOAc/ HOAc, pH 5.5	Polymer capillary coating; LOD: 2.9 fmol; adulteration detection	[105]
Manganese	Liver	Homogenization, liquid nitrogen, extraction in Tris-HCl	ICP	Q	10 mM Tris-HCl pH 7.4	Speciation study; LOD: 1.1 µg Mn/L	[106]
Glycoproteins	Plasma	Affinity chromatography, lyophilization	ESI (sheath-liquid: 2-propanol/1 M HOAc 1:1 v/v, 3 µL/min)	QIT	1 mM HOAc, 4 M urea	Characterization of glyco iso forms, coated capillaries	[107]
Oxycodone phase I and II metabolites	Urine	SPE	ESI (sheath-liquid: water-methanol 1:1 v/v, 1% formic acid, 5.0 µL/min)	IT	20 mM ammonium acetate pH 9	MS ⁿ , computer simulation of fragmentation	[108]
Benzodiazepines	Urine	SPE	ESI (sheath-liquid: MeOH-water 80:20 v/v, 2 µL/min)	IT	100 mM formic acid, 1 mM TEA	MS ² , use of dynamically coated capillaries (CEo fix); LOD: 50-100 ppb	[109]
Heterocyclic amines	Urine	LLE-SPE	ESI (sheath-liquid: MeOH-20 mM HCOOH 75:25, 5 µL/min)	IT	16 mM HCOOH/HCOONH ₄ 40 mM pH 4.5, 60% MeOH	LOD: 0.3-45 ng/mL	[25]

Imazamo x pesticide	Water	SPE	ESI (sheath-liquid: MeOH HCOONH ₄ (5 mM) 50:50 v/v, pH 3.7, 4 µL/min)	IT	10 mM HCOONH ₄ in 0.01% MeOH-water, pH 7.0	LOD: 20 ng/L	[110]
Glycosamino glycan oligosaccharides Serotonin, tryptophan and 5-hydroxytryptophan	Human embryonic kidney 293 cells	Dialysis, lyophilization, digestion SPE	ESI (sheathless)	QTOF	50 mM NH ₄ OAc pH 12.0 in water:MeOH 40:60 v/v	MS ²	[111]
	Plasma		ESI (sheath-liquid: MeOH/water 60:40 v/v, 0.2% HCOOH, 2 µL/min)	TOF	1.5% HCOOH (pH 2.07)	LOD: 0.13-3.23 µM	[18]
Pesticides	Fruit juices	SPME	ESI (sheath-liquid: isopropanol-water 65% v/v, 0.22 mL/h)	IT	0.3 M HOAc, pH 4	Chemometric optimization. LOD 40-150 µg/L	[40]
9 biogenic amines	Red and white wines	FI system	ESI (sheath-liquid: 70:30 v/v MeOH/water, 1.0% HCOOH, 4 µL/min)	Q	25 mM citric acid pH 2	LOD 0.018-0.09 µg/mL	[52]
Pesticides	Water, grape, apple, orange, tomato	SPME	ESI (sheath-liquid: 32 mM HCOONH ₄ -HCOOH pH 3.1 + 20% MeOH, 14 µL/min)	Q	32 mM HCOONH ₄ -HCOOH pH 3.1	LOQ: 0.02-5 mg/Kg	[39]
Peptides and proteins	Urine	SPE, lyophilization	ESI (sheath-liquid: 30% v/v MeOH, 0.5% v/v HCOOH, 5 µL/min)	TOF	30% v/v MeOH, 0.5% v/v HCOOH, pH 2.4	-	[112]
Dopamine and methoxycatecholamines	Urine	Enzymatic hydrolysis, cation exchange extraction, SPE	ESI (sheath-liquid: MeOH-water 80:20 v/v, 0.5 % v/v HOAc, 6 µL/min)	IT	50 mM NH ₄ OAc HOAc, pH 4	Comparison CE-UV, CE-MS with LC-EC; LOD: 0.7-1.4 µM	[113]
Alkaloids	Herbs (<i>Strychnos piaman</i> , <i>Radix aconiti praeparata</i>)	Ultrasonication	ESI (sheath-liquid: water-MeOH 1:9 v/v, 0.5% HOAc, 3 µL/min)	QIT	NH ₄ OAc, HOAc, MeOH	-	[30]
Pesticides	Wheat	Extraction with water-acetone	ESI (sheathless)	IT	1 mM NH ₄ Ac/HOAc (pH 6.3) in MeOH:water 50:50 v/v	Coated capillary; LOD: 2.5 µM	[31]
Heterocyclic aromatic amines	Urine	LLE, SPE	ESI (sheath-liquid: MeOH:20 mM HCOOH 75:25, v/v, 3 µL/min)	IT	16 mM HCOOH/HCOONH ₄ , pH 4.5, 60% MeOH	FASI. LOD: 0.8-21 ng/g	[26]
Enantiomeric drugs	Plasma	LLE	ESI (sheath-liquid: ACN/5 mM NH ₄ OAc/HCOOH 75:25:0.1 (v/v), 2 µL/min)	TOF	25% MeOH, 75% 5 mM NH ₄ OAc (pH 6), 1.0% HOAc, 0.3% HS-β-CD	Electrokinetic injection; LOQ: 10 ng/mL	[114]
Polypeptides	Dialysis fluids, urine,	Anion exchange	ESI (sheath-liquid: 30%	TOF	30% MeOH and 0.5%	Polipeptide pattern	[115]

	serum	chromatography, lyophilization	MeOH, 0.5% HCOOH, 69.5% water, pH 2.3-2.5, 10 µL/min)		HCOOH, 69.5% water, pH 2.3-2.5	stablishment	
Substituted methoxy phenols and aromatic acids	Biomass burning aerosol	Filter extract (water)	ESI (sheath-liquid: water 50% 2-propanol 50% v/v, 3 µL/min)	IT	20 mM NH ₄ OAc-10% MeOH pH 9.1; 1 M NH ₄ OH pH 11	LOD: 0.1-1.0 µM	[1 16]
Glycoalkaloids and relative aglycones	Potatoes	Extraction with MeOH	ESI (sheath-liquid: MeOH-water 1:1 v/v, 1% HOAc, 2.5 µL/min)	IT	90:10 v/v MeCN-MeOH containing 50 mM NH ₄ OAc 1.2 M HOAc	MS ² ; LOD: 10-50 µg/L	[1 17]
Irioid glycosides	Plants	Water extraction	ESI (sheath-liquid: 1.0 mM lithium acetate in water-MeOH 50:50 v/v, 200 µL/h)	IT	100 mM SDS in 20 mM ammonium acetate, pH 9.5	LOD: 15-50 mg/L; calculation of water- micelle partition coefficients	[33]
Procymidone and thiabendazole	Fruits, vegetables	Sonication, SPE	ESI (sheath-liquid: 20 mM HCOOH -12 mM HCOONH ₄ pH 3.5 with 2% MeOH, 13 µL/min)	Q	20 mM HCOOH -12 mM HCOONH ₄ pH 3.5 with 2% MeOH	LOQ: 0.005-0.05 mg/kg	[23]
Oxycodone and major metabolites	Urine	SPE, LLE	ESI(sheath-liquid: water- methanol 50:50 v/v, 1% formic acid, 5 µL/min)	IT	25 mM ammonium acetate pH 9	MS ⁿ , hydrodynamic injection (LOD:10- 300 ng/ml) and FASS (LOD: 1-50 ng/ml)	[77]
Catecholamines	Urine	SPE	ESI (sheathless)	QQQ	Various BGE containing NH ₄ OAc, water, MeOH, ethanol, HOAc, propanol	LOD: 0.48-1.30 µM	[20]
Catecholamines and metanephrines	Urine	SPE	ESI (sheath-liquid: 75:25:0.1 MeOH/water/HOAc v/v, 1.5 µL/min)	TOF	1% HOAc (pH 2.8)	Electrokinetic injection	[19]
Furosemide	Urine	SPE	ESI (sheath-liquid: MeOH-water-ammonia 50:49:1 v/v, 5 µL/min)	IT	20 mM NH ₄ OAc pH 9 with TEA	MS ²	[1 18]
Opioids	Urine	SPE, LLE	ESI (sheath-liquids: MeOH-water 60:40 v/v, 1% HOAc or 1% HCOOH, 3 µL/min or 5 µL/min)	IT	25 mM NH ₄ OAc pH 9	Use of FASS	[76]
Drugs	River water	LLE, SPE	ESI (sheath-liquid: 2- propanol-water 80:20 v/v, 0.1% v/v HOAc or 0.1% v/v TEA; 4 µL/min)	Q	20 mM NH ₄ OAc pH 5.1	LOD: 18-134 µg/L	[1 19]

Steroidal alkaloids	Leaves and seeds (<i>Solanum sodomaeum</i>), berries (<i>Solanum elaeagnifolium</i>)	Extraction with ethanol and HCl	ESI (sheath-liquid: isopropanol-water 50:50 v/v, 0.5% HCOOH; 3 µl/min)	Q	25 mM NH ₄ OAc and 1 M HOAc in MeOH-ACN 20:80 v/v	LOD: 0.05 µg/mL	[120]
Irioid glycosides	Plants	Water extraction	ESI (sheath-liquid: 1.0 lithium acetate in water-MeOH 50:50 v/v, 200 µL/h)	IT	100 mM SDS in 20 mM NH ₄ OAc, pH 9.5	LOD: 25-50 mg/L	[32]
Morphine and related opioids	Urine	LLE, SPE	ESI (sheath-liquid: MeOH:water 60:40 v/v, 1% HOAc, 3 µl/min)	IT	25 mM ammonium acetate and NH ₃ (pH 9)	MS ³ , LOD 100-200 ng/mL	[14]
Gonadotropin-releasing hormone	Serum, urine	Immunoaffinity CE	ESI (sheath-liquid: 20 mM HOAc in 50% MeOH, 0.5 ml/min)	Q	60 mM NH ₄ HCO ₃ pH 8.0, 1% v/v ACN	-	[121]
Tramadol and its main phase I metabolite	Plasma	LLE	ESI (sheath-liquid: Isopropanol-water 1:1 v/v, 0.5% formic acid, 3 µl/min)	Q	40 mM ammonium acetate buffer, pH 4.0, sulfobutyl ether β-CD (2.5 mg/ml)	Partial filling	[12]
Etodolac and its urinary phase I metabolites	Urine	LLE	ESI (sheath-liquid: ACN-10 mM ammonium formate pH 3.0 1:1 v/v, 3 µl/min)	IT	ACN-10 mM ammonium formate pH 3.0 1:1 v/v	CEC (C ₁₈ capillaries); electrokinetic injection	[13]
Nitrocatechol-type glucuronides	Urine	SPE	ESI (sheath-liquid: ACN-20mM ammonium acetate 1:1 v/v, 5 µl/min)	QQQ	20 mM ammonium acetate, pH 6.84	Stacking, LOD 7 ng/mL	[83]
Codeine, dihydrocodeine and their glucuronides	Urine	SPE	ESI (sheath-liquid: MeOH-water-acetic acid 69:39:1 v/v/v, 3 µL/min)	IT	25 mM NH ₄ OAc pH 9.	MS ² ; LOD: 100-200 ng/mL	[122]
Amphetamine and designer drugs	Urine	LLE	ESI (sheath-liquid: MeOH:water:HOAc 60/39/1 v/v/v, 3 µL/min)	IT	20 mM NH ₄ OAc, 20 mM HOAc pH 4.6	MS ²	[123]
Proteins	-	In-line digestion microchip	ESI (sheathless)	Q	10 mM (NH ₄)HCO ₃ , 100 mM HCOOH	-	[67]