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1. Introduction

Nowadays, there is a growing need for applications in food and environmental areas able to cope with the analysis of a large number of analytes in very complex matrices [1]. The new analytical procedures demand sensitivity, robustness, effectiveness and high resolution with reduced analysis time. Many of these requirements may be met to a certain extent by the total or partial automation of the conventional analytical methods, including sample preparation or sample pre-treatment coupled on-line to the analytical system. Furthermore, the recent use of ultra-high-performance liquid chromatography (UHPLC) for environmental and food chemical analysis has increased the overall sample throughput and laboratory efficiency without loss (and even with an improvement) of resolution obtained by conventional HPLC systems.

Nonetheless, despite the advances in chromatographic separations and mass spectrometry techniques, sample treatment is still one of the most important parts of the analytical process and effective sample preparation is essential for achieving good analytical results [1]. Ideal sample preparation methods should be fast, accurate, precise and must keep sample integrity. Therefore, over the last years, considerable efforts have been made to develop modern approaches in sample treatment techniques that enable the reduction of the analysis time without compromising the integrity of the extraction process. The use of on-line solid-phase extraction (SPE), which minimizes sample manipulation and provides both high pre-concentration factors and recoveries [2-5], is an increasingly powerful and rapid technique used to improve the sample throughput and overcome many of the limitations associated with the classical off-line SPE procedure. However, in most of the cases, matrix related compounds may also be co-extracted and could interfere in the analysis. Consequently, in order to minimize the effect of all these possible interferences a selective clean-up step may be required. Higher specificity and selectivity together with satisfactory extraction efficiency can be obtained using sorbents based on molecularly imprinted polymers (MIPs) [6-8].
Solid-phase extraction (SPE) based on MIPs is a highly attractive and promising approach for matrix clean-up, enrichment and selective extraction of analytes in such kind of complex samples. Another modern trend in sample preparation for multi-residue applications is the use of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method. The QuEChERS method is a recent and fascinating alternative procedure that has become particularly popular for the multi-residue analysis of pesticides in various food matrices [9,10], although this methodology is also being successfully employed for the extraction of other families of compounds [11,12]. Recently, the use of turbulent-flow chromatography (TFC) has also been reported for direct analysis of complex matrices such as honey, milk and animal tissues with reduced or without any sample manipulation [13-15].

The aim of this chapter is to discuss new trends in sample preparation techniques applied into food and environmental analysis. It includes a selection of the most interesting and promising sample treatment procedures such as on-line SPE methods, MIPs, QuEChERS, and turbulent flow chromatography. The applicability of each technique in food and environmental analysis will be discussed through the analysis of the most relevant papers recently published.

2. Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is the most popular sample preparation technique for environmental and food samples. Due to its high versatility, the SPE procedure is used for many purposes, such as purification, trace enrichment, desalting, derivatization and class fractionation. The principle of SPE is similar to that of liquid-liquid extraction (LLE). It involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid sorbent phase. Anyway, many of the problems associated with LLE, such as incomplete phase separations (emulsion), less-than-quantitative recoveries, use of expensive, breakable specialty glassware, disposal of large quantities of organic solvents, can be prevented by using SPE procedure. In addition, SPE resulted more efficient than LLE because yields quantitative extractions that are easy to perform, is rapid, and can be automated [16,17].

The general SPE procedure has to provide sample extracts that are free of interfering matrix components and concentrated enough for detection. The SPE process basically consists in four different steps: conditioning, sample addition, washing and elution (Figure 1).

First, the most suitable solid sorbent will be selected and conditioned using an appropriate solvent. During the conditioning the functional groups of the sorbent bed are solvated in order to make them able to interact with the sample. The sample addition consists in the percolation of the samples through the solid sorbent. During this step, the analytes as well as some matrix components are retained and thus concentrated on the SPE packing material. Successively, the analytes and interferents separation could be realized by the three following ways: selective extraction, selective washing or selective elution. Selective extraction is performed when the SPE procedure is used to remove the interfering components (trace enrichment). In this way, only selected components are retained. Selective washing is accomplished when the target analytes and the impurities are retained
on the sorbent bed: the impurities will be rinsed through with wash solutions that are strong enough to remove them, but weak enough to leave the analytes behind. Differently, selective elution consists in the elution of the adsorbed compounds of interest by a solvent that leaves the strongly retained impurities behind. The elution of target analytes could require different solvents, when SPE is applied in order to perform the class compound fractionation.

Figure 1. Schematic representation of SPE clean-up procedure.

To achieve optimal SPE extraction conditions, the choice of sorbent is a key factor because this can control parameters of primary importance such as selectivity, affinity and capacity [18]. This choice depends strongly on the nature of the analytes and their physical and chemical properties, which should define the interaction with the chosen sorbent. However, results can also depend heavily on the sample matrix and its interactions with both sorbent and analytes.

After the sorbent choice, the eluotropic strength of adsorption on silica and the polarity index will be helpful in order to select a suitable solvent. The polarity index is an accurate measure of solvent’s ability to interact as proton donor, proton acceptor or dipole whereas eluotropic series arranges solvents in order of decreasing elution strength for solutes from a particular sorbent.

2.1. SPE sorbents

The sorbent selectivity depends on the attractive forces between the analytes and the functional groups on the sorbent surface. The sorbent can interact with analytes by hydrophobic (non polar-non polar, van der Waals), hydrophilic (polar-polar, hydrogen bonding, dipole-dipole, dipole-induced dipole), cationic-anionic and selective antigen-antibody interactions.
Each sorbent offers a unique mix of these types of interactions.

The sorbent widely used for SPE packing can be classified into polar phases (normal phase), non-polar phases (reversed phase), ion exchange and immunoaffinity adsorbents.

**Polar phases** are used under normal phase chromatography conditions. These phases include polar adsorption media (LC Florisil, ENVI-Florisil, and LC-Alumina) and polar-functionalized bonded silica materials. The retention mechanism of an analyte is primarily due to interactions between polar functional groups of the analyte and polar groups on the sorbent surface. These include hydrogen bonding, π-π interactions, dipole-dipole interactions, and dipole-induced dipole interactions, among others. A compound adsorbed by these mechanisms is eluted by passing a solvent that disrupts the binding mechanism; usually a solvent that is more polar than the sample’s original matrix.

Polar adsorption media comprises underivatized silica material (SPE-Si), magnesium silicate (SPE-Florisil) and aluminum oxide materials (SPE-alumina).

SPE-Si is suitable to adsorb polar compounds from non-polar matrices. All samples used with this material must be relatively water-free since the functional group involved in the adsorption of compounds are the free hydroxyl groups on the surface of silica particles.

Polar-functionalized bonded silica sorbent consists of a silica material modified by bonding functional groups, such as cyano (SPE-CN), aminopropyl (SPE-NH$_2$), diol (SPE-Diol) to the surface of the SPE material. These phases are less retentive than SPE-Si toward very polar analytes and therefore permit extractions impossible to achieve with unmodified silica gel [19]. They result useful to adsorb and selectively elute compounds of very similar structure (e.g. isomers), or complex mixtures or classes of compounds such as drugs and lipids. Moreover, SPE-CN, can be used also under reversed phase conditions (with aqueous samples) to extract moderately polar compounds. The SPE-NH$_2$ can also be applied under ion exchange conditions in order to separate charged compounds.

**Non polar phases** are used under reversed phase chromatography conditions. These sorbents comprise alkyl silica and polymer based materials. Alkyl silica sorbents are manufactured by bonding alkyl or aryl functional groups, such as cyano (SPE-CN), octyl (SPE-8), octadecyl (SPE-18) and phenyl (SPE-Ph) to the silica surface. These phases are suitable for the extraction of hydrophobic or polar organic analytes from aqueous matrices. The retention of analytes is due primarily to the non-polar-non polar attractive forces between the carbon-hydrogen bonds in the analytes and the functional groups on the silica surface. The elution of adsorbed compounds is generally made by using a non polar solvent to disrupt the forces that bind the compound to the packing. Since all silica based bonded phases contain not-uncapped silanols, which can cause the strongly binding (sometime irreversibly, i.e tetracyclines) of some group of compounds, the addition of a more polar solvent may be often necessary. The main drawback of alkyl silica sorbent, especially of SPE-8 and SPE-18, is their poor water wettability. These cartridges require an initial conditioning step with a water-miscible organic solvent. When the internal surface of sorbent fails to be wetted because of the omission of the conditioning step or if the sorbent runs dry, the accessibility of sorbent surface for adsorbing
analytes is severely reduced. For instance, low recovery of analytes can be observed when SPE-18 sorbent is accidentally dried down before sample application.

Nevertheless, the narrow pH stability range of all modified silica reversed phase must be taken into account when SPE is carried in extremely acidic or basic media. For this purpose, a reversed phase polymerically bonded, such as copolymers of styrene-divinylbenzene (SPE-PS-DVB) resulted more resistant to pH extremes, and thus is more suitable for environmental applications for trapping organic compounds from acidified aqueous samples. Moreover, PS-DVB resin copolymer is a hydrophobic resin which has greater analyte retention, mainly for polar compounds, than their hydrophobic surface containing a relatively large number of active aromatic sites which allow $\pi$-$\pi$ interactions with unsaturated analytes [20]. The higher potential of PS-DVB over SPE-18 for trapping aromatic compound, especially phenols, is largely demonstrated [21,22]. Anyway, PS-DVB has some drawbacks, such as lack of selectivity and low breakthrough volumes for highly polar compounds, which leads to their incomplete extraction from predominantly aqueous matrices. Over the years, the performance of SPE-PS-DVB has been enhanced by attaching polar groups (i.e. acetyl, hydroxymethyl, benzoyl, $o$-carboxybenzoyl, sulfonate, trimethylammonium) to the aromatic ring on the polymer DVB [23] or by changing the copolymer composition. The SPE-DVB phase modified with $o$-carboxybenzoyl was useful applied for the determination of pesticides and phenolic compounds in environmental waters. The HLB sorbent, a macroporous copolymer prepared from a balance ratio of two monomers the lipophilic divinylbenzene and the hydrophilic N-vinylpyrrolidone has been formulated. It can absorb a wide range of polar and non-polar compounds [24] and its performance is unaffected by sorbent dry. It represents the most common hydrophilic sorbent used in the herbicides extraction [25,26].

Ion Exchange phases are comprised of positively (aliphatic quaternary amine, aminopropyl) or negatively (aliphatic sulfonic acid, aliphatic carboxylic acid) charged groups that are bonded to the silica surface. These sorbents are really suitable for extraction of charged analytes, such as acidic and basic compounds, from aqueous or non-polar organic samples. They exert a retention mechanism based mainly on the electrostatic attraction of the charged functional group of the analytes to the charged groups that are bonded to the silica surface. In order to retain a compound by ion exchange from an aqueous solution, the pH of the sample matrix must be one at which both the compound of interest and the functional group on the bonded silica are charged. Also, there should be few, if any, other species of the same charge as the compound in the matrix that may interfere with the adsorption of the compound of interest. A solution having a pH that neutralizes either the compound’s functional group or the functional group on the sorbent surface is used to elute the compound of interest. When one of these functional groups is neutralized, the electrostatic force that binds the two together is disrupted and the compound is eluted. Alternatively, a solution that has a high ionic strength, or that contains ionic species that displaces the adsorbed compound, is used to elute the compound.

Positively charged compounds are isolated under cation exchange conditions by using SPE sorbent containing silica linked with aliphatic sulfonic acid (SPE-SCX) or aliphatic carboxylic acid (SPE-WCX). The sulfonic acid group is strongly acidic and attracts or exchanges cationic
species in a contacting solution. It is charged over the whole pH range, and therefore can be used to isolate strong cationic (very high pKa >14) or weak cationic (moderately high pKa <12) compounds, as long as the pH of the solution is one at which the compound of interest is charged. Anyway, SPE-SCX cartridges should be used to isolate strong cations only when their recovery or elution is not desired. Weak cations can be isolated and eluted from SPE-SCX; elution is done with a solution at 2 pH units above the cation’s pKa (neutralizing the analytes), or by adding a different cation that displaces the analytes. If recovery of a strongly cationic species is desired, SPE-WCX is more suitable. The carboxylic acid group, present in SPE-WCX material, is a weak anion, and is thus considered a weak cation exchanger (WCX). It has a pKa of about 4.8, will be negatively charged in solutions of at least 2 pH units above this value, and will isolate cations if the pH is one at which they are both charged. SPE-WCX can be used to isolate and recover both strong and weak cations because the carboxylic acid functional group on the silica surface can be neutralized (2 pH units below its pKa) in order to elute the strong or weak cations. Weak cations also can be eluted from LC-WCX with a solution that neutralizes the adsorbed cations (2 pH units above its pKa), or by adding a different cation that displaces the analytes.

Negatively charged compounds can be isolated under anion exchange condition by using SPE sorbent containing silica functionalized with positively charged groups, such SPE-SAX and SPE-\(\text{NH}_2\).

SPE-SAX material presents an aliphatic quaternary amine as functional group. This is a strong base that exchanges or attracts anionic species in the contacting solution. Its pKa is very high (greater than 14), which makes the bonded functional group charged at all pHs in aqueous solution. As a result, LC-SAX is used to isolate strong anionic (very low, pKa <1) or weak anionic (moderately low, pKa >2) compounds, as long as the pH of the sample is one at which the compound of interest is charged. For an acidic compound of interest, the pH of the matrix must be 2 pH units above its pKa for it to be charged. In most cases, the compounds of interest are strong or weak acids. Because it binds so strongly, LC-SAX is used to extract strong anions only when recovery or elution of the strong anion is not desired (the compound is isolated and discarded). Weak anions can be isolated and eluted from LC-SAX because they can be either displaced by an alternative anion or eluted with an acidic solution at a pH that neutralizes the weak anion (2 pH units below its pKa). If recovery of a strongly anionic species is desired, the use SPE-\(\text{NH}_2\) is recommended. Generally, SPE-\(\text{NH}_2\) is used for normal phase separations but it is also considered to be a weak anion exchanger (WAX) when used with aqueous solutions. It has an aliphatic aminopropyl group bonded to the silica surface. The pKa of this primary amine is around 9.8. For it to be used as an anion exchanger, the sample must be applied at a pH at least 2 units below 9.8. SPE-\(\text{NH}_2\) is used to recover both strong and weak anions because the amine group can be neutralized (2 pH units above its pKa) in order to elute the strong or weak anions.

\textit{Immunooaffinity SPE} phases, also called immunosorbents (ISs), are very interesting materials because of their high selectivity. ISs cartridges are filled with antibody materials bonded onto silica gel support. They allow extraction, concentration and clean up from complex matrices in a single step, and from large sample volumes. The retention mechanism of these sorbents involves reversible and selective antigen-antibody interactions.
Due to the drawbacks of the commonly used SPE phases (previously discussed), the main current trends is the study and development of new sorbents materials. These new materials try to fulfill the requirements according to present needs, such higher specific surface area, selectively towards the target analytes, easy manipulation allowing coupling on-line configurations and higher biocompatibility, with the overall objective of the enhancement of the efficiency of the extraction process. Among them, molecularly imprinted polymers (MIPs), restricted access material (RAM), porous graphite carbon (PGC) and mixed-mode polymeric sorbent are attracting much interest [3,27-28].

MIP, which has become more and more popular in recent years, is a technology where recognition sites are created by copolymerization of a target molecule in a macromolecular matrix. This technique will be discussed in detail in section 3.

RAM materials possess a pore size that restricts big molecules from entering the interior extraction phase based on size. They have a bimodal surface topochemistry and enable the simultaneous performance of two different chromatographic processes [29]:

1. size exclusion chromatography (SEC), i.e. macromolecular sample components (>15,000 Dalton) are directly eluted to waste;
2. adsorption chromatography (e.g. reversed-phase chromatography), i.e. low-molecular-weight sample components are bound adsorptively on the internal pore surface.

Because the ability of these phases to exclude proteins, RAMs are the most suitable choice for clean-up biological and food samples. In a recent work, Chico et al. [30], evaluated the SPE-RAM clean-up for tetracyclines analysis in milk and water samples. The RAM clean-up removed large peaks that otherwise appeared in the initial time window of the chromatograms, attributed to proteins in milk samples and humic substances in water samples. Thus, quantification of analytes in real samples, especially of the most polar compounds such as oxytetracycline and tetracycline, was clearly improved.

Porous graphite carbon (PGC) material is manufactured by impregnating a high porosity LC silica gel (to provide the desired pore size) with a phenol-formaldehyde resin.

PGC behaves as a strong reversed-phase stationary phase, even stronger than SPE-18 silica phase which represents the most hydrophobic of the commonly used alkyl substituted silica phases [31]. The retention mechanism of PGC is different from that observed of reversed-phase silicas. The retention mechanism of polar analytes on PGC is a charged-induced interaction of the polar analyte with the polarizable surface of graphite [32]. The strength of interaction between a hydrophobic analyte molecule and the PGC surface largely depends on how well the molecule fit onto the flat graphite surface. PGC has been found to be particularly selective with respect to geometrical isomers and closely related substances. It was found that non-polar analytes were strongly retained on PGC.

Mixed mode polymeric sorbents combine the polymeric skeleton with ion-exchange group. It can be divided into cationic (SPE-MCX) or anionic (SPE-MAX) and as weak or strong ion exchange, depending on the ionic group linked to the resin. The retention mechanism of mixed-mode ion exchange chromatography combines the use of reversed-phase and ion-exchange modes into a single protocol on a single SPE cartridge. The mixed mode sorbents
are useful for fractionation of analytes. It can be used to isolate and separate neutral, acidic, and basic compounds from a single complex matrix. Intermediate washes with organic solvent mixtures of appropriate elution strength may be used to isolate neutral compounds, including ionizable analytes in their neutral state. Selective elution of ionically bound analytes may be attained by manipulating the charge of either the analyte (when bound to strong ion exchangers) or the sorbent (for analytes bound to weak ion exchangers).

2.2. Off-line SPE

SPE is widely used in environmental and food analysis in order to clean-up complex matrices and to isolate or/and concentrate target compounds. Two different SPE approaches are currently available: off-line and on-line procedures. In any case, the method development in SPE is accomplished by investigating pH, ionic strength, polarity and flow-rate of the elution solvent and physico-chemical characteristics of the sorbent bed. Briefly, if the target analytes are polar, normal phase extraction is indicated. When the analytes are less polar, reverse phase separation is advised. While ion exchange SPE extraction is suitable when the analytes are charged.

Some reasons for low sample recovery in SPE are: inappropriate cartridge conditioning, too-strong loading and wash solvent, too large volume (mass) of loaded, and too weak or too small volume of elution mobile phase [16].

The off-line SPE equipment is economical and uncomplicated, thus fully applicable to on-site sampling. This consists in a sorbent material, which come in different packaging (syringe barrels, microtubes-tips and discs), in a solvent system (eluent) and in a vacuum manifold. The most popular packaging format for off-line SPE results a syringe barrels which are easy to handle by using vacuum or positive-pressure manifold. However, it is not easy to control the flow-rate, and care should be taken to prevent the column from drying out prior to sample application. As it could be difficult to elute the analytes of interest from SPE syringe using minimal solvent volume unless organic solvent composition rises up to 100%, special SPE discs are typically used for these purposes. This approach is much quicker as evaporation to dryness and reconstitution are no longer necessary because elution can be performed directly by a mobile phase. A major drawback is the small sample capacity of the discs.

The off-line SPE procedure presents the following weaknesses: it is time consuming, it requires large amount of the organic solvent for the elution, and it could cause a possible loss of analytes during the evaporation steps. In addition off-line SPE provide a large manipulation of the samples thus possibility of contamination, less accuracy and precision can be found.

In spite of all disadvantages, off-line SPE approach remains useful for analyzing complex samples, because of its greater flexibility and whenever elution solvent is not compatible with the subsequent method of analysis [33].

Off-line SPE remain the widely used sample preparation technique for environmental and food analysis. Some of the most recent applications of SPE to environmental and food matrices are summarized in Table 1.
<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample</th>
<th>Detection technique</th>
<th>SPE column</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral oil saturated</td>
<td>Vegetable oils</td>
<td>GC-FID</td>
<td>Silver silica gel</td>
<td>[34]</td>
</tr>
<tr>
<td>hydrocarbons</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pesticides</td>
<td>Berry fruits</td>
<td>GC-MS</td>
<td>Envi-Carb + SPE-NH₂</td>
<td>[35]</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>Berry fruits</td>
<td>HPLC-MS</td>
<td>Amberlite XAD7</td>
<td>[36]</td>
</tr>
<tr>
<td>Sudan dyes</td>
<td>Hot chili powder</td>
<td>HPLC-DAD</td>
<td>Alumina</td>
<td>[37]</td>
</tr>
<tr>
<td>High-intensity sweeteners</td>
<td>Food products (aqueous solution)</td>
<td>HPLC-MS</td>
<td>Chromabond C18ec, Strata-X RP,</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bakerbond Octadecyl, Bakerbond SDB-1, Bakerbond</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SPE Phenyl, Oasis HLB, LiChrolut RP-18, Supelclean</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>LC-18, Discovery DSC-18, Zorbax C18</td>
<td></td>
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<tr>
<td>Melamine and cyanuric acid</td>
<td>Egg, pork, liver, kidney of pig, shrimp,</td>
<td>HPLC-MS/MS</td>
<td>Hydrophilic functional gel and</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>honey, soybean milk, soybean powder,</td>
<td></td>
<td>cation exchange sorbent</td>
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<tr>
<td></td>
<td>protein powder, milk and other dairy</td>
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<tr>
<td></td>
<td>products</td>
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<tr>
<td>Environmental analysis</td>
<td></td>
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</tr>
<tr>
<td>Nitrobenzene compounds</td>
<td>Lake water, sanitary wastewater, and</td>
<td>GC-MS</td>
<td>Phenothiazine bonded silica (PTZ-Si)</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>pond water</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Polybrominated diphenyl ethers</td>
<td>Food samples (fish, meat and vegetables)</td>
<td>GC-MS</td>
<td>Florisil; Alumina</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>environmental samples (soil or sediments)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylphenol ethoxylates; steroid</td>
<td>Wastewater</td>
<td>HPLC-MS</td>
<td>Sep-Pak Vac C05, Oasis HLB, Bond Elut-ENV,</td>
<td>[42]</td>
</tr>
<tr>
<td>hormonal; bisphenol-A; bisphenol-</td>
<td></td>
<td></td>
<td>Bond Elut Plexa, LiChrolut EN (500 mg)</td>
<td></td>
</tr>
<tr>
<td>Aromatic hydrocarbons</td>
<td>Environmental water</td>
<td>GC-MS</td>
<td>Multi-walled carbonphase</td>
<td>[43]</td>
</tr>
</tbody>
</table>

**Table 1.** Some recent examples about applications on off-line SPE
2.3. On-line SPE

To meet the ever-growing demands for sensitivity, reliability and speed, the continuous development of more efficient methods for both sample pretreatment and analysis is crucial. SPE technique can be easily coupled on-line to high performance liquid chromatography (HPLC) and gas chromatography (GC) systems. On-line systems are beneficial when the amount of sample is limited, or when very high sensitivity is required. In most cases, even though the use of an automated on-line instrument is quite straightforward, experienced personnel are required for method development and eventual trouble-shooting.

The strong differences among the solid phase extraction principle and the gas chromatography analysis made the on-line combination of SPE and GC more complicated.

Nevertheless, the combination with GC has already been successfully applied in environmental analysis [44-46]. In the on-line SPE-GC the analytes are trapped in a short column (10-20 mm×1-4.6 mm i.d.) packed with a suitable stationary phase (typically C8, C18 or styrene-divinylbenzene copolymer). The SPE procedures are essentially the same as the off-line ones. It involves conditioning of the SPE material before loading of the sample. Since water is not a good solvent for GC, primarily because it hydrolyses the siloxane bonds in GC columns causing deterioration of the column performance, the introduction of water directly to GC should be avoided. After trapping, and before elution of the analytes, the SPE column is often dried with a gas flow, or the extract is dried with a separate drying column packed with copper sulphate or silica to remove water, which is placed after the SPE column. The column can also be heated during the drying process, but this increases the risk of losing volatile analytes. The elution of the analytes is performed with a solvent suitable for the GC injector system.

On the other hand, the methods which combine SPE with HPLC are the most frequently used in environmental and food analysis, mainly to determine polar compounds in water solution. Different systems and configurations are available. The most commonly used approach involves the implementation of a small SPE column within the injection loop of a six-port rotary valve (Figure 2).

After conditioning, sample application, and eventual clean-up by means of a high-pressure pump, the SPE column is placed in front of an analytical column by switching the valve into the “inject” position. A sample is thus loaded in this SPE column, whereupon the valve is switched in order to elute the analytes out of the sorbent by the LC mobile phase and transfer them into the analytical column [3]. The SPE column is reusable. However, the reusability can cause a progressive deterioration of the column material and thus, lead to a change in their selectivity and capacity. Moreover, the SPE column must be filled with a sorbent compatible with the sorbent of the analytical column which efficiently traps the analytes. The SPE column should be as small as possible in order to prevent band broadening. Usually, the dimension of stainless steel columns is 30 mm length, 2 mm i.d. and 8 mm length, 3 mm i.d.
Recent advances in technology have made the Ultra High Performance Liquid Chromatography (UHPLC)/mass spectrometry (MS) system a perfect candidate for combination with on-line SPE. The on-line SPE-UHPLC/MS allows complete separation of high number of analytes via a single chromatographic run that takes few minutes.

Gosetti et al. [5], applied an automated on-line SPE UHPLC–MS/MS method for the identification and quantification of nine perfluorinated compounds (PFCs) in matrices of environmental, biological and food interest. The SPE protocol was performed by using an anion exchange SPE column (Poros HQ column). The separation of nine PFCs was obtained within 7 min. The limits of detection (LODs) ranged from 3 to 15 ng L\(^{-1}\) whereas the limits of quantification (LOQs) from 10 to 50 ng L\(^{-1}\).

The same authors performed a simultaneous determination of thirteen polycyclic aromatic hydrocarbons (PAHs) and twelve aldehydes in cooked food by means of an automated on-line SPE-UHPLC-MS. The resolution allowed the separation of four couples of PAH isomers. SPE treatment was made using Strata C18-E column and the extraction procedure was carefully optimized in order to apply the whole methodology to the analysis of different food matrices as salmon, frankfurter, steak, and pork chop, subjected to different cooking modes (smoked, grilled, cooked in oil or in butter). LODs values ranging from 0.028 to 0.768 µg L\(^{-1}\) for PAHs and from 0.002 to 0.125 µg L\(^{-1}\) for aldehydes were obtained [4].

Previously, PAH, such as naphthalene, biphenyl, acenaphthene, anthracene and pyrene have been determined in natural water by using on-line SPE–HPLC-UV [47]. Fluorocarbon polymer SPE sorbent was used. This application resulted in better extraction selectivity towards PAHs in comparison with several other sorbents and provided no additional peak broadening. Detection limits of method were established as 5 ng L\(^{-1}\) (biphenyl), 7 ng L\(^{-1}\) (anthracene), 8 ng L\(^{-1}\) (acenaphthene), 30 ng L\(^{-1}\) (pyrene), 40 ng L\(^{-1}\) (naphthalene).
Gallart-Ayala et al. [48], used an automated on-line SPE fast LC–MS/MS method for the simultaneous analysis of bisphenol A (BPA), bisphenol F (BPF), bisphenol E (BPE), bisphenol B (BPB) and bisphenol S (BPS) in canned soft drinks without any previous sample treatment. SPE on-line pre-concentration was performed by using a C18 cartridge. The analysis of all compounds was accomplished in 3 min. Quality parameters of the method were established and the authors obtained a simple, fast, reproducible (RSD values lower than 10%) and accurate (trueness higher than 93%) method for the analysis of bisphenols in canned soft drinks at the ng L\(^{-1}\) level using matrix-matched calibration.

Finally, in the current year, Vega-Morales et al. [2] used an on-line SPE-UHPLC-MS/MS to characterize 27 endocrine disrupting compounds (norethindrone, norgestrel, 17-alpha-ethinyloestradiol, etc.) in sewage samples. SPE treatment was performed by using Oasis HLB columns (mixed-mode sorbent). The complete analysis of each sample required less than 4 min and provided satisfactory recoveries (72–110%) and limits of detection in the order of few nanograms per liter (0.3-2.1 ng L\(^{-1}\)).

3. Molecularly imprinted polymers (MIPs)

Molecularly imprinted polymers (MIPs) are cross-linked, synthetic polymers with an artificially generated three-dimensional network able to specifically rebind a target analyte, or a class of structural analogues [1]. The principle is that a polymer network is obtained by polymerizing functional and cross-linking monomers around a template molecule. Subsequent removal of the template leaves a cavity with specific recognition sites complementary in shape, size and functional groups to the target analyte (Figure 3). These recognition sites can specifically bind target compounds in a similar way that antibodies specifically bind to an antigen, with the advantages of being very selective without suffering from stability problems associated to biological receptors [49]. All these aspects, together with the fact that MIPs synthesis is also relatively easy and cheap when compared with the purification procedure of natural antibodies, have led to a considerable growth of interest in the use of MIPs in several analytical techniques and applications.

Over the last 15 years, MIPs have been successfully applied as stationary phase on liquid chromatography, solid-phase extraction, micro-extraction, capillary electrochromatography, immunosassay determinations, and chemical sensing, with an almost exponential increase in the number of publications [50]. However, it should be pointed out that even if the interest in the area is relatively new, the concept itself has a long history. The earliest documents describing conceptually similar approaches had first been published in the early 1930s [51]. Nonetheless, today’s concept of molecular imprinting technology started back in 1972 when the groups of Klotz and Wulff independently presented the first examples of synthetic organic polymers with predetermined ligand selectivities. In both of these studies, MIP synthesis was based on a covalent linkage of the template molecule to the monomers prior to polymerization. Later on, in the early 1980s, the group of Mosbach has reported for the first time a general non-covalent approach for producing organic imprinted polymers [52,53]. This important development has broadened the scope of molecularly imprinting polymers, improving considerably the versatility and the number of possible applications for this type of materials.
3.1. Applications of MIPs to SPE

Out of all the MIPs applications, the use of MIPs as selective sorbents for solid-phase extraction (MIP-SPE) represents the most important application area in the field of analytical separation sciences [54]. Solid-phase extraction (SPE) is a well-established method routinely used for clean-up and pre-concentration of analytes in a wide range of environmental, pharmaceutical, agricultural and food analysis [1]. Nevertheless, sorbents used in conventional SPE often lack selectivity resulting in co-extraction of interfering matrix components. Therefore, specificity, selectivity and sensitivity together with high extraction efficiency can be obtained using sorbents based on molecularly imprinted polymers (MIPs) [8].

To assess the potential of MIPs in terms of selectivity, we have compared the ability of MIP-SPE for selective extraction of zearalenone from cereal sample extracts with that of a commercial immunoaffinity column (IAC). Figure 4 shows the similarity of the behavior of these two types of selective sorbents, resulted in high degrees of clean-up. In both cases, very reliable baselines and similar recoveries were obtained, proving that the high selectivity of immunoaffinity sorbents also can be achieved with molecularly imprinted polymers SPE. Furthermore, previous studies have found MIP-SPE to have a similar selectivity but a higher capacity than commercial IAC columns [49,55].
Figure 4. HPLC-FLD chromatograms of wheat sample spiked with ZON at level of 100 µg kg\(^{-1}\) after extract clean-up with (—) MIP-SPE (AFFINIMIP\(^\text{®}\) SPE ZEARALENONE; Polyintell) and (—) immunoaffinity column (IAC).

These aspects are highly attractive for matrix clean-up, enrichment and selective extraction of analytes in difficult samples that are very common to food and environmental analyses. Hence, several examples of MIP-SPE applications have been described in the literature, as exemplified in Table 2, which presents a selection of the most recently published scientific research.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sample</th>
<th>Analysis</th>
<th>Recovery Rates (%)</th>
<th>Analytical features</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food Analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan I</td>
<td>Chilli Sauce</td>
<td>HPLC-UV</td>
<td>87.5 to 103.4 %</td>
<td>LOD ≥ 3.3 µg kg(^{-1})</td>
<td>[56]</td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>Wheat Samples</td>
<td>MISPE-FLD</td>
<td>92.1 to 104 %</td>
<td>LOD ≥ 1.2 ng mL(^{-1})</td>
<td>[57]</td>
</tr>
<tr>
<td>Domoic acid</td>
<td>Seafood Samples</td>
<td>HPLC-PDA</td>
<td>93.4 to 96.7 %</td>
<td>LOQ ≥ 0.1 mg L(^{-1})</td>
<td>[58]</td>
</tr>
<tr>
<td>Catechins</td>
<td>Tea, Cocoa, Grape</td>
<td>HPLC-PDA-FL</td>
<td>50 to 100%</td>
<td>--</td>
<td>[59]</td>
</tr>
<tr>
<td>Zearamone</td>
<td>Cereal Samples</td>
<td>HPLC-UV</td>
<td>82 to 90%</td>
<td>--</td>
<td>[49]</td>
</tr>
<tr>
<td>Mycohenolic acid</td>
<td>Maize</td>
<td>HPLC-MS-MS</td>
<td>49 to 84%</td>
<td>LOD ≥ 0.17 µg kg(^{-1})</td>
<td>[60]</td>
</tr>
<tr>
<td>Tetracycline Antibiotics</td>
<td>Egg Samples</td>
<td>HPLC-PDA</td>
<td>91.6 to 107.6%</td>
<td>LOQ ≥ 0.8 ng g(^{-1})</td>
<td>[6]</td>
</tr>
<tr>
<td>Thiamphenicol</td>
<td>Milk and Honey Samples</td>
<td>HPLC-PDA</td>
<td>92.9 to 99.3 %</td>
<td>LOD ≥ 0.003 µg mL(^{-1})</td>
<td>[61]</td>
</tr>
<tr>
<td><strong>Environmental Analysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>Ultrapure, Tap, Drinking, River Water Samples</td>
<td>HPLC-FLD</td>
<td>84.7 to 93.8%</td>
<td>LOD ≥ 2.50 pg mL(^{-1})</td>
<td>[62]</td>
</tr>
<tr>
<td>Natural and Synthetic Estrogens</td>
<td>River and Tap Water Samples</td>
<td>UHPLC-MS-MS</td>
<td>LOD ≥ 4.50 ng L⁻¹</td>
<td>LOQ ≥ 14.9 ng L⁻¹</td>
<td>[8]</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------------------</td>
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<td>------------------</td>
<td>-----------------</td>
<td>-----</td>
</tr>
<tr>
<td>Pyrethroid Insecticides</td>
<td>Aquaculture Seawater</td>
<td>GC-ECD</td>
<td>LOD ≥ 16.6 ng L⁻¹</td>
<td>LOQ ≥ 55.3 ng L⁻¹</td>
<td>[63]</td>
</tr>
<tr>
<td>Water-Soluble Acid Dyes</td>
<td>Wastewater and Soft Drink Samples</td>
<td>HPLC-PDA</td>
<td>LOD ≥ 0.095 µg L⁻¹</td>
<td></td>
<td>[64]</td>
</tr>
<tr>
<td>Levonorgestrel</td>
<td>River and WWTP influent and effluent samples</td>
<td>HPLC-UV</td>
<td>79.9 to 132.7 %</td>
<td>--</td>
<td>[65]</td>
</tr>
<tr>
<td>Methamidophos</td>
<td>Soil Samples, Tap and River Water Samples</td>
<td>GC-NPD</td>
<td>95.4 to 96.1 %</td>
<td>LOD ≥ 10 ng L⁻¹</td>
<td>LOD ≥ 3.8 ng g⁻¹</td>
</tr>
<tr>
<td>Dibutyl Phthalate</td>
<td>Aqueous Environment Samples</td>
<td>GC-MS</td>
<td>94.7 to 101.9 %</td>
<td>LOD ≥ 5.49 ng L⁻¹</td>
<td>[67]</td>
</tr>
<tr>
<td>Atrazine Herbicide</td>
<td>Aqueous Environment Samples</td>
<td>HPLC-PDA</td>
<td>94 to 99 %</td>
<td>LOD ≥ 80 ng L⁻¹</td>
<td>[68]</td>
</tr>
<tr>
<td>Chlorsulfuron</td>
<td>Water, Soil, and Wheat Plant Samples</td>
<td>HPLC-UV</td>
<td>82.3 to 94.7%</td>
<td>--</td>
<td>[69]</td>
</tr>
<tr>
<td>Parabens</td>
<td>Soil and Sediment Samples</td>
<td>HPLC-UV</td>
<td>80 to 90 %</td>
<td>LOD &lt; 1 ng g⁻¹</td>
<td>[70]</td>
</tr>
<tr>
<td>Fluoroquinolone antimicrobials</td>
<td>Water Samples</td>
<td>HPLC-FLD</td>
<td>62 to 102%</td>
<td>LOD ≥ 1 ng L⁻¹</td>
<td>[7]</td>
</tr>
</tbody>
</table>

Table 2. Some recent applications of MIP-SPE in food and environmental analysis. LOD= Limit of detection; LOQ= Limit of quantification

Regarding the analytical method, MIP-SPE procedure is based on the same main four steps as conventional SPE such as pre-conditioning of the sorbent, sample loading, interferences wash step and elution of the target compounds. Therefore, to obtain optimal recovery rates and selectivity, each step of the extraction procedure must be properly optimized.

MIP-SPE can be basically used in both the reversed phase and normal phase modes. In the normal phase approach, the sample is usually percolated though the MIP-SPE column using the same solvent that was used as porogen for the MIP synthesis. Under this condition, the target analyte develops specific interactions with the monomer residues present in the polymer cavities, resulting in selective adsorption and molecular recognition by MIP due to the well-known solvent “memory” effect [54].
Nonetheless, in some common situations, a loading step based on direct percolation of aqueous sample through the MIP-SPE cartridge is highly desirable since most environmental or biological samples exist in an aqueous matrix [71]. Under this reversed-phase condition, the target analytes as well as non-polar interfering compounds are mainly retained by non-specific hydrophobic interactions. Thus, to generate specific interactions between the target compounds and the MIP and to disrupt the non-specific interactions between the residual monomers located at the surface of the polymer and matrix components, a selective washing step using low-to-medium polarity organic solvents, such as dichloromethane, chloroform, toluene or acetonitrile, is usually required [49]. It should be pointed out, however, that in some cases this selective washing step can be problematic because of the low polarity of common solvents used, which may give rise to miscibility problems and/or losses of the analyte [54]. Consequently, a drying step prior to this organic washing process becomes mandatory [8].

Once matrix interferences are removed, the analytes can be eluted from the column with a pure solvent, solvent that contains a small amount of modifier such as acetic acid or a combination of solvents with different polarities that must possess an elution strength sufficiently high to disrupt the specific interactions of the target analytes with the polymer, in minimal elution volumes.

As example of the successful application of a MIP-SPE compatible with aqueous samples, Figure 5 shows the HPLC-FLD chromatogram (red line) corresponding to the injection of the elution fraction obtained after the purification of 100mL of Seine river water spiked with 0.5 ng mL$^{-1}$ of 17β-estradiol.

![Figure 5. HPLC-FLD chromatograms obtained after extracts clean-up with MIP-SPE (AFFINIMIP® SPE Estrogens; Polyintell) of 100mL of Seine water spiked at 0.5 ng mL$^{-1}$ with 17β-estradiol (—) and before MIP clean-up (—).](image-url)
The chromatogram obtained (red line) clearly illustrates the efficiency of the MIP-SPE procedure (extraction rate of about 90%) and the advantages of both the concentration and sample clean-up with very low background and no interferences close to the retention time of 17β-estradiol. As a result, the use of such kind of selective sorbent allowed the successful detection of estradiol present at very low concentration without the need for a more selective and sensitive method of data acquisition such as mass spectrometry (MS) detection.

Regarding different operation modes, MIP-SPE has been used both in on-line and off-line modes prior to various detection techniques. Most of the MIP-SPE applications reported so far have been developed in the off-line mode because of its simplicity, ease-of-use and high flexibility. In addition, the drying step required in most of the off-line procedures is not compatible with on-line operations. Nonetheless, in the last years, there has been a considerably increase in the number of applications that use MIPs as sorbent for on-line SPE because provides higher pre-concentration factors with reduced analysis time and sample manipulation.

In the off-line mode, MIP–SPE has been applied for the selective extraction and pre-concentration of a wide range of analytes, such as mycotoxins in cereal samples [55], thiamphenicol in milk and honey samples [61], domoic acid from seafood [58], ofloxacin and lomefloxacin in chicken muscle [72], pyrethroid insecticides in aquaculture seawater [63] and parabens in soil and sediment samples [70]. Furthermore, MIP-SPE has been applied not only for the extraction of single target analyte but also for the simultaneous isolation of a class of structurally related compounds such as catechins or estrogens from real samples [8,59]. Luo et al. [64], developed a sensitive and selective off-line MIP-SPE based method to determine five water-soluble acid dyes in wastewater and soft drink samples. The precision and accuracy of the method were satisfactory and it gave average recoveries between 89.1 and 91.0%. In a similar way, Qi et al. [65], prepared a MIP by conventional bulk polymerization for the extraction of levonorgestrel from water samples. The synthesized MIPs not only displayed high specific recognition for levonorgestrel (recoveries > 79%), but also showed high cross-reactivity values for structurally related contraceptive drugs, suggesting that MIPs could be used as broad specific recognition absorbent.

On-line MIP-SPE protocol has been successfully used to extract benzimidazole fungicides in water samples [73], ochratoxin A in wheat samples [57], and bisphenol A in environmental water samples [74]. An automated on-line SPE using microspherical monodispersed molecularly imprinted particles coupled to HPLC-fluorescence detector was successfully applied to the simultaneous multi-residue analysis of six fluoroquinolone antimicrobials (enrofloxacin, ciprofloxacin, norfloxacin, levofloxacin, danofloxacin, and sarafloxacin) in water samples [7]. In this work, polymer particles prepared via precipitation polymerization were used as SPE sorbent. High recoveries with good precision (RSDs <5%) were obtained for the different fluoroquinolones tested, with values ranging from 91 to 102% in drinking and fish farm water samples. The detection limits were between 1-11 and 1-12 ng L⁻¹ for drinking and fish farm water samples, respectively.
On-line MIP-SPE pre-concentration methodology has also recently been used by Jing et al. [6], for the determination of trace tetracycline antibiotics (TCs) in egg samples. This approach affords high-throughput analysis (18 min per sample), and also provides high sensitivity and selectivity with recoveries ranging between 91.6 and 107.6%, showing that efforts should continue to be made in this promising research area.

4. QuEChERS

4.1. QuEChERS procedure

The need for a simple, rapid, cost-effective and multi-residue method able to provide high quality of analytical results led Anastassiades et al. to develop in the years 2001 and 2002 a new sample treatment method called “QuEChERS”. Initially, the methodology was developed for the analysis of veterinary drugs (anthelmintics and thyreostats) in animal tissues, but after realizing its great potential in the extraction of polar and particularly basic compounds, it was also tested with great success on pesticide residue analysis in plant material. The detailed method was first published in 2003 [75].

QuEChERS, acronym of “Quick, Easy, Cheap, Effective, Rugged and Safe”, is a sample preparation technique entailing solvent extraction with acetonitrile and partitioning with magnesium sulfate alone or in combination with other salts followed by a clean-up step using dispersive solid-phase extraction (d-SPE). This last step is performed by adding small amounts of bulk SPE packing sorbents to the extract. This procedure has attracted the attention of pesticides laboratories worldwide and it is the most commonly employed sample treatment methodology for the multi-residue analysis of pesticides in fruits and vegetables [9,10,76-80]. But today, this methodology is not limited to the analysis of pesticides and its use for the extraction of other families of compounds is tremendously increasing.

The different steps on a typical QuEChERS procedure for the multi-residue analysis of pesticides are shown in Figure 6.

**Figure 6.** Schematic view of a typical analytical QuEChERS procedure for the analysis of pesticides as described in [75].
The idea of the QuEChERS procedure was to reduce complicated, laborious and time-consuming multi-residue sample treatment methods that required high amount of solvents and were therefore expensive. Moreover, some basic, acidic and very polar compounds cannot be satisfactorily extracted with common multi-residue methods. Thus, in order to cover all these analytes, laboratories have to perform specific analysis, and as a consequence, some of these compounds were not being monitored.

The first two steps of a typical QuEChERS procedure consist in weighing an appropriate amount of sample previously processed and homogenized (for instance 10 g) in a 50 mL Teflon tube (Step 1) and the addition of a solvent for the extraction (Step 2), in general acetonitrile, although the use of other organic solvents such as acetone, THF or ethyl acetate have been described [81].

Then, an extraction-partitioning step takes place by the addition of magnesium sulfate alone or in combination with other salts, generally sodium chloride (Step 3). Acetonitrile is the recommended solvent for QuEChERS because, upon the addition of salts, it is easily separated from water than, for instance, acetone. Ethyl acetate has the advantage of a partial miscibility with water but it can also co-extract lipids and provides lower recoveries during the dispersive SPE. The extraction of lipophilic materials is lower with acetonitrile but this solvent can form two phases with water when samples with high sugar content are manipulated [75]. The addition of salts in Step 3 helps to induce the phase separation. This salting-out effect also influences analyte partition, which of course is also dependent upon the solvent used for extraction. The concentration of salt can also influence the percentage of water in the organic phase and can play an important role in adjusting its polarity. Magnesium sulfate acts as a drying salt to reduce the water phase, thereby helping to improve recoveries by promoting partitioning of the pesticides (or other target compounds) into the organic layer while sodium chloride helps to control the polarity of the extraction solvent. With this, a single extraction-partitioning step is carried out (similarly to an “on-line” approach) which simplifies the necessity of multiple partitioning steps required in other multi-residue methods. Moreover, this extraction-partitioning step is produced by shaking vigorously for a few minutes, thus preventing more time-consuming steps such as sample blending. At this point, internal standards can be added to the system if necessary (Step 4), followed by shaking again the solution and a centrifugation step that help to separate salts. The use of internal standards can minimize the error generated in the multiple steps of the QuEChERS method. Sometimes the use of more than one internal standard is recommended especially with samples with high fat content because the excessive fat can form an additional layer into which the analytes can also partition [82]. Another advantage of QuEChERS procedure is the fact that, once the extraction-partitioning step is carried out, an aliquot of the extract is used for the next steps, minimizing also the separation or the transfer of the entire extracts frequently employed in other multi-residue methods.

Then, a dispersive solid-phase extraction (d-SPE) clean-up procedure is carried out with an aliquot (for instance 1 mL) of the extract which is placed in a vial containing again magnesium sulfate and small amounts of bulk SPE sorbent materials (Step 5). The vial is
then shaken vigorously or mixed on a vortex mixer to distribute the SPE material and facilitate the clean-up process. This d-SPE step is quite similar to matrix solid-phase dispersion developed by Barker [83, 84], but in d-SPE the sorbent is added to an aliquot of the extract rather than to the original solid sample. Moreover, small amounts of sorbents are used because only a small portion of the extract is subjected to the clean-up procedure, and compared to conventional SPE clean-up methods, d-SPE is less laborious and time-consuming. Magnesium sulfate is again used in this step as a drying agent to remove water and improve analyte partitioning to provide better clean-up. Primary secondary amine (PSA) is the most common SPE sorbent used in QuEChERS procedure for pesticide analysis. The idea is to use a sorbent able to retain matrix components, but not the analytes of interest. However, depending on the sample matrix, other SPE sorbents can also be used alone or combined with PSA, such as C18, OASIS HLB, and graphitized carbon black sorbents. For instance, for samples with high fat content, PSA mixed with C18 is recommended [85] while for samples with moderate or high levels of chlorophyll and carotenoids (for example carrots), PSA mixed with graphitized carbon black is frequently used [86-88]. After the clean-up, the extract is centrifuged and an aliquot of the supernatant can be concentrated or directly analyzed usually by means of Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry (LC-MS) techniques (Step 6).

Although QuEChERS is quite a simple procedure as can be seen from Figure 8, in some cases method development will be necessary depending on the family of compounds to be analyzed, and many modifications over the QuEChERS procedure are being proposed. At the end, compromises will be required to ensure simplicity, speed, broad applicability, high recovery and selectivity. For instance, the control of pH in the extraction step is an important factor when analyzing pesticides in order to ensure an efficient extraction of pH-dependent compounds such as phenocycaloanoc acids and to minimize degradation of labile pesticides under alkaline or acidic conditions. Buffering with citrate salts has been introduced in the first extraction/partitioning step to adjust the pH at around 5 where most labile pesticides under acidic or alkaline conditions are sufficiently stabilized. The pH control can also be very important in other steps of the QuEChERS procedure to prevent degradation of some compounds. For instance, to improve the stability of alkaline-labile compounds after the PSA clean-up step the final sample extract is slightly acidified by the addition of small amounts of formic acid. Of great importance was the introduction of acetate buffering to achieve a pH value of 6 in order to improve recoveries of pH-dependant analytes [89]. This approach resulted in the official method AOAC 2007.01.

The use of analyte protectants is also often proposed as an optional step previous to GC analysis for those compounds that might tail or breakdown on the capillary GC column interior surfaces, on the inlet liner or on the guard column. A combination of sorbitol, gulonolactone, and ethylglycerol was found to be the most effective analyte protectant to cover the whole range of pesticide compounds [90]. The hydroxyl groups of these protectants can interact with active sites on the chromatographic column and in the flowstream and enhanced the pesticide analyte response. Those protectants are of course not required when LC methods are employed for analysis.
4.2. Applications of QuEChERS

As commented before, QuEChERS procedure is the sample treatment of choice for the multi-residue analysis of pesticides in fruits and vegetables, and many works can be found in the literature dealing with the simultaneous extraction and clean-up of more than 100 pesticides with acceptable recoveries [75,77,79,80,91]. As an example, Woo Lee et al. developed a new QuEChERS method based on dry ice for the determination of 168 pesticides in paprika [91]. For this purpose, extraction was carried out by using 30 mL of acetonitrile and 10 mL of water, and approximately 10 g of dry ice granules were poured and maintained until layer separation. Clean-up was then carried out by using PSA and GCB sorbents. The separation of the sample extract was induced via the sublimation of dry ice, which occurs at -78.5°C at atmospheric pressure (1 atm). After some minutes of dry ice sublimation with the sample extract, the reduced temperatures of acetonitrile and water ranged from -4.0 to -5.0°C and from -6.0 to -6.5°C, respectively, and water was then iced and super cooled. The negative temperatures of the two solvents may reduce their entropies and allowed them to separate. As densities of ice and supercooled water were heavier than that of acetonitrile at 0°C, water changed to ice and the supercooled water was separated with an acetonitrile layer from the mixed solution. This methodology improved the extraction for flonicamid and its metabolites which was not satisfactory enough using citrate-buffering QuEChERS method.

Recently, the use of carbon-based nanoparticles has also been described as clean-up sorbent for the analysis of pesticides [10]. In this work, multi-walled carbon nanotubes (MWCNTs) were proposed as reversed-dispersive solid phase extraction (r-DSPE) material for the analysis of 30 pesticides in fruits and vegetables. The amount of MWCNTs influenced the clean-up performance and the recoveries, but the use of only 10 mg MWCNTs was suitable for cleaning up all analyzed matrices and showed to be a good alternative to PSA sorbent. The method was validated for different matrices such as spinach, orange and cabbage with recoveries in the range of 71-100% for all 30 pesticides. QuEChERS has also been proposed for the extraction and clean-up of pesticide residues in other food matrices such as milk [85]. In this last work, recovery of 14 different pesticides residues in milk was investigated to respect the amounts of sodium acetate, PSA and C18 used on the clean-up step. Recoveries for hydrophilic pesticides such as myclobutanil ranged from 82 to 99% while lower values (<80%) were obtained for lipophilic pesticides because they were partially removed by C18 along with other fatty compounds.

But today, one of the most important features of QuEChERS may be its application to other families of compounds in a variety of matrices different than fruits and vegetables. Some examples of the use of QuEChERS for the extraction of compounds other than pesticides are given in Table 3. QuEChERS methodology has already been applied to the analysis of polycyclic aromatic hydrocarbons (PAHs) in fish and shrimp samples [92,93]. Forsbeg et al. developed and validated a modified QuEChERS method for the determination of 33 parent and substituted PAHs in high-fat smoked salmon that greatly enhanced analyte recovery compared to traditional QuEChERS procedure [93]. For this purpose, a mixture of acetone, ethyl acetate and isooctane instead of acetonitrile was employed for the extraction, and different kinds of salts were used for partitioning. The proposed modified QuEChERS
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sample</th>
<th>QuEChERS procedure</th>
<th>Analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycyclic Aromatic Hydrocarbons</td>
<td>High-Fat Salmon</td>
<td>2 mL acetone:ethyl acetate:isooctane (2:2:1 v/v/v) + 6 g MgSO₄ + 1.5 g NaC₆H₅O₇</td>
<td>150 mg MgSO₄ + 50 mg PSA</td>
<td>GC-MS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mL acetone:ethyl acetate:isooctane (2:2:1 v/v/v) + 4 g MgSO₄ + 1 g NaCl + 1 g NaC₆H₅O₇ + 0.5 g NaC₆H₅O₇</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrylamide</td>
<td>Foodstuffs</td>
<td>5 mL of hexane (only for high fatty matrices) + 10 mL water + 10 mL acetonitrile + 4 g MgSO₄ + 0.5 g NaCl</td>
<td>150 mg MgSO₄ + 50 mg PSA</td>
<td>LC-MS or GC-MS</td>
</tr>
<tr>
<td>Veterinary drug residues</td>
<td>Milk</td>
<td>10 mL acetonitrile (1% acetic acid) + 10 mL 0.1M Na₂EDTA solution + 4 g MgSO₄ + 1 g sodium acetate</td>
<td>--</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Persistent organic pollutants: 22 organochlorine pesticides + 7 polychlorinated biphenyls (PCBs)</td>
<td>Fish tissue</td>
<td>10 mL acetonitrile + 10 mL water + 4 g MgSO₄ + 1 g NaCl + 0.5 sodium citrate dibasic + 1 g sodium citrate tribasic</td>
<td>pre-frozen step (2 hours) 1 g calcium chloride + 900 mg MgSO₄ + 150 mg PSA</td>
<td>GC-MS</td>
</tr>
<tr>
<td>10 mycotoxins</td>
<td>Eggs</td>
<td>10 mL methanol:water (80:20 v/v) with 1% acetic acid + 4 g MgSO₄ + 1 g sodium acetate</td>
<td>(C18 or Oasis HLB SPE)</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>Phytohormones</td>
<td>Vegetables</td>
<td>10 mL acetonitrile with 1% acetic acid + 4 g MgSO₄ + 1 g NaCl + 1g sodium citrate + 0.5 disodium citrate</td>
<td>--</td>
<td>UHPLC-MS/MS</td>
</tr>
<tr>
<td>UV Ink Photoinitiators</td>
<td>Packaged food (baby food, fruit juices, wine)</td>
<td>12 mL acetonitrile + 4 g MgSO₄ + 1.5 g NaCl</td>
<td>250 mg MgSO₄ + 750 mg PSA</td>
<td>LC-MS/MS</td>
</tr>
</tbody>
</table>

Table 3. Application of QuEChERS procedure for the extraction of different kinds of analytes.
substantially improved average recovery of 15 PAHs by roughly 38% and led to individual
gains of 50-125% for some PAHs such as naphthalene and anthracene among others when
compared to traditional Soxhlet extraction with hexane. Acrylamide has also been extracted
from various food matrices such as chocolate, peanut butter, and coffee [94]. An accurate
determination of acrylamide in foodstuffs was possible using QuEChERS since the use of
salt and the PSA sorbent increased the selectivity of the method by reducing the content of
more polar matrix coextractives. Hexane was required for high fatty samples such as peanut
butter.

The extraction of veterinary drugs residues from animal tissues [98] and from milk
[95,99,100] has also been described. For instance in [95] the use of QuEChERS was proposed
as a fast sample treatment for a rapid screening method in the identification of 21 veterinary
drug residues in milk. In this case, 1% acetic acid in acetonitrile together with a 0.1 M
Na2EDTA solution was proposed as extraction solvent, using for partitioning magnesium
sulfate and sodium acetate, but no further clean-up step was necessary to attain good
results. The analysis of persistent organic pollutants (POPs) such as organochloride
pesticides and polychlorinated biphenyls (PCBs) in fish tissues was recently reported using
conventional QuEChERS procedure but with the addition of a pre-frozen step for 2 hours at
-24°C (by means of a homemade freezing device) before the PSA clean-up step for removal
of lipids [96]. After this freezing step, between 60 to 70% of lipids were removed. The
reduction of co-extractives increased up to 96% by treatment with calcium chloride and
PSA. Extraction of mycotoxins and phytohormones from eggs [12] and vegetables [97],
respectively, has also been described using QuEChERS with no further clean-up steps,
although in the case of mycotoxins a SPE clean-up step using C18 or Oasis HLB cartridges
was sometimes proposed. An interesting application of QuEChERS was recently reported
by Gallart-Ayala et al. for the analysis of contaminants migrating into food from food
packaging materials [11]. In this case, the extraction of UV Ink photo initiators such as
benzophenone and isopropylthioxanthone (ITX) in several foodstuffs (baby food, fruit juices
and wine) packaged in tetra brick containers was performed using a common QuEChERS
procedure with PSA sorbent for clean-up. The extraction method proposed showed
comparable results in terms of method limits of quantification, run-to-run and day-to-day
precisions, and quantification results than a previous SPE method reported for the analysis
of ITX, with the advantage of being 12 times faster (per sample).

Summarizing, QuEChERS approach appears to have a bright future not only for the analysis
of pesticide residues in foods and other agricultural products but also for the analysis of
different families of contaminants either in food or even in other matrices. For instance,
QuEChERS has also been proposed in environmental analysis for the extraction of
chlorinated compounds from soil samples [101]. The simplicity of its use and the great range
of modifications that can be applied, make QuEChERS an ideal extraction procedure to
think about when dealing with the extraction of any kind of analytes, and the number of
publications using QuEChERS will considerably increase in the future.
5. Turbulent-flow chromatography (TFC)

For a long time the determination of small drug molecules in biological fluids was a very challenging task due to both the complexity of biological samples and the requirement of long chromatographic separations because of the presence of endogenous interferences. The recent implementation of automated on-line extraction procedures has allowed fast sample clean-up in bioanalytical applications, and turbulent-flow (or TurboFlow) chromatography (TFC) appears as one of the most interesting ones in this area [102-105].

The on-line set-up for a typical TFC-LC-MS method is shown in Figure 7. TFC methods are based on the direct injection of biological samples without previous extraction or any treatment into a column packed with large particles. These large particles could have some stationary phase bonded to them, adding an additional selectivity to the extraction procedure. Once the sample have been injected (Figure 7a) onto a TurboFlow column, an extraction solution is pumped into the column at a high flow rate (between 1.5 to 5.0 mL min⁻¹) generating turbulent flow conditions inside the column (Figure 7b). In general, 100% aqueous mobile buffers are used for this purpose. Under these conditions, small analyte molecules are retained via diffusion processes into the particle pores, while big molecules such as proteins are washed out from the column. In this way, the compounds of interest are extracted from the biological matrix and then eluted from the TurboFlow column onto the analytical column with a volume of solvent which was stored in a holding loop or pumped directly from the chromatographic LC system (Figure 7c). In general organic mobile phases or pH buffered solutions are used for the elution of the compounds of interest depending on the chromatographic separation used after extraction, but the elution volume must be at least ten times that of the TurboFlow column in order to guarantee a complete elution. The analytes are released from the TurboFlow column at a considerably lower flow rate than the one used during extraction into the analytical system where they are mixed with the chromatographic mobile phase and introduced into the chromatographic column, being focused into a sharp band at the head of the HPLC column. When the transfer of analytes is complete, the TurboFlow column could be washed for the next extraction while a regular gradient or isocratic elution is taking place in parallel on the analytical column. The optimization of the different on-line extraction steps is crucial and parameters like mobile phase composition, flow rates and extraction time windows will affect recovery or extraction efficiency in general.

The theory of turbulent flow in open tubes has been discovered and studied for decades. Nevertheless, its application to LC packed columns was only patented in 1997 [106]. The challenge at the moment was to design a chromatographic platform using turbulent flow properties to isolate small analytes from macromolecules present in complex matrices such as biological fluids.

TFC has been used mainly in the handling of biological samples containing a large amount of proteins, such as blood plasma [107,108]. For instance, Michopoulos et al. compared the use of TFC for the metabonomic analysis of human plasma with protein precipitation showing that TFC could be effectively used with the benefit that off-line sample handling was significantly reduced [107]. However, the analysis of the data obtained with TFC for
human plasma revealed substantial differences in the overall metabolite profiles compared to methanol-precipitated HPLC-MS, probably due to greatly reduced amounts of phospholipids (ca. 10 fold reduction) with TFC methodology compared to protein-precipitated samples. TFC seems to be also more efficient at removing proteins based on their size than restricted access media (RAM) or solid phase extraction (SPE) [1,109].

Figure 7. On-line set-up of a typical TFC-LC-MS system. The position of the various valves to perform the sample clean-up and subsequent analysis of the samples is shown. a) Filling the sample loop; b) transfer from the sample loop to the TFC column followed by washing; c) elution from the TFC column to the Chromatographic column using gradient elution. 1) sample; 2) ASPEC system with a syringe pump; 3) sample loop (10 mL); 4) Waste; 5) HPLC Pump (4 mL min⁻¹); 6) TFC column; 7) Waste; 8) HPLC Pump (gradient); 9) HPLC column; 10) MS analyzer. Reproduced from [111].

TFC shows also a big potential in clinical applications, where the increased emphasis on both drug safety and translational biology, e.g. the need to understand how pre-clinical efficacy models are representative of human pharmacology, has considerably modified the expectations for what needs to be measured routinely in biological samples. Moreover, sometimes it is necessary to monitor in the same sample not only the drug levels but also its potential active/reactive metabolites as well as the biomarkers associated with the mechanism of action of the drug. Those biomarkers could span from a very small and polar compounds
such as a neurotransmitter to a very large and hydrophobic entity like fatty acids. So, amongst the key analytical challenges with biomarkers is generally the sampling procedure as well as the sample volume available. TFC has some intrinsic capabilities that facilitate the analysis of biomarkers and metabolites [103]. First, it provides high sensitivity assays without the need for high sample volume. In addition, the on-line extraction approach removes the need for lengthy sample preparation procedures, hence reducing sample degradation issues frequently observed in biomarker analysis. As an example, Mueller et al. proposed a fully automated toxicological LC-MSn screening system in urine using on-line extraction with TFC [110].

Very recently the use of TFC couple to tandem mass spectrometry has been reported for the automated analysis of perfluorinated compounds (PFCs) in human hair and urine samples [112]. The method allowed the extraction and analysis of 21 PFCs with recoveries between 60 to 105%.

But today, TFC is being used in other fields of applications, such as food or even environmental analysis. Table 4 shows some examples of these TFC applications.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Sample</th>
<th>TFC column</th>
<th>Flow-rate / Injection volume</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinolones</td>
<td>Honey</td>
<td>Cyclone HTLC, 50 x 0.5 mm, 60 µm (Thermo Fisher Scientific)</td>
<td>1.5 mL min⁻¹ / 160 µL</td>
<td>LC-ESI-MS/MS</td>
<td>[13]</td>
</tr>
<tr>
<td>Quinolones (Enrofloxacin and Ciprofloxacin)</td>
<td>Edible tissues (cattle, pig, turkey, rabbit)</td>
<td>Cyclone HTLC, 50 x 1.0 mm, 50 µm (Thermo Fisher Scientific)</td>
<td>5 mL min⁻¹ / 20 µL</td>
<td>LC-ESI-MS/MS</td>
<td>[14]</td>
</tr>
<tr>
<td>Veterinary drugs</td>
<td>Milk</td>
<td>Cyclone – Cyclone P connected in tandem, 50 x 0.5 mm, 60 µm (Thermo Fisher Scientific)</td>
<td>1.5 mL min⁻¹ / 50 µL</td>
<td>LC-ESI-MS/MS</td>
<td>[15]</td>
</tr>
<tr>
<td>Flavonoids and resveratrol</td>
<td>Wine</td>
<td>50 x 1.0 mm, 60 µm C18 (Thermo Fisher Scientific)</td>
<td>4 mL min⁻¹ / 10 mL</td>
<td>LC-ESI-MS/MS, LC-APCI-MS</td>
<td>[111]</td>
</tr>
<tr>
<td>PFOS</td>
<td>River water</td>
<td>50 x 1.0 mm, 50 µm C18 (Cohesive Technologies)</td>
<td>1 mL min⁻¹ / 1 mL</td>
<td>LC-APPI-MS</td>
<td>[113]</td>
</tr>
<tr>
<td>Anti-infectives</td>
<td>Wastewater</td>
<td>50 x 1.0 mm, 50 µm C18 SL (Cohesive Technologies)</td>
<td>3 mL min⁻¹ / 1 mL</td>
<td>LC-ESI-MS/MS</td>
<td>[114]</td>
</tr>
<tr>
<td>Pesticides</td>
<td>Surface, drinking water</td>
<td>50 x 1.0 mm, 35 µm Oasis HLB (Waters)</td>
<td>5 mL min⁻¹ / 10 mL</td>
<td>LC-APPI-MS/MS</td>
<td>[115]</td>
</tr>
</tbody>
</table>

Table 4. Relevant examples of the application of turbulent flow chromatography.
In a recently published review dedicated to sample preparation methodologies for the isolation of veterinary drugs and growth promoters from food, Kinsella et al. described turbulent flow chromatography as a technique that eliminates time-consuming sample clean-up, increases productivity and reduces solvent consumption without sacrificing sensitivity [116]. Food matrices have a high content of fat and proteins, which makes TFC an ideal sample treatment technique for the determination of a specific class of contaminants in various matrices such as honey, tissues and milk [117]. Some examples are described in the literature concerning the determination of veterinary drugs such as quinolones in honey and animal tissue [13-14]. For instance, turbulent flow chromatography coupled to LC-MS/MS was proposed for the quantitative high-throughput analysis of 4 quinolones and 12 fluoroquinolones in honey [13]. The manual sample preparation was limited to a simple dilution of the honey test portion with water followed by a filtration. The extract was then on-line purified on a large particle size TFC column where the sample matrix was washed away while the analytes were retained. Recoveries of 85-127% were obtained, while matrix effects were still observed which led to the use of standard addition for calibration. The proposed methodology has also shown good robustness, with over 400 injections of honey extracts without any TFC column deterioration, with the consumption of 44 mL of solvent per sample. The authors described that TFC showed a strong potential as an alternative extraction and clean-up sample method compared to those making use of off-line sample preparation, in terms of both increasing the analysis throughput and obtaining higher reproducibility linked to automation to ensure the absence of contaminants in honey samples. In the case of animal tissues TFC was used for sample preparation in the analysis of two quinolones (enrofloxacin and its metabolite ciprofloxacin) [14]. Sample was extracted with a mixture of acetonitrile/water 1:1 acidified with 0.01% formic acid. Mean recovery rates for the tissues of the different species (cattle, pig, turkey and rabbit) were in the range of 72-105% in a run time of only 4 min.

Presta et al. [111] described the use of TFC coupled to LC-MS for the determination of flavonoids and resveratrol in wines. 10 mL of sample (diluted wine) was passed over the TFC column, after which the retained analytes were separated by reversed-phase LC. The method proved to be fast, non-laborious, robust and sensitive.

Turboflow chromatography has also been described for sample treatment in the screening of eight veterinary drugs in milk [15]. Protein precipitation was induced before analyzing samples of whole, skimmed and semi-skimmed milk samples. While matrix effects – ion suppression and enhancement – were obtained for all analytes, the method has proved to be useful for screening purposes because of its sensitivity (0.1 to 5.2 µg L⁻¹), linearity and repeatability (RSD ≤ 12%). As an example, Figure 8 shows the chromatographic separation of a non-fat milk sample spiked with target veterinary drugs and analyzed by TFC-LC-(ESI)-MS/MS.

This sample treatment technique has also been applied successfully to environmental samples. For instance, anti-infectives analysis in wastewater has been reported with good recovery (86-141%) and limits of quantification (45-122 ng L⁻¹) [114]. Signal distortion,
represented as matrix effect, was still observed probably due to the fact that small molecules (below 1000 Da) present in wastewater samples will have affinity for the stationary phase and will not be completely removed in the clean-up step. Takino et al. have minimized the matrix effect observed by using atmospheric pressure photoionization (APPI) instead of electrospray (ESI) as ionization source [113]. In this case, a simple, fast and sensitive LC/APPI-MS method, with automated on-line extraction using TFC was developed for the determination of perfluorooctane sulfonate (PFOS) in river water. TFC columns packed with organic polymers or graphitized carbons were also found to be highly capable for enrichment of trace pesticides from drinking and surface water samples [115].

In summary, turbulent flow chromatography appears as a very useful approach for sample treatment because it possesses greater efficiency in removing proteins based on their size than restricted access media or SPE procedures and combines high-throughput and high reproducibility by means of separating analytes from various matrices with reduced sample handling. The advantages of this sample extraction and clean-up procedure is unquestionable in bioanalytical applications, and although not many applications in other fields such as food and environmental analysis are yet available, it will surely become a very

Figure 8. Representative SRM chromatograms of a non-fat milk sample spiked with the mixture of antibiotics standards at 100 µg L⁻¹ level and analyzed by TFC-LC-(ESI)-MS/MS. Reproduced from [15] with permission from Springer.
useful method in the area of food analysis, especially in matrices with a high content of fat and proteins such as milk.

6. Conclusion

There is an increasing demand for high-throughput chromatographic separations in food and environmental analysis where highly heterogeneous and difficult matrices may be analyzed. Despite the important advances in chromatographic separations, food and environmental matrices are very complex samples, and sample extraction and clean-up treatments are usually required. Therefore, sample treatment is still one of the most important parts of whole analytical method and effective sample preparation is crucial in achieving accurate analytical results. Food and environmental analysis generally requires several steps such as extraction from the sample of interest, removal of co-extracted matrix components, analytes enrichment and their subsequent quantification. Thus, the availability of robust, sensitive, selective and rapid analysis methods is of primary importance. The most recently introduced sample treatment methodologies in food and environmental applications have been discussed in this chapter, such as on-line SPE methods, QuEChERS, MIPs as selective sorbents for SPE, and the use of turbulent-flow chromatography.

On-line SPE is a viable and increasingly popular technique used to improve the sample throughput by reducing sample preparation time and overcome many of the limitations associated with the classical off-line SPE procedure.

For sensitive and selective determination of compounds in very complex matrices, the use of polymers with recognition sites able to specifically bind a particular substance or a group of structural analogues has attracted increased attention due to their outstanding advantages, i.e., high specificity, selectivity and capacity.

QuEChERS appeared as satisfactory, simple, rapid and inexpensive sample extraction and clean-up multi-residue methods especially employed in the analysis of pesticides. However, this methodology is also being successfully employed for the extraction of other families of compounds in food and environmental matrices such as acrylamide, mycotoxins, PAHs and chlorinated compounds.

The use of turbulent-flow chromatography represents a highly attractive and promising approach for removing proteins based on their size better than RAM or SPE procedures. TFC has been satisfactorily applied to the direct analysis of complex matrices with reduced or without any sample manipulation and, even not many applications in food and environmental samples are yet available, it will become a very useful method due to its great potential for the analysis of protein- and fat-rich matrices.

Finally, future developments in all areas of analytical sample preparation are expected to continue in order to improve accuracy, sensitivity, specificity, and reproducibility of the sample treatment technique together with reduced analysis time and sample manipulation.
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