Research Article

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A review of recent developments and trends in the QuEChERS sample preparation approach

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Abstract: A comprehensive review is presented on the recent developments and trends in the QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation approach. This technique involves liquid-liquid partitioning using acetonitrile and purifying the extract using dispersive solid-phase extraction (d-SPE). Originally, the QuEChERS was introduced for pesticides residues analysis in high moisture fruits and vegetables, but more recently it is gaining significant popularity in the analysis of a broad spectrum of analytes in huge variety of samples. The wide range of the technique applications is possible due to introducing various modifications based on the use of different extraction solvent and salt formulation and buffer additions for salting-out partitioning step and the application of various d-SPE sorbents for clean-up step. Therefore, the QuEChERS approach is useful for analysis of, among others pesticides, veterinary drugs and other pharmaceuticals, mycotoxins, polycyclic aromatic hydrocarbons (PAHs), dyes, acrylamide, synthetic musks and UV filters, bisphenols, polybrominated diphenyl ethers and other flame retardants, endocrine disruptors, and other chemical compounds. Thanks to the QuEChERS approach, high-throughput multiresidue methods operate in a routine contaminant control of food products, feedstuffs, and environmental samples.

Keywords: QuEChERS, d-SPE sorbents, matrix effect, food control, LC-MS (MS/MS) or GC-MS (MS/MS)

1 Introduction

Nowadays, there is a growing demand for high-throughput multiresidue methods (MRMs), which should be easy to perform, rapid and of low cost, require a minimum volumes of solvents, provide a high selectivity without complicated clean-up solutions, and allow analysing broad range of analytes. To accomplish the goal, QuEChERS as a quick, easy, cheap, effective, rugged, and safe multiclass, multiresidue analytical approach was introduced. It was for the first time presented at the 4th European Pesticide Residue Workshop in Rome in 2002 by Anastassiades, Lehotay, Stajnbaher, and Schenck [1] and then the detailed method was published in 2003 [2]. This technique involves liquid-liquid partitioning using acetonitrile (MeCN) and purifying the extract using dispersive solid-phase extraction (d-SPE) [2]. Since its development and until November 2014, about 900 papers on using QuEChERS methods have been published, according to the Web of Science. Originally, QuEChERS was introduced for pesticides residues analysis in fruits and vegetables with high water content. However, more recently it is gaining significant popularity in the analysis of pesticides and other compounds in a huge variety of food products and other with different types of matrices. QuEChERS method has important advantages over most traditional extraction methods. It enables yielding high recovery rates for a wide range of analytes and is characterized by very accurate (true and precise) results thanks to the use of an internal standard (IS) for elimination of problematic commodity differences [3]. Internal standard addition is also important for minimization of error generation in the multiple steps of the QuEChERS [4]. Another important advantage of the QuEChERS technique is its rapid character and high sample throughput. Using this method, a batch of 10–20 samples could be extracted in 30–40 min by a single analyst [3]. QuEChERS approach is also in accordance with so-called green chemistry due to low solvent consumption and absence of chlorinated solvents and a very small waste generation [5]. These arguments
and the need of using only basic laboratory devices make this sample preparation technique relatively inexpensive in comparison to most traditional extraction methods [3]. The QuEChERS method and its modifications are now rapidly developing beyond its original scope of application. In our paper we focused on developments of QuEChERS accumulated since the dawn of the technique to the beginning of September 2014, according to a literature overview performed using Elsevier, Springer, Willey, ACS, PubMed and Google search engines. In this review paper most recent achievements with application of the QuEChERS, as e.g., a sample treatment for analysis of different compounds classes in various food sample types are presented. In comparison, Bruzzoniti et al. described a critical review on QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices such as soils, sediments and water [6]. The novelty of our article may be noticed especially in comprehensive description of extracts purification and ‘complicated analytes’ analysis, as well. We also made an assessment of actual trends and perspectives of application of QuEChERS taking into account attempts related to automation of its subsequent steps. Potential readers can gain practical information about introducing various modifications to QuEChERS and can efficiently optimize procedures in the light of the scope of their research.

2 QuEChERS – general information

The QuEChERS procedure entails a number of simple analytical steps and is thus fast and easy to perform. In brief, QuEChERS involves an acetonitrile salting-out extraction of a solid sample in an aqueous environment followed by dispersive solid phase extraction (d-SPE) to remove a majority of the remaining matrix interferences [7]. The final extract concentration of the method in MeCN is 1 g mL⁻¹. In order to obtain lower value of 10 ng g⁻¹ limit of quantitation (LOQ) in modern gas chromatography coupled with mass spectrometry (GC-MS), large volume injection (LVI) of 8 µL is generally required [3]. The final sample for GC-MS analysis can be reconstituted in the other more suitable solvent like toluene (4 g mL⁻¹), in which 2 µL splitless injection provides the anticipated degree of sensitivity [3].

During the development of the method authors have to deal with some fundamental aspects. The major aspect considered in initial extraction and extraction/partitioning stage were: the choice of extraction solvent and sample/solvent ratio, sample amount, influence of sample pH on recoveries; type and amount of salts used for phase separation induction and use of internal standard [2,8]. As for clean-up stage by d-SPE, type and amount of sorbent and MgSO₄ and their selectivity were the main problematic issues [2,7,9,10]. In case of final instrumental analysis, influence of clean-up step on matrix effects and application of analytes protectants for GC were studied.

Liquid-liquid extraction (LLE) has long been an effective method of separating compounds having different solubilities in two immiscible liquids [4]. Furthermore, the addition of an inorganic salt into a mixture of water and a water-miscible organic solvent causes a separation of the solvent from the mixture and formation of a two-phase system. Observations of salting out extraction/partitioning were made for a number of water-miscible organics such as: acetone, ethyl acetate, methanol, ethanol, and acetonitrile. Various salts and their different concentrations caused different degrees of phase separation. High polarity solvents used in salting out systems have been investigated for extraction or concentration of many analytes that cannot be extracted by conventional LLE solvents [4]. The choice of acetonitrile as a solvent for the first step of the QuEChERS was made on the basis of its selectivity, which means that only few co-extractives from matrix were extracted but still broad scope of pesticides (analytes) was covered [2,10]. Another advantage of acetonitrile is its compatibility with the chromatographic applications, although it tends to give a large solvent expansion volume during GC vaporization, interferes with nitrogen-specific GC detectors, and is less volatile than the other common organic solvents, thus making evaporative concentration steps more time consuming [10,11]. Moreover, the solubility of lipids in acetonitrile is limited, thus lipid co-extraction with this solvent is relatively low, but problems of accessibility of pesticides from lipids may occur. This results in losses of non-polar pesticides and their recoveries drop proportionally to lipid/solvent partition coefficient [10]. Other non-halogenated solvents such as acetone and ethyl acetate may be used [4], but acetonitrile is recommended for QuEChERS, because upon the addition of salts, it is separated more easily from water than acetone. The polarity of acetonitrile is higher than that of acetone and ethyl acetate, therefore, the medium to high polar pesticides have much better solubility and hence higher recoveries when MeCN is used [4]. In comparison, ethyl acetate has some other disadvantages of: (I) possibility to extract lipids and waxes stronger than acetonitrile; (II) lower recoveries for acid/base pesticides and (III) lower clean-up efficiency in d-SPE [4]. QuEChERS inventors claim that miniaturization of sample amounts improve
efficiency of extraction and contributing less material consumption and costs reduction as well. Very important is also appropriate homogenization of the samples, e.g., by usage of dry ice for blending. The procedure was therefore optimized for 10 g well-homogenized subsamples by using cryogenic milling to maximize surface area and to ensure better extraction efficiencies [2,10]. The use of dry ice during the homogenization step is highly recommended also due to loss of the more volatile analytes prevention [2,11]. A homogenization procedure is essential to have confidence that 10 g subsample is representative of the original tested commodity sample [2,11]. In order to achieve the final extract concentration equal 1 g mL⁻¹, the sample/solvent ratio for initial extraction was established to 1:1 (w/vol), which still allows obtaining good recoveries of studied pesticides residues without any evaporation step with application of modern chromatographic instruments [2,10]. Different type and amounts of salts used in salting-out step affect recovery rates. It is well known that concentration of salt can influence the percentage of water in the organic layer and therefore can adjust its “polarity” [2,11]. Anastassiades and co-workers conducted experiments with deuterated solvents and nuclear magnetic resonance to investigate the influence of different salt additions on recovery and other extraction parameters [2,11]. Among tested salts ammonium formate application to induce phase separation is also promising in monitoring of GC- and LC-amenable pesticides [12]. Recent reports demonstrated that ammonium formate application to induce phase separation is also promising in monitoring of GC- and LC-amenable pesticides [12].

For minimization of error generation in multiple steps of the QuEChERS process, an internal standard is frequently added. In the original development method, authors applied triphenylphosphate (TPP) for this purpose, which could undergo quantitative extraction from low-fat matrices [2]. A more complete study of various internal standards was undertaken by Anastassiades, in which he proved that the application of more than one internal standard as a quality control determinant allows recognition of errors due to mis-pipetting or discrimination during partitioning or clean-up [4,13]. As for this study, the internal standard is generally employed at an early stage of the experiment. However, in the case of samples with high fat content, the excessive fat can form an additional layer into which analytes can partition and get lost. In the presence of high fat amounts (for example, higher than 0.3 g of fat per 10 mL of acetonitrile), it was recommended to employ the internal standard at the end of the procedure [13].

The next problem to solve was the conditions under which d-SPE clean-up step needs to be conducted. To perform traditional solid phase extraction (SPE), cartridges containing various amounts and types of sorbents are used. The principle of SPE is similar to that of LLE, involving a partitioning of analytes between two phases, but instead of two immiscible liquid phases, as in LLE, SPE involves partitioning between a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase [14]. In d-SPE, an aliquot of sample extract is added to a centrifuge tube containing a relatively small amount of SPE sorbent and the mixture is shaken to increase distribute the SPE material and facilitate the clean-up process. Next, centrifugation of the sample enables separation of the sorbent and an aliquot of nascent supernatant can be analyzed. The sorbent in d-SPE clean-up step is chosen to retain undesired, co-extracted compounds from the matrix and to allow the analytes of interest remain in the liquid phase [4]. Dispersive SPE shows few advantages against classical solid phase extraction like: (I) no need of use SPE manifold and vacuum/pressure devices, (II) no conditioning step needed, (III) no problems with channeling, flow control, drying-out, (IV) no elution step needed, (V) no dilution of extract and therefore no evaporation needed (VI), (VII) less sorbent expenditure, (VIII) faster and cheaper and (IX) no experience to perform needed [10]. Magnesium sulfate is added simultaneously with the d-SPE sorbent to remove the majority of the undesirable water and improve analyte partitioning to provide better clean-up [2,11]. In the originally developed QuEChERS method, Anastassiades and his co-workers used 150 mg anhydrous magnesium sulfate and sodium chloride (MgSO₄·7H₂O), which he proved that the application of more than one internal standard as a quality control determinant allows recognition of errors due to mis-pipetting or discrimination during partitioning or clean-up [4,13]. As for this study, the internal standard is generally employed at an early stage of the procedure [13].

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MgSO₄ and 25 mg primary secondary amine (PSA) sorbent for 1 mL of acetonitrile extract for removal of residual water and simultaneously performed the clean-up [2]. PSA is a sorbent commonly applied for the removal of sugars and fatty acids, organic acids, lipids and some pigments from the preliminary extract [15]. As a substitution for 25 mg PSA, Lehotay proposed the use of 75 mg aminoethyl SPE sorbent per mL of extract [3]. Various d-SPE sorbent formulations, not included in the official methods, were tested to be useful in d-SPE clean-up step of the QuEChERS method.

The optional step of the QuEChERS procedure is to apply some compounds, which could act as so-called analyte protectants in GC analysis for analytes that might tail or breakdown on the capillary GC column interior surfaces, on sorbed nonvolatile compounds from previous injection, and on the inlet liner or on the precolumn (guard column). In this case, analyte protectants are added to the extracts before GC [4]. According to Anastassiades, various compounds were tested for their “protective potential” and best protection provides polyhydroxy-compounds like: sugars and their derivatives, e.g. sorbitol, ethylglycerol and δ-gulonolactone [10]. The use of analyte protectants allows minimizing errors related to matrix-induced enhancements, which is clearly demonstrated in Fig. 1 [16]. Further improvements were directed towards pH-issues, selectivity issue and expanding matrix scope. Modification of original method for improvement stability of pH-labile compounds and recoveries of ionizable compounds are reviewed in 2.2.1 subsection of this paper. Selectivity of extraction and clean-up step issues for analysis of contaminant residues in difficult matrices (e.g. fatty, dried and highly pigmented commodities) are considered in 2.2.2 subsection of the paper.

2.1 General procedure – main steps

The originally published QuEChERS procedure as a simple, fast and inexpensive method for the determination of pesticide residues in fruits and vegetables enabling a researcher to achieve recoveries between 85 and 101% and repeatability – expressed as %RSD – were typically below 5% for a wide range of fortified pesticides [2]. The procedure entails several successive steps. First step is to weigh 10 g of the well-chopped, homogenized sample into a 40 mL polypropylene (PP) centrifuge tube followed by addition of 10 mL of acetonitrile and shaking the sample vigorously for approximately 1 minute. Next, an addition of 4 g anhydrous MgSO₄ and 1 g NaCl is followed by intense agitation. After that, an internal standard for GC-MS (ISTD) is added and next whole sample is shaken for 30 s and centrifuged. Afterwards, a 1 mL aliquot of the upper acetonitrile layer is transferred into a centrifuge vial containing 25 mg of PSA sorbent and 150 mg of anhydrous MgSO₄. Then, the sample is shaken by hand or with the vortex mixer for 30 s and centrifuged. The obtained supernatant is taken from the centrifuge vial and as a final extract can be analyzed directly by GC- and/or LC-techniques coupled with mass spectrometry detectors [2]. The schematic flow chart for main steps of originally developed QuEChERS is presented in Fig. 2 [17,18].

2.2 Standardized methods and their modifications

Lehotay in 2005 conducted a validation experiments of the QuEChERS method for the determination of residues from 229 pesticides in fruits and vegetables using gas and liquid chromatography and mass spectrometric detection [19]. The 15 g lettuce and orange samples were fortified at 10–100 ng g⁻¹. Next, extraction using 15 mL acetonitrile followed by a liquid-liquid partitioning step performed by adding 6 g anhydrous MgSO₄ and 1.5 g NaCl was conducted. After centrifugation, the extract was decanted into a tube containing 300 mg PSA sorbent and 1.8 g anhydrous MgSO₄. The obtained purified final extracts were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) with an ion trap (IT) instrument and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) with a triple quadrupole (QqQ) instrument using electrospray ionization [19]. In this study, recoveries for almost all of the pesticides in at least one of the matrices ranged between 70–120% (90–110% for 206 pesticides), and repeatabilities typically below 10% were achieved for a wide range of analytes, including methamidophos, spinosad, imidacloprid, and imazalil [19]. The results demonstrated that the clean-up step with PSA sorbent retained carboxylic acids (e.g., daminozide), and below 50% recoveries were obtained for asulam, pyridate, dicrofol, thiram, and chlorothalonil [19]. Another occurring problem was that in nonacidic matrices, like lettuce, pesticides sensitive to a basic pH, such as captan, folpet, chlorothalonil and dichlofluanid, were degraded [19]. Consequently, some modifications to the originally published method had to be introduced to ensure efficient extraction of pH-dependant compounds and to expand the spectrum of matrices covered. Lehotay et al. and Anastassiades et al. realized that introduction of buffering salts to improve recoveries of pH-dependant analytes was necessary [20,21]. The buffering at pH between 5 and 5.5
Figure 1: The use of analyte protectants to minimize errors related to matrix-induced enhancements. The error is considered to be the difference between the relative signal obtained from cucumber extracts and the signal obtained from a standard in pure solvent containing the same concentrations of pesticides. The errors are given as absolute values. PCB-138 was used as the ISTD. With permission from [16].
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during extraction provided the optimum balance to attain sufficiently high recoveries (higher than 70%) for some pH-dependent pesticides (e.g. pymetrozine, imazalil, thiabendazole) independent of the fruit/vegetable matrix [20-22]. Lehotay et al. implemented the use of relatively strong acetate buffering conditions [20] and Anastassiades et al. decided to use weaker citrate buffering conditions [21] in terms of ionic strength. The solution prepared by Lehotay et al. involves the extraction of the sample with acetonitrile containing 1% acetic acid (HOAc) and simultaneous liquid-liquid partitioning formed by adding anhydrous MgSO\textsubscript{4} and sodium acetate (NaAc) [20].

**Figure 2:** Schematic flow chart for main steps of three primary QuEChERS methods: Original QuEChERS Method [2], AOAC 2007.01 Official Method [17], EN 15662 The European Official Method [18] (abbreviations used: GCB – graphitized carbon black; MgSO\textsubscript{4} – magnesium sulfate anhydrous; MeCN – acetonitrile; HOAc – acetic acid; NaOAc – sodium acetate; NaCl – sodium chloride; Na\textsubscript{3}Citrate – sodium citrate tribasic dehydrate; Na\textsubscript{2}HCitr – sodium citrate dibasic sesquihydrate; PSA – primary secondary amine sorbent; TPP – triphenyl phosphate).
The obtained recoveries were 95% (± 10%), even for some problematic pesticides [20]. Anastassiades et al. regarded acetate buffering as beneficial due to the requirement of the addition of only one solid component and thus keeping whole procedure simple [21]. Taking into consideration that parts of acetate buffer are evidently partitioning into the organic phase and exhibit there a strong buffering activity the application of this kind of buffering procedure results in virtually constant pH values of the acetonitrile extract [21]. As for stability of alkaline-sensitive pesticides this seems to be an advantage, but on the other hand it can be considered a disadvantage regarding the clean-up efficiency of PSA in d-SPE step. The strong buffer activity of the acetate resulting in visibly worse clean-up performance of PSA compared to the original QuEChERS method [21]. Anastassiades et al. finally chose a mixture of disodium and trisodium citrate as the best solution to adjust the pH of various samples to desired range without negative impact on the subsequent PSA clean-up step [21]. An additional relevant pH-dependent issue with significant influence on pesticides determination and quantification is the degradation of analytes in the final sample extracts. After PSA clean-up step, the measured pH of the final extracts reaches values typically in the range from 8 to 9, which endanger the stability of base sensitive pesticides such as captan, folpet, dichlofluanid, tolylfluanid, pyridate, methiocarb sulfone and chlorothalonil [21]. Adjustment of the extracts after QuEChERS procedure to a pH value about 5 was deemed to be a satisfactory compromise for most of pH-related degradation susceptible analytes [21]. The addition of formic acid (5% in MeCN) bring pH of extracts to the value about 5 and seems to be the easiest solution to the problem [10]. Both, introduction of buffering salts to improve recoveries of pH-dependant analytes and ongoing carrying out validation studies led to elaboration of two official QuEChERS methods. The approach conducted by Lehotay et al. resulted in the official method of AOAC 2007.01 [17]. The citrate buffered method introduced by Anastassiades et al. at the CVUA Stuttgart effected in the European Standard EN 15662 method published in 2008 [18]. In accordance to the European Standard EN 15662 protocol, Payá et al. conducted study for safe multiresidue method for validation of the extraction of 80 pesticides belonging to various chemical classes from various types of representative commodities with low lipid contents such as cucumber, wheat flour, raisins, orange, lemon, red grapes and red wine [16]. Obtained mean recovery values mostly ranged between 70 and 110% (98% on average), and relative standard deviations (RSD) were generally below 10% (4.3% on average) [16]. It was also demonstrated that in LC-MS/MS analysis, matrix effects resulting from certain commodity/pesticide combinations cannot be neglected and should be taken into account in order to avoid incorrect results [16]. As for GC-MS/MS analysis, the concept of analyte protectants proved to be an successful approach to minimizing the errors linked to the use of standards in pure solvent [16]. General steps of both official methods [17,18] are presented in Fig. 2.

### 2.2.1. Modification of procedure based on properties of analytes

While most analytes (especially pesticides) give satisfactory recoveries using the official methods, some show poor extraction efficiency and require some certain modifications or even separate procedures. Very polar, acidic or basic analytes might be troublesome, thus if such analytes are within the scope of analysis, some improvements should be made [21].

#### 2.2.1.1. Basic and acidic analytes

In terms of acidic and basic compounds considered as pH-dependent analytes some modification of QuEChERS might be needed to achieve satisfactory recoveries. Some pesticides get ionized at low or high pH-values in dependence to their physic-chemical properties (pKa values). It is well known that ionic form prefers to remain in the water phase during QuEChERS extraction/partitioning step [22]. For acidic analytes pKa value corresponds to the pH above, which compounds stay in deprotonized form and for bases pKa value is equal pH below which compounds lay predominantly in protonized form. Taking into consideration the pH-range of agricultural samples spanning between about 2.5 for some citrus fruits or juice to 7 for e.g. asparagus, ionization of some pesticides is inevitable [22]. Despite that fact, it was shown that for basic pesticides an effect of pH on recoveries is insignificant [10]. Regardless of theoretically adverse pH, the basic pesticides still prefer to be partitioned into the acetonitrile layer, which can be explained in that the MeCN phase after partitioning still contains a appreciable amount of water [10]. In the case of acidic pesticides, influence of pH on recovery is substantial and the QuEChERS method has to be modified, in order to include such compounds in analysis [22]. Anastassiades et al. list 32 acidic pesticides and some of them like 2,4-D and fluazifop are relatively often found in food samples [22]. For acidic pesticides a recovery drop of an alarming degree at pH 6 is observed [10].
for exemplary pesticide residues in the light of their pKa values is shown in Fig. 3 [22]. The use of buffers is crucial for the inclusion of acidic pesticides in the analysis spectrum [23]. The results obtained by Lehotay et al. [23] demonstrate that the QuEChERS version using the strong acetate buffering at pH 4.8 [20] more often gave higher and more consistent recoveries for the problematic, pH-dependent pesticides than the unbuffered method (as expected) and the citrate-buffered version, which uses citrate buffering of weaker strength and slightly higher pH of 5–5.5 [21].

Acidic analytes are also frequently covalently bound to matrix components and thus their concentration are often underestimated [22]. For conjugates disruption and conversion of all possible residues to free acid, alkaline hydrolysis is proposed as a suitable solution. Alkaline hydrolysis can be carried out by an addition of 5 N NaOH solution to the sample (leaving the sample for 30 min at room temperature) and subsequent neutralization by addition of 5 N H2SO4 solution [22]. Exemplary protocol for analysis of acidic pesticides in wheat four samples by LC-MS/(MS) using the QuEChERS method including optional alkaline hydrolysis to release covalently bound compounds is elaborated by Anastassiades [24]. Another occurring problem during acidic analytes extraction with the classic QuECHERS method is significant losses of acidic compounds after clean-up step due to their interaction with PSA sorbent [22]. Therefore, clean-up step with PSA sorbent should be avoided and instead of raw extract should be analyzed if possible. An optional freeze-out step over night, for removal of co-extracted fats as well as other components with limited solubility in acetonitrile, could be performed [24].

2.2.1.2 ‘Complicated’ analytes
Buffering with acetate or citrate salts in the first extraction/partitioning step has been introduced to adjust the pH to a compromise value, where most analytes, labile under acidic or alkaline conditions, are sufficiently stabilized. This issue has been widely discussed in previous subsections (2.2.–2.2.1.). The case of degradation of base-labile compounds such as captan, folpet, dichlofluanid and others in QuEChERS extracts after PSA clean-up was also described earlier (2.2.). Briefly, the extracts have to be acidified using formic acid to a pH of about 5 to stabilize these alkaline-labile analytes [21]. A little different situation occurs in case of highly acid-labile analytes such as ethoxyquin and pymetrozine, which degrade at a pH of 5 [25]. Analytical measurement of such compounds should be performed immediately or alternatively directly from the non-acidified extract. The recoveries of the acid-labile pesticides could be improved if 1.5 g of trisodium citrate is used, instead of using 1 g of the di- and 0.5 g
of the trisodium citrate [25]. Additionally, keeping low temperatures is helpful especially for ethoxyquin [25].

Another ‘complicated’ analyte, which shows poor extraction efficiency and requires separate procedure is a widely employed non-systemic fungicide – chlorothalonil [26]. Analysis of this compound with multiresidue methods is highly challenging due to its tendency to exhibit losses during sample preparation, storage of sample extracts and standard solutions as well as during GC measurements. The susceptibility of chlorothalonil to losses largely depends on the pH value as well as on the commodity type. For example, allium and brassica crops, containing components that reportedly undergo reactions with chlorothalonil having a particularly negative impact on its stability [26]. Specific modifications of the QuEChERS method are required for accurate determination and quantification of chlorothalonil. At the beginning of the extraction procedure, acidification of the analytical sample with sulphuric acid to pH about 1 is applied. Moreover, buffer salts and d-SPE clean-up step should be avoided. Determinative analysis is performed via GC-MS or LC-MS/MS in the APCI negative mode [26].

Some highly polar analytes (with log $K_{ow}$ below -2), due to very low recoveries caused by poor or no partition into the organic phase, are considered as non-QuEChERS-amenable and require different procedures [27]. Among such analytes, one may mention for example pesticides such as chlormequat, mepiquat and glyphosate [21].

Nicotine is another analyte, which require some improvements for effective determination [28]. Taking into account its physic-chemical properties pH adjustment is necessary for low recoveries correction. Bringing pH of the sample to 10–11 by addition of 5 N NaOH results in satisfactory recovery rates for nicotine residues analysis in mushrooms (Fig. 4) [28].

2.2.2 Modification of procedure based on properties of matrices

The original QuEChERS method [2] only focused on high water and low fat containing commodities such as high moisture fruits and vegetables and juices. Other types of food samples often require some improvements. Especially challenging for analysis are food products with intermediate or high fat content and highly pigmented commodities or that with high chlorophyll content. The dried foodstuffs, feeds and other products with very complicated matrices, having a lot of co-extractives, like herbs, are also difficult to handle. Commodities with less than 80% of water content generally require the addition of water to reach the total mass in the sample approximately 10 g [21] in order to weaken interactions of analytes with matrix and to ensure adequate partitioning [10]. The EN 15662 Method guidelines of adding water into commodities with low water content are presented in Table 1.

Examples of fatty foods include commodities like olives, oil seeds, oils, milk and other dairy products, fish and meat. For these commodities specific modifications of QuEChERS method are crucial to obtain good recovery values and satisfactory purification of the extract. Fatty foods are challenging because some of the lipids are co-extracted with acetonitrile and may cause difficulties in subsequent analysis or some fat-soluble nonpolar analytes may persist in fatty food sample and give poor extraction efficiency rates. Tailoring the use of QuEChERS
d-SPE sorbent for sample clean-up is most important for analysis of pesticides in complicated matrices with high lipid content [29]. Generally, co-extracted fat are removed by freezing out or C18 sorbent in d-SPE clean-up step.

Very acidic foods like strawberries, pineapple and raspberries as well as very acidic citrus fruits are also difficult for contaminants testing. As it was mentioned earlier, lower pH samples will produce extracts with higher co-extracted interferences [10]. The buffering capacity of existing methods cannot adequately correct for the low pH of citrus fruits. Therefore, application of the EN method recommendations requires the addition of 600 µL of 5 N sodium hydroxide solution for citrus fruits and 200 µL for raspberries [18,30]. Although the pH of citrus fruits can be modified, further complication is that citrus fruit peel contain compounds like pectin and flavonoids and oil consisting of monoterpenes, sesquiterpenes and aliphatic hydrocarbons and even waxes and dyes applied to the peel surface for preventing dehydration, increasing shelf life and to ensure a shiny, brightly coloured appearance. The presence of these compounds could cause interferences and strong matrix effects [30]. The matrix effect of an analyte is the difference in signal in a solvent solution compared with signal in matrix. A matrix effect of 100% indicates the signals are the same and no observable change to the signal occurs in sample. Values of 100 ± 20% are considered suitable values indicating small matrix effects. Misselwitz and coworkers from Restek Corporation performed experiments for celery, kale, avocado and lime fortified with about 100 pesticides and tested the samples by LC-MS/MS in order to determine the matrix effect [31]. QuEChERS method application resulted in low matrix effects for only 22% of analytes for the lime sample. This indicates co-extractives remaining in the final extract caused overwhelming matrix effects. In an attempt to improve performance, removal of the peel oil can be done by freezing the extract before the QuEChERS d-SPE clean-up step [30,31]. Lehotay et al. conducted comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in fruits and vegetables [23]. Authors evaluated the LC-MS/MS matrix effect in the originally developed, acetate-buffered and citrate-buffered QuEChERS versions for commodities such as apple-blueberry sauce, peas and limes. In the case of limes, clear matrix suppression effects occurred for all of the pesticides in the LC-MS/MS analyses from 12% (dichlorvos) to 80% (imazalil). The complete results are presented in Fig. 5 [23]. Matrix-matched calibration, especially for difficult matrices, is necessary for obtaining acceptably accurate quantitative results [32].

### 2.2.2.1 Types of samples and their purification in the d-SPE step

Selectivity of the d-SPE clean-up step is crucial for obtaining satisfactory and accurate results. Various sorbents are commonly used for co-extractives removal depending on the different sample type. More than 50 varied SPE sorbents were tested in the terms of their selectivity and applicability [10]. During ongoing experiments, it was found that different dispersive sorbents had a significant influence on the purification and recovery rates of analytes. Amino-sorbents and alumina allows removal of organic acids (including some fatty acids), sugars and some pigments (anthocyanes, chlorophyll), but these sorbents cause significant losses of acidic analytes [10]. Among these kind of d-SPE sorbents the most commonly used in the QuEChERS methods is PSA with main function to remove co-extracted constituents such as fatty acids, sugars and ionic-lipids making PSA suitable for variety of plant-based commodities [2]. Carbon-based sorbents are useful for purification of carotinoids, chlorophyll, sterols with disadvantage of losses of planar analytes [10]. Sorbents applied in reversed-phase system, such as octadecyl silica (C18), provides good results in the purification of samples with significant fat and waxes.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample weight</th>
<th>Water addition</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruits and vegetables with water content over 80%</td>
<td>10 g</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fruits and vegetables with 25–80% water content</td>
<td>10 g</td>
<td>X g</td>
<td>X = 10 g – water content in 10 g sample</td>
</tr>
<tr>
<td>Cereals</td>
<td>5 g</td>
<td>10 g</td>
<td></td>
</tr>
<tr>
<td>Dried fruits</td>
<td>5 g</td>
<td>7.5 g</td>
<td>Water can be added during homogenization step</td>
</tr>
<tr>
<td>Honey</td>
<td>5 g</td>
<td>10 g</td>
<td>–</td>
</tr>
<tr>
<td>Species</td>
<td>2 g</td>
<td>10 g</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1: The CEN 15662 Method guidelines for the addition of water into commodities with low water content.
content but recoveries of the more lipophilic pesticides may suffer [29]. PSA sorbent gives not satisfying results in the case of samples with high contents of carotenoids or chlorophyll. Small amounts of GCB in combination with PSA proved to be the best solution in handling with such matrices, but due to high affinity of planar pesticides towards GCB (e.g. hexachlorobenzene, chlorothalonil, thiabendazole) it shows significant limitations [10]. What is important is that chlorophyll has higher affinity than all pesticides, thus final extract should remain slightly coloured to ensure that planar pesticides were not significantly affected [10]. In some cases, the use of CaCl$_2$ instead of MgSO$_4$ in clean-up step is beneficial. Calcium chloride allows for more water removal and thus interactions of matrix components with the PSA sorbent (e.g., ionic or H-binding) become stronger and better purification may be obtained [33]. However, CaCl$_2$ is an appropriate solution only if polar pesticides are not
Table 2: Recommended application of common d-SPE sorbents for the QuEChERS clean-up step of various matrix types for AOAC Official Method and EN 15662 Method.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>d-SPE clean-up purpose</th>
<th>AOAC Method</th>
<th>EN Method</th>
<th>e.g. commodities</th>
</tr>
</thead>
<tbody>
<tr>
<td>General fruits and vegetables</td>
<td>Removal of polar organic acids, some sugars and lipids</td>
<td>50 mg PSA 150 mg MgSO₄</td>
<td>25 mg PSA 150 mg MgSO₄</td>
<td>apple, papaya, peach, strawberry, grapes, tomato, celery, radish</td>
</tr>
<tr>
<td>Fruits and vegetables with fats and waxes</td>
<td>Removal of polar organic acids, some sugars, more lipids and sterols</td>
<td>50 mg PSA 50 mg C18 150 mg MgSO₄</td>
<td>25 mg PSA 25 mg C18 150 mg MgSO₄</td>
<td>avocado, almonds, olives, nuts, oil seeds, orange peel</td>
</tr>
<tr>
<td>Pigmented fruits and vegetables</td>
<td>Removal of polar organic acids, some sugars and lipids, and carotenoids and chlorophyll; not for use with planar pesticides</td>
<td>50 mg PSA 50 mg GCB 150 mg MgSO₄</td>
<td>25 mg PSA 2.5 mg GCB 150 mg MgSO₄</td>
<td>red grapes, raspberries, redburrant, carrot, paprika</td>
</tr>
<tr>
<td>Highly pigmented fruits and vegetables</td>
<td>Removal of polar organic acids, some sugars and lipids, high levels of carotenoids and chlorophyll; not for use with planar pesticides</td>
<td>50 mg PSA 50 mg GCB 150 mg MgSO₄</td>
<td>25 mg PSA 7.5 mg GCB 150 mg MgSO₄</td>
<td>blackberries, blueberries, blackcurrant, spinach</td>
</tr>
<tr>
<td>Fruits and vegetables with pigments and fats</td>
<td>Removal of polar organic acids, some sugars and lipids, carotenoids and chlorophyll; not for use with planar pesticides</td>
<td>50 mg PSA 50 mg GCB 150 mg MgSO₄</td>
<td>—</td>
<td>avocado, black olives, eggplant</td>
</tr>
</tbody>
</table>

In the scope of analysis, because with this salt recoveries of such analytes drop significantly [10,34]. Commonly used sorbents recommended for purification purposes of different kinds of samples are listed in Table 2.

2.2.2.2 Application of alternative sorbents in the d-SPE clean-up steps for samples with complicated matrices

In order to enhance sample clean-up for complex matrices by effectively removing interferences and overcome existing problems for traditional QuEChERS dispersive phases, new d-SPE sorbents are developed. Therefore, beside the commonly used sorbents in the QuEChERS d-SPE clean-up step, there are literature reports of new alternative ones.

Chlorophyll is one of the most problematic matrix co-extractives in pesticide residue analysis due to its non-volatile characteristics. Graphitized carbon black (GCB) is widely applied to remove chlorophyll from samples, but its significant disadvantage is strong adsorption of planar analytes resulting in low recoveries. To resolve this issue, UCT has developed a novel sorbent (ChloroFilTr®) for efficient removal of chlorophyll from QuEChERS extracts without loss of planar analytes [15]. ChloroFilTr® has been tested against hundreds of pesticides and herbicides and has been shown greater than 82% reduction of chlorophyll, without loss of planar analytes. However, ChloroFilTr® should not be used for mycotoxin and hexachlorobenzene analysis [15]. Wang demonstrated that recoveries of some planar analytes such as carbendazim, thiabendazole, pyrimethanil and cyprodinil were adversely affected by GCB, especially thiabendazole with markedly lower recovery of 55.9% compared to 93.2% obtained by ChloroFilTr® [35]. Taking all this into account, ChloroFilTr® offers a successful substitution for GCB in chlorophyll removal.

Other novel commercially available sorbents are Z-Sep and Z-Sep Plus offered by Supelco [36]. The Z-Sep is a sorbent based on modified silica gel with zirconium oxide and the Z-Sep Plus sorbent consists of both zirconia and C18 dual bonded on the same silica particles. These innovative dispersive phases demonstrate ability to extract more fat and pigment than traditional PSA and C18 sorbents and show greater analyte recovery and better reproducibility [36]. Sapozhnikova and Lehotay evaluated three different sets of sorbents for d-SPE clean-up of 1 mL of initial catfish extract [37]. In each case, authors used 50 mg of sorbent (C18/PSA; Z-Sep; Z-Sep Plus) in combination with 150 mg anhydrous MgSO₄. The obtained results demonstrated that C18 + PSA in d-SPE removed most of the co-extractive materials from the extract by weight, but the Z-Sep d-SPE TIC chromatogram showed the lowest background levels chromatographically (Fig. 6) [36,37]. While all three sorbent combinations provided satisfactory recoveries, the purification with Z-Sep showed the best values (70–120%) with the maximum standard deviation (SD) of 13%, indicating good repeatability of the method [37]. Geis-Asteggiante et al. conducted experiments for evaluation various clean-up
sorbents and their combinations for extracts purification in multiresidue method for monitoring 127 veterinary drug residues in bovine meat using UHPLC-MS/MS [38]. Z-Sep and Z-Sep Plus allowed effective getting rid of co-extractives, but in the light of obtained veterinary drugs recoveries were inappropriate. Tetracyclines, fluoroquinolones, and macrolides were the three groups of drugs most retained by both Z-Sep Plus and Z-Sep with hexane. Additionally, Z-Sep and hexane strongly retained the β-lactams investigated by authors. Evaluation of zirconium dioxide-based sorbents to decrease the matrix effect in avocado and almond multiresidue pesticide analysis followed by GC-MS/MS was considered by Lozano et. al. [39]. In this study, the QuEChERS method with Z-Sep sorbent ensured better removal of co-extracted matrix compounds and higher recoveries than Z-Sep Plus or PSA/C18. Tuzimski and Rejczak demonstrated better clean-up efficiency of Z-Sep Plus than C18 sorbent in sunflower seeds samples [40].

Chitin obtained from shrimp shell waste was applied in the d-SPE clean-up step in methodology elaborated for organic contaminants analysis in drinking water treatment sludge by Cerqueira et al. [41]. This approach allowed authors to obtain most satisfactory recovery rates in comparison to other sorbents used for purification such as C18, PSA, PSA and C18, and GCB. An additional asset of chitin as a dispersive clean-up phase was the significant reduction of the method costs [41].

Another solution for extract purification was proposed by Hou et al. [42]. A modified QuEChERS method used multi-walled carbon nanotubes (MWCNTs) as a dispersive solid phase extraction adsorbent, which was then applied by the authors for analysis of 78 pesticide residues in tea. With 6 mg of MWCNTs, recoveries of the targeted pesticides were mainly within the acceptable range from 70 to 120%. This amount showed comparable purification efficiency with traditional QuEChERS method with PSA clean up. Authors demonstrated that MWCNTs mixed with PSA resulted in further improvement of the performance of d-SPE step [42]. The photography of raw and purified extracts using different sorbents is shown in Fig. 7.

Deng et al. tested amine-functionalised magnetic nanoparticles and multiwalled carbon nanotubes (MNPs/MWCNTs) composites as an adsorbent for rapid clean-up of acetonitrile extracts of tea samples prior to analysing eight pesticide residues by GC-MS [43]. Amine functionalized MNPs may promote strong interaction with various polar organic acids, due to their weak anion

Figure 6: Figure shows: a – total ion chromatograms (TICs) of catfish extracts after d-SPE clean-up with C18 + PSA, Z-Sep, and Z-Sep Plus; 1) deoxysperqualin; 2) oleic acid; 3) octadecadeinoyl chloride; 4) octadecynoic acid; 5) eicosatrienoic acid; 6) pyrrolizidine-one-5-ol, ethyl ether; 7) 17-octadecynoic acid; 8) cis-5,8,11,14,17-eicosapentaenoic acid; 9) 7,10,13-eicosatrienoic acid, methyl ester; 10) 25-hydroxycholesterol. With permission from [37]. b – the co-extractives removal efficiency (%) for d-SPE of catfish extracts (error bars represent standard deviations; n = 3). With permission from [37]. c – proposed mechanism of interferences retention on Z-Sep sorbent. Adopted from [36].
A review of recent developments and trends in the QuEChERS sample preparation approach

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exchange property and high content of pigments and sterols could be adsorbed by MWCNTs. Comparatively, commercial adsorbents including C18 and PSA/GCB were used in the clean-up procedure. The obtained results showed that recoveries for the pesticides obtained by MNPs/MWCNTs composites, C18, and PSA/GCB were above 85% except for parathion-methyl. However, the extracts obtained by using MNPs/MWCNTs are cleaner than those obtained by using C18 [43].

The ability of amine modified graphene to clean-up fatty acids and other interfering substances from acetonitrile extracts of oil crops has been evaluated by Guan et al. [44]. Authors conducted experiments to compare amine-modified graphene (NH$_2$-G, CH$_3$NH-G, and nBuNH-G) with G, PSA, MWCNTs and GCB as well as to evaluate their ability for interfering substances removal. The greatest reduction of fatty acids in rapeseed extract among the tested sorbents occurred after CH$_3$NH-G clean-up procedure. Overall average recoveries of most pesticides were between 70.5 and 100% and %RSD values below 13%. CH$_3$NH-G proved to be a new type of reverse-d-SPE sorbent material and is expected to be widely applied in pesticide monitoring [44].

3 Application of technique QuEChERS in analysis of different groups of analytes

More recently, QuEChERS has been gaining significant popularity in the analysis of pesticides and other compounds in huge variety of food products and other samples. The spectrum of different analytes covered by QuEChERS methodologies is still broadening. The QuEChERS approach is useful for analysis, among others, of pesticides, veterinary drugs and other pharmaceuticals, mycotoxins, polycyclic aromatic hydrocarbons, dyes, acrylamide, synthetic musks and UV filters, bisphenols, polybrominated diphenyl ethers and other flame retardants, endocrine disruptors and other chemical compounds.

3.1 QuEChERS in pesticides analysis

The rapid growth in agricultural production, observed in recent decades as a result of the implementation of new technologies and the use of chemical pesticides, can pose significant health risks to consumers. Residues of pesticides applied in agronomy may persist until the harvest stage, causing the presence of trace amounts of pesticides in agricultural crops and processed food products. Pesticide residue analysis plays an important role in food quality for evaluating food safety and possible risk to human health. Simultaneous analysis can be performed for hundreds of pesticides using GC-MS (/MS) and LC-MS/MS systems. Since the QuEChERS introduction in 2003 [2] and official methods development [17,18], it is gaining worldwide acceptance in routine pesticide residues testing. In literature there are many research papers employing this analytical approach for analysis of multiclass multiresidue pesticide contaminants in huge variety of foodstuffs. The described below examples of the QuEChERS method application in pesticides residues extraction are summarized in Table 3.

Cherta et al. developed a method applying a GC-(QqQ)/MS with APCI for the determination of 142 pesticide residues in fruits and vegetables according to the official 2007.01AOAC QuEChERS procedure [45]. The elaborated method was successfully validated for the simultaneous identification and quantification of 142 pesticides in orange, tomato and carrot matrices at 0.01 and 0.1 mg kg$^{-1}$ with satisfactory recoveries ranging between 70% and 120% for most investigated compounds in all the sample matrices [45]. Applying a GC-MS/MS technique, Hou et al. developed a method for the determination of 124 pesticides in rice by modified QuEChERS extraction [46]. Authors compared the efficiency of citrate-buffered and unbuffered method in liquid extraction procedure. It was demonstrated that buffered extraction method contains more co-extracts than unbuffered version, but citrate buffering improved recoveries of both basic-sensitive and
Table 3: Examples of the QuEChERS-based methods application in pesticides extraction from various sample types.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Matrice type</th>
<th>QuEChERS specification</th>
<th>Recoveries and Repeatabilities</th>
<th>LOQ [mg kg(^{-1})]</th>
<th>Analysis and Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>142 pesticides</td>
<td>orange, tomato and carrot</td>
<td>AOAC official method 2007.01</td>
<td>70–120%; RSD% &lt; 10% (for most analyzed compounds)</td>
<td>&lt; 0.01</td>
<td>GC-MS/MS</td>
<td>[45]</td>
</tr>
<tr>
<td>124 pesticides</td>
<td>rice</td>
<td>citrate-buffered and unbuffered method comparison; d-SPE with 375 mg PSA and 750 mg anhydrous MgSO(_4) (other clean-up variants tested)</td>
<td>70–130%; %RSD &lt; 20%</td>
<td>&lt; 0.025</td>
<td>GC-MS/MS</td>
<td>[46]</td>
</tr>
<tr>
<td>insecticides, fungicides and herbicides of 32 different chemical groups</td>
<td>olive oil and olives</td>
<td>freezing-out step; additional clean-up with 150 mg of PSA, 12.5 mg GCB and 900 mg of MgSO(_4)</td>
<td>70–120%; %RSD &lt; 20–25%</td>
<td>≤ 0.01</td>
<td>GC-MS/MS and LC-MS/MS</td>
<td>[47]</td>
</tr>
<tr>
<td>168 pesticides</td>
<td>paprika</td>
<td>dry ice-partitioning QuEChERS method and citrate-buffered method comparison; clean-up with 125 mg of PSA, 750 mg of MgSO(_4), and 15 mg of GCB</td>
<td>&gt; 76%; %RSD &lt; 20%</td>
<td>-</td>
<td>LC-MS/MS</td>
<td>[48]</td>
</tr>
<tr>
<td>&gt; 180 pesticides</td>
<td>blackcurrant</td>
<td>clean-up with 0.5 g MgSO(_4), 0.125 g of PSA or 0.5 g MgSO(_4), 0.125 g of PSA and 0.250 g C18;</td>
<td>70–116%; %RSD 3% – 19%</td>
<td>0.01</td>
<td>GC-MS/MS</td>
<td>[49]</td>
</tr>
<tr>
<td>172 pesticides</td>
<td>tea leaves and brewed tea</td>
<td>acetate buffered QuEChERS method; clean-up with 900 mg of MgSO(_4), 150 mg of GCB, and 300 mg of PSA (tea leaves) and 900 mg of MgSO(_4), 150 mg of C18, and 300 mg of PSA (brewed tea)</td>
<td>81–110%, intermediate precision ≤ 20% (for most studied analytes)</td>
<td>-</td>
<td>UHPLC/ESI-MS/MS</td>
<td>[50]</td>
</tr>
<tr>
<td>653 pesticides and chemical pollutants</td>
<td>tea</td>
<td>clean-up with GCB and PSA sorbents</td>
<td>70–110%; %RSD &lt; 15% (for most studied analytes)</td>
<td>-</td>
<td>GC/MS or GC/MS/MS</td>
<td>[51]</td>
</tr>
<tr>
<td>159 pesticides</td>
<td>tobacco</td>
<td>citrate-buffered acetonitrile extraction and toluene dilution; d-SPE clean-up using PSA (50 mg) and C18 (50 mg) sorbents and MgSO(_4) (150 mg)</td>
<td>69–141%; %RSD 2% – 27%</td>
<td>0.05</td>
<td>GC-MS/MS</td>
<td>[52]</td>
</tr>
<tr>
<td>&gt; 140 pesticides</td>
<td>catfish</td>
<td>extraction with acetonitrile and d-SPE clean-up with zirconium-based sorbent (Z-Sep)</td>
<td>70–120%; SD &lt; 20% (for most studied compounds)</td>
<td>-</td>
<td>LP-GC-MS/MS</td>
<td>[53]</td>
</tr>
<tr>
<td>51 pesticides</td>
<td>beeswax</td>
<td>liquid–liquid partitioning between acetonitrile and melted wax (=80°C), followed by freeze-out and PSA dispersive clean-up</td>
<td>70–120%; %RSD &lt; 20% (for most studied compounds)</td>
<td>1.1–0.1</td>
<td>LC-MS/MS and GCxGC-TOF</td>
<td>[54]</td>
</tr>
<tr>
<td>51 pesticides, including isomers and degradation products</td>
<td>green coffee beans</td>
<td>d-SPE clan-up with PSA and C18 separate or as a mixture was evaluated</td>
<td>70–120%; %RSD ≤ 20% (for 75% of analytes)</td>
<td>0.01–0.05</td>
<td>GC-NCl-MS</td>
<td>[55]</td>
</tr>
<tr>
<td>113 pesticides</td>
<td>avocado and almond</td>
<td>comparison of various d-SPE clean-up procedures (Z-Sep, Z-Sep+, PSA + C18 and silica) with miniLuke and ethyl acetate extraction methods; protocol with Z-Sep provided the highest number of pesticides with recoveries ranged between 70% and 120% (%RSD &lt; 15%)</td>
<td>-</td>
<td>0.01 (for most LC-MS/MS analytes)</td>
<td>[56]</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Examples of the QuEChERS-based methods application in pesticides extraction from various sample types.

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>Matrice type</th>
<th>QuEChERS specification</th>
<th>Recoveries and Repeatabilities</th>
<th>LOQ [mg kg(^{-1})]</th>
<th>Analysis and Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>148 pesticides</td>
<td>nutraceutical products obtained from green tea</td>
<td>extraction with acidified acetonitrile (acetic acid 1%, v/v) and a clean-up step using PSA (50 mg), GCB (100 mg) and MgSO(_4), (200 mg)</td>
<td>70–120%; %RSD &lt; 25%</td>
<td>0.01</td>
<td>GC-MS/MS</td>
<td>[58]</td>
</tr>
<tr>
<td>150 pesticides</td>
<td>broccoli, cantaloupe, lemon and sweet potato</td>
<td>acetate-buffered extraction/ partitioning; d-SPE clean-up with 150 mg of MgSO(_4), 50 mg PSA, 50 mg C18 and 7.5 mg GCB</td>
<td>70–120% (for 144 of the pesticides); %RSD &lt; 20% (for all but 4 analytes)</td>
<td>&lt; 0.01</td>
<td>LP-GC-MS/MS</td>
<td>[59]</td>
</tr>
<tr>
<td>14 biopesticides and piperonyl butoxide (PBO)</td>
<td>pepper, cucumber, tomato, orange, strawberry</td>
<td>acetate-buffered extraction/ partitioning; no clean-up required</td>
<td>70–112%; %RSD &lt; 28%</td>
<td>&lt; 0.003</td>
<td>UPLC-MS/MS</td>
<td>[60]</td>
</tr>
<tr>
<td>quaternary ammonium biocides</td>
<td>Oranges and cucumber</td>
<td>acetate-buffered extraction/ partitioning; no clean-up required</td>
<td>81–115%; %RSD &lt; 17%</td>
<td>0.01–0.04</td>
<td>UPLC-MS/MS</td>
<td>[61]</td>
</tr>
</tbody>
</table>

Acid-sensitive pesticides in rice. However, in this study recoveries of all targeted analytes ranged from 70% to 130%, obtained both by citrate-buffered and unbuffered QuEChERS versions with no obvious difference between those two methods [46]. An easy multiresidue method for the determination of residues of insecticides, fungicides and herbicides of 32 different chemical groups using GC-MS/MS and LC-MS/MS in olive oil and olives was developed and validated by Anagnostopoulos and Miliadis [47]. Authors found freezing-out step as critical for fat removal. Additional clean-up was also performed with PSA an GCB for cleaning of co-extracted pigments. The elaborated method showed good sensitivity and selectivity with limits of quantification at 10 μg kg\(^{-1}\). All investigated pesticides had recoveries in the range of 70–120%, with relative standard deviation values less than 20–25%. Authors applied this method to 262 samples of olive oil and olives from the Greek market, 7% of which were found positive for the presence of pesticide [47]. Lee et al. described a new QuEChERS method referred as the dry ice-partitioning QuEChERS method for the determination of 168 pesticides in paprika using tandem mass spectrometry [48]. The dry ice-partitioning QuEChERS method consists of extraction method A (for detection of the acetonitrile layer) and extraction method B (for detection of both acetonitrile and aqueous layers). The extraction efficiency was then compared with the citrate-buffered QuEChERS method in terms of recovery rates. Satisfactory extraction efficiency for most analytes was achieved by both methods. However, at a fortification level of 0.25 mg kg\(^{-1}\), the recovery values of benfuracarb (3%), carbosulfan (32%), dichlofluoruanid (31%), probenazole (20%), and tolylfluanid (47%) in the citrate-buffered QuEChERS method were increased dramatically to 121, 96, 83, 113, and 88%, respectively via the dry ice-partitioning QuEChERS method (extraction method A). In turn, the application of extraction method B improved the recovery rates to acceptable ranges for some analytes, including propamocarb, pymetrozine and metabolites of flonicamid (TFNA and TFG) due to the analysis of the aqueous layer containing the remainder of their residues. The dry ice-partitioning QuEChERS method can be employed to detect analytes within a broad polarity range and may be worth considering as a multiresidue analytical method for pesticides testing in foods as clearly cheaper and more eco-friendly, due to the fact that it not require salting-out and buffering reagents during the extraction and partitioning steps [48]. Walorczyk conducted experiments on development and validation of a QuEChERS-based gas chromatographic–tandem mass spectrometric method for multiresidue pesticide analysis in blackcurrants [49]. To reduce matrix co-extractives in the final extract, the supernatant was purified by d-SPE with a mixture of sorbents: PSA, octadecyl (C18) and GCB. The application of this sorbents led to removal of co-extracted pigments and resulted in occurrence of negligible matrix effects (± 20%) for over 90% of studied compounds. The obtained recoveries for over 180 pesticides spanned between 70% and 116% with relative standard deviation between 7% and 19% except for chlorothalonil (23%). The elaborated method was applied for analysis of real samples and revealed a high frequency of the pesticide residues presence above their
legislative MRLs, as well as the presence of pesticides unapproved for the use on blackcurrants [49]. Wang et al. made an assessment of pesticide residue percentage transfer rates from dried tea leaves to brewed tea [50]. Pesticides were extracted from dried tea leaves (5 g per sample) and brewed tea (10 mL per sample) following the acetate buffered QuEChERS method. Dispersive clean-up step was performed with anhydrous MgSO₄, PSA, and GCB for dried tea leaves or MgSO₄, PSA, and C18 for brewed tea. Among the 172 investigated pesticides, 12 were detected in 44 different dried tea leaves samples. Of these pesticides, imidacloprid, carbendazim, and methomyl were the most frequently found analytes. Transfer rates of detected pesticides ranged from 49.7 to 99.8%. Considering that pesticide residues were transferred from tea leaves to drinking tea during the brewing process, a significant risk to consumers is factual [50]. Many different research papers deal with pesticides testing using for extraction QuEChERS approach. The scope of pesticides covered by these methods is broad and huge variety of food products may be easily tested. Fan et al. developed high-throughput analytical methodology for determination of residues of 653 multiclass pesticides and chemical pollutants in tea [51]. Other multiresidue QuEChERS or QuEChERS-based methods, which allow proper determination and quantification of pesticide contaminant in tobacco [52], fish [53], beeswax [54], coffee beans [55], high oil commodities [56], soybeans and pulses [57], nutraceutical products [58], different fruits and vegetables [59] and more are developed.

More recently, biopesticides are gaining popularity in crop protection, especially in organic production. Despite the fact that biopesticides are naturally occurring substances, there is some evidence that not always these compounds are safe for consumers. For example, recent studies suggested a possible relationship between biopesticides such as rotenone and deguelin and Parkinson’s disease and between pyrethrins and adverse respiratory effects [60]. Therefore, it is important to provide sensitive analytical methods for determination of biopesticide residues in organic produce. This problem is included in the Romero-González et al. scope of research, who developed a method for the determination of 14 biopesticides and piperonyl butoxide (PBO), often applied in organic farming, in vegetables and fruits [60]. Studied analytes were extracted from cucumber, tomato, pepper, strawberry and orange samples using the acetate-buffered QuEChERS extraction/partitioning step. The determination of these compounds was carried out by UPLC/MS/MS without any extract clean-up. The method elaborated by authors allowed yielding recoveries from 70% to 120% for all analytes with %RSD values below 28%. The proposed method can be successfully applied in routine analysis of this type of compounds in fruits and vegetables [60]. Quaternary ammonium compounds, which are widely used as biocides, pesticides, disinfectants, and additives for technical applications in the modern food industry were analyzed in cucumber and orange samples by Arrebola-Liébanas et al. with application of the QuEChERS-based extraction followed by UPLC-MS/MS [61]. The developed method demonstrated good performance in terms of recoveries and repeatabilities and was employed in real samples analysis with positive findings of tested quaternary ammonium compounds.

3.2 Veterinary drugs analysis

Veterinary drugs (VDs) are chemicals widely used in farming to increase production, to treat infections, for prophylactic reasons or even as growth promoters for intensive animal production. However, VDs can be accumulated in animal tissues or transferred to food products, therefore potential presence of their residues is an important problem in the field of foodstuff safety. The presence of veterinary drugs in food may have a potential risk for the consumers, because they can provoke allergic reactions or induce pathogen resistance to antibiotics used in human medicine. The use of veterinary drugs is heavily regulated in the European Union (EU) by different Regulations and Directives [62,63]. Veterinary drugs may be classified according to their chemical or therapeutic properties but from an analytical perspective their physic-chemical properties are the most important consideration [64]. Sample preparation is the major restriction in any analytical procedure for the determination of trace levels contaminants residues in foodstuffs. The QuEChERS approach noticeably shows its potential outside of pesticide analysis and has already been applied to the determination of different VDs (Table 4). Stubbings and Bigwood developed a multiclass LC-MS/MS procedure for the determination of veterinary drug residues in chicken muscle using QuEChERS approach [64]. The optimal procedure, which used 1% (v/v) acetic acid in acetonitrile as extraction solvent with anhydrous sodium sulphate as drying agent followed by dispersive-SPE with NH₂ sorbent, was validated according to European Commission guidelines. An additional clean-up using strong cation exchange (SCX) cartridge was necessary for the determination of nitroimidazoles. According to authors, the method is adaptable and can be easily tailored to cope with new matrices through
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León et al. developed a method for wide-range screening of veterinary drugs in bovine urine by UHPLC-(HR)MS/MS [65]. The method currently covers 87 analytes belonging to different families such as steroid hormones, β-agonists, resorcylic acid lactones (RAL), stilbenes, tranquilizers, nitroimidazoles, corticosteroids, NSAIDs, amphenicoles, thyreostatics and other substances such as dapsone. After evaluating different sample preparation procedures (dilution, SPE, QuEChERS), QuEChERS was selected as the most appropriate methodology, because all of the studied VDs were correctly detected and identified. The amount of sorbents (400 mg of both PSA and C18) applied in the d-SPE clean-up step was sufficient to retain matrix components and thus led to a decrease of ion suppression phenomenon and an improvement of analyte detection and values of their recoveries. In all cases, the detection capability (CCβ) levels achieved by authors were equal or lower than the recommended concentrations established by EU reference laboratories [65]. Kinsella et al. describes a method for the detection and quantification of 38 residues of the most widely used anthelmintics (including 26 veterinary drugs belonging to the benzimidazole, macrocyclic lactone and flukicide classes) in bovine liver. In this work two different d-SPE protocols were used to purify extracts depending on the concentration level. In the low level method (2 µg kg⁻¹), the entire supernatant was poured into a centrifuge tube containing anhydrous MgSO₄ (1.5 g) and C18 sorbent (0.5 g). For MRL concentrations, the purification of 1 mL of supernatant was performed with 150 mg of MgSO₄ and 50 mg of C18. The method was accredited to ISO17025 standard and its robustness has been tested.

Table 4: Examples of the QuEChERS-based methods application in veterinary drugs extraction from various sample types.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Matrice type</th>
<th>QuEChERS specification</th>
<th>Recoveries and Repeatabilities</th>
<th>LOQ [mg kg⁻¹]</th>
<th>Analysis and Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>nitroimidazoles, sulphonamides, fluoroquinolones, quinolones, ionophores and dinitrocarbanilide</td>
<td>chicken breasts</td>
<td>different variants tested; optimal procedure used 1% (v/v) acetic acid in acetonitrile as extraction solvent with anh. MgSO₄ as drying agent followed by d-SPE clean-up with NH₂ sorbent; additional clean-up using strong cation exchange (SCX) cartridge was necessary for the determination of nitroimidazoles</td>
<td>optimal recoveries obtained for clean-up with 500 mg of NH₂ sorbent; 57–93% for following analytes sulphonamides, nitroimidazoles, dicarbanilide and ionophores; 37–95% for quinolone and fluoroquinolones</td>
<td>0.003</td>
<td>LC-MS/MS</td>
<td>[64]</td>
</tr>
<tr>
<td>87 analytes</td>
<td>bovine urine</td>
<td>d-SPE clean-up with PSA (400 mg) and C18 (400 mg) sorbents</td>
<td>64–116%</td>
<td>-</td>
<td>UHPLC-MS</td>
<td>[65]</td>
</tr>
<tr>
<td>38 residues of anthelmintics</td>
<td>bovine liver</td>
<td>d-SPE purification with MgSO₄ and C18</td>
<td>90–110%; CEs typically &lt; 23% (according to the Horwitz equation)</td>
<td>&lt; MRL values</td>
<td>LC-MS/MS</td>
<td>[66]</td>
</tr>
<tr>
<td>tetracyclines, macrolides, quinolones, sulphonamides and anthelmintics</td>
<td>eggs</td>
<td>buffered QuEChERS method with the clean-up step using 25 mg of PSA per 1 mL of the extract; EDTA addition was necessary to avoid the complexation of macrolides and tetracyclines with cations from the sample or from used reagents</td>
<td>only 12 studied veterinary drugs presented recoveries higher than 60% with %RSD &lt; 20%</td>
<td>&lt; 0.005</td>
<td>UHPLC-MS/MS</td>
<td>[67]</td>
</tr>
<tr>
<td>antibiotics and other drugs</td>
<td>shrimps</td>
<td>clean-up with 250 mg of PSA and 750 mg of MgSO₄</td>
<td>58–133%; %RSD &lt; 15%</td>
<td>&lt; 0.007</td>
<td>LC-TOFMS</td>
<td>[68]</td>
</tr>
<tr>
<td>17 veterinary hormones</td>
<td>powdered ingredients derived from bovine milk</td>
<td>clean-up with 150 mg of MgSO₄, 25 mg of PSA and 25 mg of C18 sorbent</td>
<td>62–82%; %RSD &lt; 20%</td>
<td>&lt; 0.01</td>
<td>LC-ESI-MS/MS</td>
<td>[69]</td>
</tr>
<tr>
<td>20 prohibited veterinary drugs</td>
<td>feedstuffs</td>
<td>ultrasonic-assisted extraction with a mixture of methanol–acetonitrile (50:50, v/v); clean-up using a d-SPE with PSA (150 mg)</td>
<td>56.7–103%; %RSD &lt; 10%</td>
<td>-</td>
<td>LC-MS/MS</td>
<td>[70]</td>
</tr>
</tbody>
</table>
3.3 Mycotoxins analysis

Mycotoxins are toxic substances naturally produced by fungi as their secondary metabolites, mainly by species of *Fusarium*, *Aspergillus*, *Penicillium* and *Claviceps* genus [71]. By the reason of the widespread distribution of molds in the environment, thousands of different mycotoxins are present, but only a few cause considerable food safety hazards. The most prominent mycotoxins are aflatoxins, deoxynivalenol, zearalenone, ochratoxin, fumonisins, and patulin. These compounds lead to unfavorable health problems such as kidney and liver damage, mutagenic and teratogenic effects, birth defects, and cancers that result in symptoms ranging from skin irritation to immunosuppression, neurotoxicity and death. Mycotoxin toxicity occurs at very low concentrations, therefore sensitive and reliable analytical methods for the detection and quantification of these toxins in complex and difficult matrices are required [72]. Selected examples of the QuEChERS method application in mycotoxins residues extraction are summarized in Table 5.

Cunha and Fernandes developed and validated analytical method for the rapid and simultaneous determination of five mycotoxins (zearalenone, deoxynivalenol, Fusarenon X, 15-acetyldexoxyvalenol and nivalenol) in breakfast cereals and flours by heart-cutting GC-MS [73]. Mycotoxins were extracted from the samples using a procedure based on the QuEChERS methodology with some modifications. For cereals and other samples containing less than 25% of water, authors reduced the size of the sample to 5 g and the additional water (20 mL) before the extraction was added [73]. An additional improvement was the washing of the sample (two times with 5 mL of n-hexane) after its mechanical mixing (for about 15 min) [73]. In this work, several types of d-SPE clean-up sorbents were tested, namely MgSO₄, C18, PSA, Florisil and the mixture of MgSO₄ with C18 [73]. PSA and Florisil were discarded due to low recoveries and clean-up efficiencies. Among the other sorbents assayed the mixture of MgSO₄ and C18 was the best clean-up solution that allowed retaining interfering compounds without significant loss of studied analytes [73]. Cunha and Fernandes achieved acceptable recoveries from 67 to 101% and from 52 to 103% for nearly all mycotoxins in breakfast cereals and in flour, respectively, with good repeatability.
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LOQ values (from 5 to 50 mg kg\(^{-1}\)) were lower than the maximum limit established by EU [73]. Taking all this into account, the method could be useful in routine analysis of multi-mycotoxins in complex foodstuffs. Ferreira et al. successfully adapted this elaborated method [73] for the determination of multi-mycotoxins in unpopped and popped popcorn [74].

Arroyo-Manzanares et al. developed a sensitive, simple and rapid method for the determination of fourteen mycotoxins in edible nuts and seeds (including almonds, peanuts, sunflower seeds, pumpkin seeds, walnuts, macadamia nuts, pistachios, hazelnuts and pine nuts) using UHPLC-MS/MS [75]. The sample treatment was based on simplified QuEChERS procedure and its graphical diagram is shown in Fig. 8 [75]. For the proper determination of fumonisin B1, fumonisin B2, deoxynivalenol, fusarenon-X, T-2 and HT-2 toxin, citrinin, sterigmatocystin, zearalenone and ochratoxin A only QuEChERS based extraction/partitioning step is necessary [75]. However, in the case of the analysis of aflatoxins (AFB1, AFB2, AFG1 and AFG2) a subsequent clean-up step, based on the dispersive liquid–liquid microextraction (DLLME), was required [75]. Arroyo-

Table 5: Examples of the QuEChERS-based methods application in mycotoxins extraction from various sample types.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Analytes</th>
<th>Matrice type</th>
<th>QuEChERS specification</th>
<th>Recoveries and Repeatabilities</th>
<th>LOQ [mg kg(^{-1})]</th>
<th>Analysis and Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>zearalenone, deoxynivalenol, fusarenon X, 15-acetyl-deoxynivalenol and nivalenol</td>
<td>cereal products</td>
<td>d-SPE clean-up with 900 mg of MgSO(_4) and 300 mg of C18</td>
<td>67–101%; %RSD 11–16%</td>
<td>0.005–0.05</td>
<td>GC-MS</td>
<td>[73]</td>
<td></td>
</tr>
<tr>
<td>deoxynivalenol, nivalenol, 15-acetyl-deoxynivalenol, fusarenon X and zearalenone</td>
<td>unpopped and popped popcorn</td>
<td>d-SPE clean-up with 900 mg of MgSO(_4) and 300 mg of C18</td>
<td>61–118% in unpopped popcorn; 65–89% in popped popcorn; %RSD &lt; 18%</td>
<td>&lt; 0.196</td>
<td>GC-MS</td>
<td>[74]</td>
<td></td>
</tr>
<tr>
<td>14 mycotoxins</td>
<td>edible nuts and seeds</td>
<td>citrate buffered QuEChERS extraction with 5% formic acid in MeCN; additional clean-up step based on DLLME for aflatoxins</td>
<td>60.7–104.3%; %RSD &lt; 11%</td>
<td>0.057–0.15</td>
<td>UHPLC-MS/MS</td>
<td>[75]</td>
<td></td>
</tr>
<tr>
<td>14 mycotoxins</td>
<td>rice</td>
<td>citrate buffered method with acidified MeCN; d-SPE clean-up step with MgSO(_4) (1.2 g), C18 (0.25 g), Al-N (0.25 g) and PSA (0.4 g)</td>
<td>70–98%; %RSD ≤ 7% for most analytes</td>
<td>0.017–0.05</td>
<td>UPLC-MS/MS</td>
<td>[76]</td>
<td></td>
</tr>
<tr>
<td>36 mycotoxins</td>
<td>wines</td>
<td>various sorbents and their mixtures were studied</td>
<td>70–120%; %RSD &lt; 20% for most analytes</td>
<td>0.001–0.5</td>
<td>UPLC-MS/MS</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>15 mycotoxins</td>
<td>beer-based drinks</td>
<td>Mycotoxins were extracted from samples using acetonitrile with sodium chloride, anhydrous magnesium sulfate, and sodium citrate, and were then purified with a solid phase extraction (SPE) cartridge including C18.</td>
<td>70.3 to 110.7%; %RSD &lt; 14.6%</td>
<td>&lt; 0.005*</td>
<td>UHPLC/MS/MS</td>
<td>[78]</td>
<td></td>
</tr>
<tr>
<td>27 mycotoxins</td>
<td>human breast milk</td>
<td>simplified QuEChERS procedure without clean-up step</td>
<td>69–110%; %RSD ≤ 205</td>
<td>-</td>
<td>UHPLC-MS</td>
<td>[79]</td>
<td></td>
</tr>
<tr>
<td>10 mycotoxins</td>
<td>eggs</td>
<td>simplified QuEChERS procedure without clean-up step</td>
<td>70–110%, except for ochratoxin A and aflatoxin G1; %RSD &lt; 25%</td>
<td>0.001–0.01</td>
<td>UHPLC-MS/MS</td>
<td>[80]</td>
<td></td>
</tr>
<tr>
<td>17 mycotoxins</td>
<td>spices</td>
<td>simplified QuEChERS procedure without clean-up step</td>
<td>75–117%; %RSD 4–22%</td>
<td>0.002–0.146</td>
<td>HPLC-MS/MS</td>
<td>[81]</td>
<td></td>
</tr>
<tr>
<td>56 mycotoxins</td>
<td>feed matrices</td>
<td>different extraction variants tested</td>
<td>70–120%; %RSD &lt; 10% for most analytes</td>
<td>0.001–0.5</td>
<td>UHPLC-MS/MS</td>
<td>[82]</td>
<td></td>
</tr>
</tbody>
</table>

*(a = mg mL\(^{-1}\))
Manzanares et al. proposed this clean-up solution as an alternative to dispersive SPE in order to avoid losses of mycotoxins in terms of recovery [75]. Achieved precision (repeatability and intermediate precision) was lower than 11% in all cases, and recoveries were between 60.7 and 104.3% so that elaborated procedure was efficiently employed for analysis of mycotoxins in commercially available commodities, with some positive findings [75].

Koesukwiwat et al. evaluated a modified QuEChERS method for analysis of 14 mycotoxins in rice [76]. Authors used 10% formic acid in MeCN for extraction and the partitioning step was accomplished with citrate buffered QuEChERS version. Purification of the 8 mL of the MeCN extracts was carried out with 1.2 g of anhydrous MgSO₄, 0.25 g C₁₈, 0.25 g Al-N (neutral alumina sorbent) and 0.4 g PSA. Optimal analytical results were obtained, most analytes showed average recoveries in the acceptable range of 70–98% and the repeatability (RSD) for all analytes was consistently below 7% for each spiking level. Mycotoxins such as citrinin and fumonisin B₁ presented relatively low overall recoveries at 56 and 66%, respectively. This small variation suggests that losses occurred during the extraction, in relation to the insufficient extraction with solvent or the use of PSA and Al-N sorbents in the d-SPE clean-up [76]. Numerous publications have already dealt with QuEChERS-based extraction for determination of mycotoxins in various types of samples. Pizzutti et al. developed, optimized and validated a multiresidue method for the determination of 36 mycotoxins in wines by LC-MS/MS [77]. Multi-mycotoxin analysis in beer-based drinks by a modified QuEChERS method and UHPLC-MS/MS was conducted by Tamura et al. [78]. Evaluation of mycotoxins and their metabolites in human breast milk by a newly developed method based on QuEChERS extraction and UHPLC-HRMS detection was carried out by Rubert et al. [79]. Frenich et al. described a QuEChERS-based extraction procedure for multi-mycotoxin analysis in eggs [80]. Yogendrarajah et al. developed and validated a QuEChERS-based method for the determination of multiple mycotoxins in different spices by LC-MS/MS [81]. Dzuman et al. elaborated high throughput method for reliable detection and quantification of 56 mycotoxins in a wide range of animal feed samples represented by cereals, complex compound feeds, extracted oilcakes, fermented silages,
malt sprouts or dried distillers’ grains with solubles [82]. In this study, authors tested three extraction approaches (acetonitrile, acetonitrile/water, and QuEChERS) and the QuEChERS-based method was selected as the best in terms of analytes recoveries and low matrix effects [82].

### 3.4 Polycyclic aromatic hydrocarbons (PAHs) analysis

Polycyclic aromatic hydrocarbons (PAHs) represent a diverse class of organic compounds, containing two or more aromatic rings. Hundreds of different PAHs may be formed and released during a variety of combustion and pyrolysis processes and thus the natural and anthropogenic sources of PAHs in the environment seem to be numerous. For the general population, the major routes of exposure are from food and inhaled air, while in smokers, the contributions from smoking and food may be of a comparable magnitude. Food can be contaminated by environmental PAHs that are present in air, soil or water, by industrial food processing methods (e.g. heating, drying and smoking processes) and by home food preparation (e.g. grilling and roasting processes) [83]. PAHs have been classified as important environmental pollutants because they may interfere with the normal function of DNA, therefore environmental and food quality control is indispensable. Since trace amounts of PAHs are present in the complex food matrix, the analysis of this compounds has been difficult [83]. Traditional methods of extraction of PAHs were often based on extraction with nonpolar or low-polar solvents, such as hexane or methylene chloride, or with the Soxhlet method, followed by saponification or liquid–liquid partition to remove soluble in water impurities and purification using SPE cartridges with silica gel or octadecyl sorbent. Nevertheless, these methods are laborious, time-consuming, and often insufficient purification led to interferences with chromatographic analysis, resulting in low recovery [84,85].

Hua Kao et al. evaluated an analysis of 16 PAHs by combining the QuEChERS method with GC/MS and their formation in different poultry meat as affected by marinating and frying [85]. An amount of 5 g of meat subsample was mixed with 10 mL of deionized water in a centrifuged tube and shaken vigorously for 1 min, after which 10 mL of acetonitrile was added and shaken again for 1 min. Next, the QuEChERS method containing 6 g of magnesium sulfate and 1.5 g of sodium acetate was added, followed by shaking for 1 min and centrifuging at 4000 rpm for 5 min. Then, 6 mL of collected supernatant was purified with 400 mg of PSA, 1200 mg of MgSO₄, and 400 mg of endcaped C18 [85]. Obtained in this study recovery of 16 PAHs ranged from 71.2 to 104.0% in poultry meat samples. The quantitation limits of studied PAHs were from 0.02 to 1 ng mL⁻¹, with the intraday variability being from 2.4 to 6.6% (%RSD) and interday variability ranging from 3.3 to 7.1% (%RSD) [85]. In terms of d-SPE clean-up step, only a slight difference in recovery between purified and nonpurified meat sample was observed. The purification step by the QuEChERS method was considered to be obligatory to extend column life [85]. In an analogous study, using 10 mL of acetonitrile for PAHs extraction from 5 g of fish meat, Ramalhosa et al. demonstrated any significant differences in extraction efficiency (also without application d-SPE) [86]. Determination of 33 PAHs in high-fat smoked salmon using a modified QuEChERS extraction, d-SPE by GC-MS analysis was conducted by Forsberg et al. [87]. Authors demonstrated that newly elaborated modified QuEChERS version greatly enhances analyte recovery compared to traditional QuEChERS procedures. Crucial modification was implementation of a three-component extraction solvent system (consisting of acetone, ethyl acetate and isooctane in a 2:2:1 (v/v/v) ratio). According to authors, the advantages of the applied solvent system involve the ability to disrupt strong associations between planar hydrophobic PAHs and fatty components of biological matrices such as waxes, lipids, steroids and pigments. These extraction conditions gave enhanced recoveries values. A solvent’s ability to disrupt such interactions may be assessed by comparison of solvent and PAH octanol-water partition coefficients (log $K_{ow}$), where solvents with coefficients similar to PAHs should display better selectivity [87]. Dispersive SPE for all samples in these experiments was performed using 50 mg of PSA, 50 mg C18 and 150 mg MgSO₄ (Sampli-Q AOAC fatty sample dispersive SPE tubes). With reference to the results obtained by Forsberg et al., recoveries of some 2-, 3- and 5-ring PAHs were improved over traditional methods, while average recovery across all PAHs was improved by 67%. Method precision was satisfactory with RSD values below 10%, detection limits were in the low ng g⁻¹ range [87]. Johnson developed and validated a high-throughput method of analysis of PAHs in 4 seafood matrices (crab, finfish, oyster, and shrimp) using QuEChERS-based extraction and GC-MS/MS [88]. The effectiveness of d-SPE clean-up was examined in a pilot study and on its basis 150 mg of MgSO₄, 50 mg of PSA and 50 mg of C18 were applied for the purification of acetonitrile extracts. It was shown that increase of PSA amount from 25 to 50 mg allows removal of the interfering
peaks in the mid retention time range (mostly fatty acids), but for getting rid of strong peaks at retention time around 30–37 min (sterols) the use of C18 sorbent was crucial. However, application of C18 in the clean-up step may cause reduction of recovery values of strongly nonpolar PAHs [88]. Johnson, as well as Forsberg and others reported some PAH contamination in QuEChERS products [87,88]. The accepted explanation of this problem is that residual PAHs leach out from the polypropylene centrifuge tubes due to endothermic reaction between MgSO₄ and water in sample. Therefore, the levels of contaminants obtained in reagent blanks should be subtracted from the sample results of validation studies [41]. The Johnson’s method provides mass spectrum based analyte identification with detection limits at sub to low ppb levels, recoveries ranged from 72 to 116% with RSDs below 20%, thus could be used for seafood safety assessment. The summation of the QuEChERS method application in PAHs residues extraction is presented Table 6.

### 3.5 Dyes analysis

Dyes are widely used as food additives to compensate for the loss of natural colors, which are destroyed during processing and storage, and to provide the desired colored appearance of foodstuffs [89]. An increasing number of evidences in recent years indicates that dyes and their metabolites pose potential health risk to human, including allergy and asthmatic reaction, DNA damage, hyperactivity and carcinogenesis, etc [90]. According to the Commission Regulation 1333/2008, all food additives authorized for use in the (EU) before 20 January 2009 should be subjected to a new risk assessment by the EFSA [91]. Due to the high occurrence of dyes in food, they are a global concern and monitoring food programs include their analysis. Different physicochemical properties of dyes cause that development of analytical methods for their simultaneous determination is very difficult [89]. Modified QuEChERS method were reported in literature for an effective dyes extraction.

Table 6: Examples of the QuEChERS-based methods application in polycyclic aromatic hydrocarbons and dyes from various sample types.

<table>
<thead>
<tr>
<th>Polycyclic aromatic hydrocarbons (PAHs)</th>
<th>Analytes</th>
<th>Matrice type</th>
<th>QuEChERS specification</th>
<th>Recovery and Repeatabilities</th>
<th>LOQ [mg kg⁻¹]</th>
<th>Analysis and Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 PAHs</td>
<td>poultry meat</td>
<td>QuEChERS method with acetonitrile and 6 g of MgSO₄, and 1.5 g of sodium acetate; d-SPE with 400 mg of PSA, 1200 mg of MgSO₄, and 400 mg of C18</td>
<td>71.2–104%; %RSD 2.4-6.6%</td>
<td>0.02–1</td>
<td>GC-MS</td>
<td>[85]</td>
<td></td>
</tr>
<tr>
<td>16 PAHs</td>
<td>fish</td>
<td>QuEChERS method with acetonitrile and 6 g MgSO₄, and 1.5 g C₂H₄NaO₂, and clean-up with 900 mg MgSO₄, 300 mg PSA and 150 mg C18</td>
<td>84.8–110.5%; %RSD &lt; 4%</td>
<td>0.12–1.90</td>
<td>HPLC-FLD</td>
<td>[86]</td>
<td></td>
</tr>
<tr>
<td>33 PAHs</td>
<td>salmon</td>
<td>different variants of QuEChERS method tested; implementation of a three-component acetone, ethyl acetate and isooctane extraction solvent in a 2:2:1 (v/v/v) ratio gave the best results</td>
<td>&gt; 70% for most analytes; %RSD &lt; 10%</td>
<td>0.002–0.010a</td>
<td>GC-MS</td>
<td>[87]</td>
<td></td>
</tr>
<tr>
<td>20 PAHs</td>
<td>edible seafood</td>
<td>clean-up step with 900 mg MgSO₄, 150 mg PSA and 150 mg C18;</td>
<td>72–116% ; %RSD &lt; 20%,</td>
<td>0.0001–0.025</td>
<td>GC-MS/MS</td>
<td>[88]</td>
<td></td>
</tr>
</tbody>
</table>

Dyes

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Matrice type</th>
<th>QuEChERS specification</th>
<th>Recovery and Repeatabilities</th>
<th>LOQ [mg kg⁻¹]</th>
<th>Analysis and Detection</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>69 dyes</td>
<td>wines</td>
<td>Acetate buffered QuEChERS method with acetonitrile as a solvent; optimal clean-up with 107 mg of PSA and 96 mg C18</td>
<td>87.2–107.4%; %RSD &lt; 6.4%</td>
<td>-</td>
<td>UHPLC-MS</td>
<td>[89]</td>
</tr>
<tr>
<td>8 dyes</td>
<td>worcestershire sauce</td>
<td>AOAC buffered extraction procedure; clean-up with AOAC d-SPE for general fruits and vegetables</td>
<td>64–133%; %RSD 1.3-26%</td>
<td>-</td>
<td>LC-MS/MS</td>
<td>[93]</td>
</tr>
</tbody>
</table>

**Note:**

- `a` – ng mL⁻¹
- `b` – MDL (method detection limit)
from foodstuffs (Table 6). Hashimoto et al. developed and validated a simple method for the determination of malachite green and leucomalachite green residues in fish by a modified QuEChERS extraction and LC-MS/MS [92]. Authors achieved satisfactory performance for their method, characterized by high accuracy values ranged between 95 and 107%, precision values lower than 11.2% and limits of quantification below than 2 g g⁻¹ [92]. Stevens developed a method for determination of banned dyes (Sudan I, II, III, and IV) in food product by QuEChERS and LC-MS/MS analysis [93]. The best recovery values of dyes from Worcestershire sauce samples, were obtained with application of PSA and MgSO₄, [93]. Jia et al. elaborated a simultaneous determination of 69 dyes in wines by HPLC-(Q-Orbitrap)MS [89]. Authors tested three different solvents (acetonitrile, ethyl acetate and acetone) and acetonitrile provided the best extraction efficiency for all 69 target analytes. The best ratio of mass (g) of sample per volume (mL) of extraction solvent was found to be 1.50 because of the most suitable dispersion and the best homogenization between the wine and the extraction solvent were obtained. The amount of sodium acetate was an important factor affecting the recoveries of the pH-dependent compounds. The use of PSA and C18 for purification purposes reduces the peak area of interfering compounds but also reduces the peak area of the analytes affecting the recovery values. Jia et al. attempted to find a compromise between each individual extraction optimum conditions to perform the simultaneous analysis of multiple dyes from a complex wine matrix. For this purpose, statistical tools like response surface seems to be useful, which is presented in Fig. 9 [89] for canacert indigo carmine extraction efficiency optimization by evaluating the effects of the varying amounts of PSA and C18. Acceptable values of recoveries (in the range of 87.2–107.4%) for 69 of the target analytes were obtained by described procedure [89]. Jia et al. developed an accurate and highly sensitive method by combining a QuEChERS extraction procedure and UHPLC-ESI-Q-Orbitrap-MS, which can be implemented for routine screening dyes in foodstuffs.

### 3.6 Other applications

How powerful the QuEChERS approach is reflected in increasing number of its various application. The QuEChERS-based sample preparation methodologies have also been used for a wide variety of contaminants analysis other than those described in the previous subsections such as acrylamide [94], UV filters and synthetic musks [95], bisphenol A and B [96], polybrominated diphenyl ethers and other flame retardants [37], endocrine disruptors [97] and others. The scope of analytes being covered by the QuEChERS approach is still broadening.

### 3.7 Natural compounds analysis

Most of the QuEChERS applications are focused on food or environmental contaminants testing. However, there are also examples of adoptions of this approach for extraction of compounds such as isoflavones and carotenoids (fucoxanthin), which are naturally occurring substances deemed as beneficial to health. Delgado-Zamarreño et al. developed an analytical method for the determination of isoflavones in legumes using LC-MS/MS [98]. A modified QuEChERS was used by authors to extract the analytes from the food samples such as chickpeas, lentils, and beans. Type and volume of extraction solvent, the sample amount, the extraction time, salting-out, and clean-up were the parameters evaluated in this work. In order to achieve best results, a two-step extraction was applied. Firstly, extraction of more polar analytes was conducted with MeCN/H₂O (70:30, v/v). Secondly, extraction of less polar analytes was performed with MeCN. Considering the fact that the isoflavones are present in very low concentrations, authors tested whether the increase of the extraction time might result in better efficiency of the process. Accordingly, an extraction time of 5 min was chosen as a compromise between the recoveries yields and reproducibility. For the samples of chickpeas and white beans, analyte extraction was improved in the presence of citrate buffer. However, in the case of lentils, the addition of citrate buffer produced a decrease in the signal. The clean-up step using d-SPE with PSA or C18 was also tested, but it was found that neither the resolution of the chromatograms nor recovery was improved. Under these conditions, the clean-up step was not necessary. The method proposed by Delgado-Zamarreño et al. was precise, selective and not time-consuming, with recoveries ranging from 72 to 119% and standard deviations lower than 25% for the inter-day precision [98]. Piovan et al. applied the QuEChERS method to obtain fucoxanthin extracts from Undaria pinnatifida, a seaweed rich in this carotenoid [99]. Taking into account a growing evidence from in vitro and in vivo studies, suggesting that fucoxanthin has health promoting effects because of its strong anti-oxidant properties, the authors aim was to determine the photostability of this carotenoid in extracts with different chemical profiles. With application of conventional liquid solvent extraction procedures a fucoxanthin purity was below 50%, whereas...
after QuEChERS-based liquid-liquid partitioning, PSA clean-up, and PSA and GCB clean-up fucoxanthin purity increased to 70, 86, and 94%, respectively [99]. Although in the acetone extract the initial concentration of fucoxanthin was the highest, results demonstrated that co-extractives play an important role in enhancing the rate of carotenoid photodegradation. After light exposure, the conventional extracts lost around 90% of the initial fucoxanthin content. The extracts obtained by the QuEChERS method showed significantly higher photostability. After application of PSA or PSA and GCB, around 60 or 70% of the initial concentration was retained. The results was comparable to the photostability of fucoxanthin standard. Piovan et al. suggested that the QuEChERS method could be used and further improved to obtain more purified and stable fucoxanthin extracts from *U. pinnatifida* [99].

### 4 New trends and perspectives on QuEChERS methodology

Making assessment of actual trends, it can be stated with a high dose of certainty that the QuEChERS approach will be further expanding in terms of the scope of analytes and matrices. On one side, simple and sensitive analytical methods are needed to monitor the contaminants residues in foodstuff and ensure that it is safe for consumption, on the other, determining of persistent organic pollutants (POPs) is crucial for increasing accuracy of the description of the state of the natural environment. The best solution is to develop analytical methods, which are able to determine as many different chemical groups of contaminants as possible in one single run. The QuEChERS extraction method followed by HPLC-MS/MS or GC-MS/MS analysis seems to be capable of meeting this challenge. Multiclass, multiresidue methods (MMMs) that allow simultaneous identification and quantification of analytes in complex matrices are forthcoming. Great example of such work is a method for the analysis of 13 novel flame retardants, 18 representative pesticides, 14 polychlorinated biphenyl (PCB) congeners, 16 polycyclic aromatic hydrocarbons (PAHs), and 7 polybrominated diphenyl ether (PBDE) congeners in catfish muscle using fast low pressure GC-(QqQ)MS/MS developed and evaluated by Sapozhnikova and Lehotay [37].

Another future perspective on QuEChERS approach could be its automation. Taking into account the fact that laboratories are now encountering large numbers of samples and that the QuEChERS technique is still a manual procedure with lots of shaking and sample manipulations steps, automation can be a convenience. To accomplish the goal, Gerstel is working with DPX Labs and others to automate QuEChERS. Certain configurations are possible with application of disposable pipette extraction (DPX), which is a d-SPE technique that can...
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be fully automated and applied instead of typically used d-SPE involving centrifugation. It was demonstrated that DPX used in the QuEChERS clean-up step resulted in comparable efficiency of co-extractives removal and recovery values [100]. Automation of DPX clean-up step with application of Gerstel Dual rail MPS-2 Prepstation with DPX option was evaluated in analysis of over 200 pesticides residues in carrots, tomatoes, green beans, broccoli, and celery by GC-MS by Kaewsuya et al. [101]. Authors obtained satisfactory results with high recoveries (70−117%) and good reproducibilities (< 12%). The ability to automate the dispersive SPE clean-up of the QuEChERS extracts and combine it with direct introduction of the purified extract to the LC-MS/MS was also demonstrated (Fig. 10) [102,103]. Automation of the clean-up procedure helps save cost while improving productivity, throughput and reproducibility. The full automation approach of QuEChERS extraction procedure of pre-weighted samples was made by Teledyne Tekmar [104]. The AutoMate-Q40 system automates the following sample preparation functions such as liquid dispensing/pipetting, vortex mixing, vial shaking, opening/closing sample vials, addition of solid reagents (salts, buffers), identifying liquid levels, decanting, centrifugation, matrix spiking and d-SPE clean-up.

5 Conclusions

Due to its great flexibility and rapid character the QuEChERS should be considered rather as a sample preparation concept (methodology) than a specified method. The possibility of introducing modifications based on the application of different solvents, salts, buffers and sorbents, allows the QuEChERS methodology to be implemented in the analysis of broad spectrum of analytes and matrices. The simultaneous analysis can be performed for hundreds of pesticides using GC-MS/MS and LC-MS/MS systems. An increasing number of researchers have successfully applied the QuEChERS for analysis of analytes other than pesticides. Scope of applications of this analytical approach is constantly expanding. Recent advances in separation and detection provided by the UHPLC-MS/MS or GC-MS/MS instruments permit analysis at desired detection limits without intensive sample preparation, hence, the QuEChERS procedures provide just clean enough extracts. Although the required LC-MS/MS or GC-MS/MS instrumentation is costly, the ability to simultaneously detect so many pesticides and other analytes, combined with the QuEChERS sample preparation procedures, makes the overall analysis cost-effective.
Abbreviations

Al-N – neutral alumina sorbent
APCI – atmospheric pressure chemical ionization
C18 – octadecyl sorbent
CCβ – detection capability levels
DLLME – dispersive liquid-liquid microextraction
DPX – disposable pipette extraction
d-SPE – dispersive solid-phase extraction
EDTA – ethylenediaminetetraacetic acid
EFSA – European Food Safety Authority
GC – gas chromatography
GCB – graphitized carbon black
GC-MS – gas chromatography – mass spectrometry
GC-MS/MS – gas chromatography – tandem mass spectrometry
GC-MSD – gas chromatography – mass selective detector
GC-NCI-MS – gas chromatography – negative chemical ionization mass spectrometry
GCxGC-TOF – comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometer
HOAc – acetic acid
HPLC-FLD – high-performance liquid chromatography with fluorescence detection
IS – internal standard
ISTD – internal standard for gas chromatography
IT – ion trap
LC – liquid chromatography
LC-MS/MS – liquid chromatography – tandem mass spectrometry
LLE – liquid-liquid extraction
LOQ – limit of quantification
LP-GC/MS-MS – low pressure gas chromatography – triple quadrupole tandem mass spectrometry
LVI – large volume injection
MeCN – acetonitrile
MgSO₄ – magnesium sulfate anhydrous
MMMs – multiclass, multiresidue methods
MNPs/MWCNTs – amine-functionalised magnetic nanoparticles and multiwalled carbon nanotubes
MRL – maximum residue limit
MRM – multiple-reaction-monitoring mode
MRMs – multiresidue methods
MSPD – matrix solid-phase dispersion
MWCNTs – multi-walled carbon nanotubes
Na₂HCit – sodium citrate dibasic sesquihydrate
Na₃Citrate – sodium citrate tribasic dehydrate
NaCl – sodium chloride
NaOAc – sodium acetate
NH₂-G, CH₃NH-G, and tBuNH-G – amine modified graphene
PAHs – polycyclic aromatic hydrocarbons
PCB – polychlorinated biphenyl
POPs – persistent organic pollutants
PP – polypropylene
PSA – primary secondary amine
Q-Orbitrap MS – quadrupole orbitrap mass spectrometry
QuEChERS – quick, easy, cheap, effective, rugged, and safe
RSD – relative standard deviations
SCX – strong cation exchange
SPE – solid phase extraction
TIC – total ion chromatogram
TPP – triphenylphosphate
UHPLC-MS – ultra-high performance liquid chromatography – mass spectrometry
UHPLC-MS/MS – ultra-high performance liquid chromatography – tandem mass spectrometry
UHPLC-(HR)MS/MS – ultra-high performance liquid chromatography – high resolution tandem mass spectrometry
UPLC-MS/MS – ultra performance liquid chromatography – tandem mass spectrometry
VDs – veterinary drugs
Z-Sep – sorbent based on modified silica gel with zirconia oxide
Z-Sep Plus – sorbent consists of both zirconi and C18 dual bonded on the same silica particles

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