SPECIAL GUEST EDITOR SECTION

New Trends in Pesticide Residue Analysis in Cereals, Nutraceuticals, Baby Foods, and Related Processed Consumer Products

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Pesticide residue methods have been developed for a wide variety of food products including cereal-based foods, nutraceuticals and related plant products, and baby foods. These cereal, fruit, vegetable, and plant-based products provide the basis for many processed consumer products. For cereal and nutraceuticals, which are dry sample products, a modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method has been used with additional steps to allow wetting of the dry sample matrix and subsequent cleanup using dispersive or cartridge format SPE to reduce matrix effects. More processed foods may have lower pesticide concentrations but higher co-extracts that can lead to signal suppression or enhancement with MS detection. For complex matrixes, GC/MS/MS or LC/electrospray ionization (positive or negative ion)-MS/MS is more frequently used. The extraction and cleanup methods vary with different sample types particularly for cereal-based products, and these different approaches are discussed in this review. General instrument considerations are also discussed.

This review includes an evaluation of methods for pesticide analysis used for cereal-based products, nutraceuticals, and baby foods. The range of analytes for these methods has also been extented from pesticides to mycotoxins, alkaloids, and/or veterinary drugs in cereal-based products, nutraceuticals, and traditional, organic, or hypoallergenic baby foods (1–9). Processed foods also include fruit, vegetable, cereal, or meat-based baby foods that have higher water content, as well as processed cereal-based products such as breads, doughs, and pasta. As with other food analysis, matrix-matched standards and use of isotopically labeled standards is recommended for all pesticide analysis to compensate for matrix effects caused by co-extracts not removed during sample cleanup. Matrix effects are generally evaluated by comparison of the slope of calibration curves from matrix-matched standards to those of standards in solvent. Isobaric interferences in one or more multiple reaction monitoring (MRM) transitions or ions selected for GC with the selected ion monitoring mode (SIM) can be due to presence of co-extracts and lead to MS signal suppression or enhancement. The GC/SIM-MS, GC/MS/MS, LC/MS/MS, and LC/quadrupole time of flight (qTOF)-MS approaches were similar to other food product analysis and will be discussed together.

Pesticide methods for these food products are largely applications/modifications of methods used for the analysis of fresh fruits and vegetables (10–14) for which QuEChERS (quick, easy, cheap, effective, rugged and safe) is one of the most popular sample preparation approaches. Among the possible extraction approaches, modified QuEChERS methods were also the most common for dried samples with parameters optimized for specific co-extracts or sample matrix type. Figure 1 shows a comparison of the typical parameters used in the modified QuEChERS methods for dried sample types reviewed here. One important step for dry products including cereals and nutraceuticals was the addition of a wetting step prior to or in combination with the acetonitrile salt-out extraction. Addition of a combination of $MgSO_4$ and NaCl induces the phase separation of water and acetonitrile layers. For baby foods that typically have higher water content, a larger range of extraction approaches has been used. When QuEChERS was used for extraction of pesticides from baby foods, typically there was no wetting of the homogenized sample prior to extraction with buffered acetonitrile.

As complexity of the matrix within each of these samples types increased, the need for cleanup with dispersive SPE (dSPE) or other approaches was required and will be discussed further. As can be seen in Figure 1, common dSPE sorbents were primary secondary amine (PSA) and graphitized carbon black (GCB) to remove fatty acids or pigments from sample extracts, and/or C18 (for removal of lipids and commonly used for foods with higher fat content) with a few more recent methods also using Z-Sep+. Problematic pesticides include those prone to degradation and those with high affinity to PSA or low MS signal intensity. PSA is also good for removing saccharides and organic acids. Florisil removes polar and low-fat co-extracts; C18 or Z-Sep+ removes lipids. Falvones, alkaloids, starch, polysaccharides, and volatile oils have been removed with a combination of PSA and GCB (15). Salts such as NaCl promote organic phase and water phase separation by the salt-out effect. $MgSO_4$ is added to extracts to absorb moisture.

A limitation of the QuEChERS procedure for these sample types was the requirement for use of matrix-matched standards, particularly for laboratories analyzing a larger number of samples or different types of samples. It has also been found

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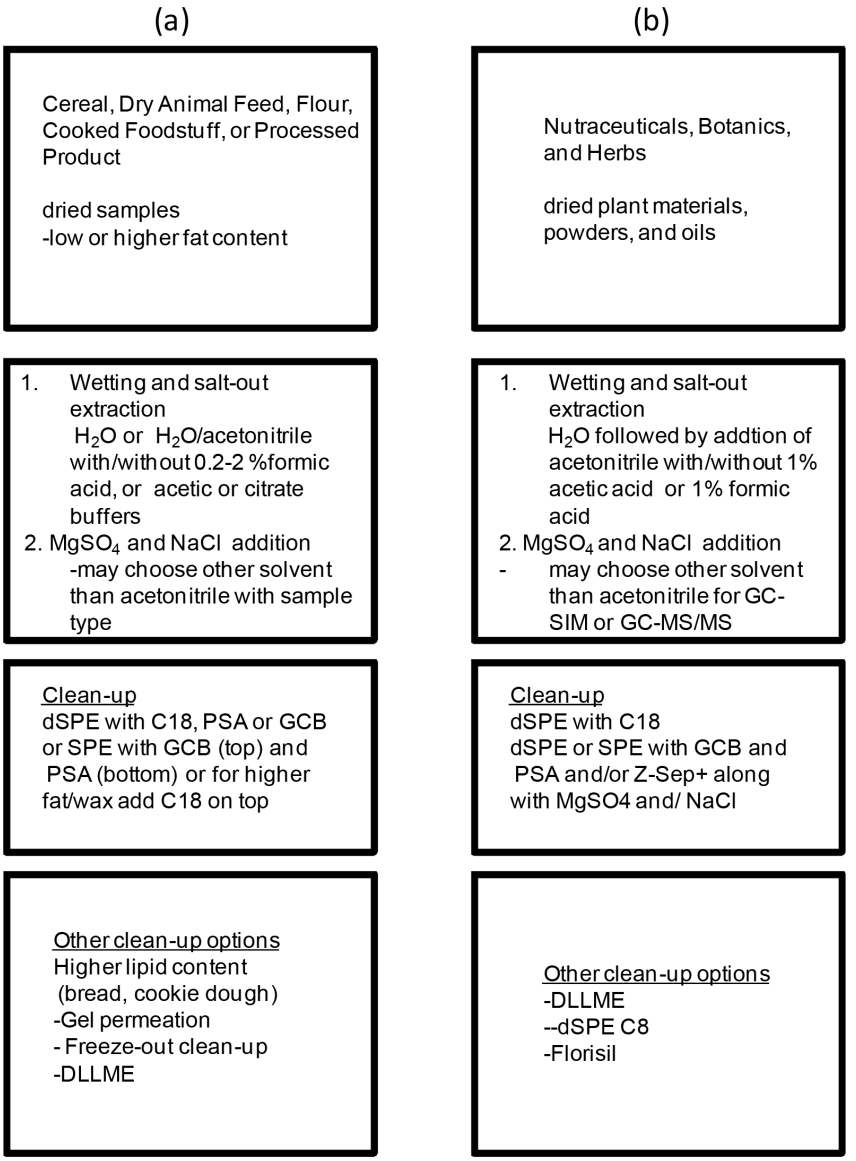

Figure 1. Modified QuEChERS methods for dried samples with main parameters for optimization noted: (a) cereal-based products, (b) nutraceutical products and related plant materials.

that for fruits and vegetables matrix-matched calibration with one fruit does not correct results for other samples even from the same fruit (16). Standard addition has also been used with a single level calibration standard in solvent and appropriate level of dilution of fruit and vegetable extracts (16), but it was not used for these sample types reviewed.

Cereal-Based Products

Cereal products include cereal grains, flour, bran, baked goods, doughs or muffin mixes, cooked foodstuff, dry animal feed, and food and feed crops (wheat, rice, maize, soybeans, and potatoes) from which pesticides were commonly extracted using modifications or extensions of the QuEChERS method (1–3, 17–29). Cereal and dry animal feed have more severe matrix issues due to high starch, protein, and fat contents as well as added vitamins, minerals, or milling byproducts that impact extraction and LC/MS/MS or GC/MS instrument performance. Processed food products of cereal origin are often dried or have low-water content, and major co-extracts include fatty acids with higher fat content than cereal grains. For dietary studies cooking oils and condiments can also be used in food preparation, which are expected to increase the complexity and fat content of extracts. Feed, corn, and soy-based products generally have more severe matrix effects (3, 17, 18). Fatty acids such as linoleic, oleic, and palmitic are extracted in the acetonitrile used in the QuEChERS method, and their presence is more problematic in corn relative to rice and wheat flour (17, 18). Other challenges with cereal-based products and processed foods based on cereal grains is that pesticide levels are often lower in more processed foods although matrix effects can be more severe. Pesticide levels decline with storage, milling, baking of bread, cookie processing, or processing method (hot or cold pressing; 30–33), and frequency of application of pesticides, rate of application, and preharvest intervals can impact pesticide levels in cereals (34). Carryover of pesticides

from wheat to spaghetti ranges from 1 to 10% depending on the specific organophosphorus (OP) pesticide (35).

The QuEChERS method was originally developed to deal with fruits and vegetables where there is a high moisture content and low fat content (3). Both an acetate-buffered and citrate-buffered version have been used (3, 4). In addition to changing the buffer system (acetate or citrate), lowering the sample to solvent ratio and choice of sorbents for dSPE has been used to reduce matrix effects prior to LC/MS/MS or GC/MS/MS analysis (17).

The amount of PSA in the dSPE step is often increased to compensate for the presence of buffer that reduces the capacity of sorbents (18). Adding acid to acetonitrile improves the recoveries for base-sensitive pesticides. The QuEChERS method for dried samples such as cereals, flour, cooked foodstuff, and processed products is modified to allow for swelling of the dry food matrix by addition of water to give a slurry sample for subsequent steps (3, 17, 18, 21–26). The water may be added prior to the addition of acetonitrile or at the same time and vortexed prior to the next steps. Some pesticides are prone to hydrolysis during the prolonged wetting step (30 min to 2 h), and as such use of a water–acetonitrile mixture (such as 2 + 3, v/v, ratio) allows for wetting of the dry sample matrix and analyte extraction (27). The greatest number of modifications to the QuEChERS method were done for cereal-based products with choice of acid added (formic, acetic, or citric) either to the water during wetting or acetonitrile (or acetonitrile–water mixture) extraction and its concentration being important considerations. Use of acidified water with 0.2–2% (v/v) formic acid has been used to improve stability of base-sensitive pesticides (captan, folpet, dichlofluanid, and tolylfluanid) that undergo hydrolysis at high pH and, along with use of longer swelling and/or extraction times (30 min), provides acceptable recoveries (1, 2). Low recoveries have been observed for polar or acidic pesticides (clopyralid, dicamba, and fumonisins) when formic acid was present at 0.5% (v/v), while most compounds had acceptable recoveries (70–120%) when 1 or 2% (v/v) formic acid in water was used (2). Optimal formic acid concentration was 1% (v/v) added to the organic solvent, with acetonitrile showing better recoveries than acetone or methanol (3). A formate buffered QuEChERS method was also reported to provide good recoveries for some GC-amenable pesticides in wheat grain (29). Maize flour, a difficult sample matrix, showed acceptable recoveries in all solvents (methanol, acetone, and acetonitrile) for >90% of the analytes (3). With the use of acetonitrile as the organic solvent, the method could be further modified to eliminate the salt-out partitioning step that can discriminate very polar and pH-dependent pesticides important to LC/MS/MS methods (3).

The use of acetate buffer in the QuEChERS method relative to conventional unbuffered methods was shown to improve recoveries for pH-dependent pesticides such as azole fungicides (22). Citrate-buffered systems have also been preferred for use for sample matrixes with fatty acid co-extracts such as for analysis of organochlorines, pyrethroids, and OPs (23, 27). Acetonitrile with acetic acid and sodium acetate can lead to signal enhancement in commodities with large starch content due to co-extraction of fatty acids (23). Comparison of the QuEChERS method with acetate buffer (AOAC Official Method^{SM} **2007.01**) and citric acid [European Committee for Standardization (CEN) Standard Method EN 15662)] showed that acetonitrile extraction with acetate buffer gave poor recoveries (<70%) for a number of acidic pesticides, while the recoveries with citrate buffer were in the acceptable range (2). The pH selected for the extraction should be based upon the target analytes. Not all basic analytes have poor recoveries at low pH, and what co-extracts are present may also be a factor. Selected highly polar basic compounds such as pymetrozine can remain in the water phase at low pH and have improved recoveries at pH >6 (2).

The QuEChERS method has also been modified for cereal samples to include use of a homogenizer to improve the extraction efficiency along with a C18 SPE column (to remove lipids) prior to GCB plus PSA to remove fatty acids or pigments from samples (21, 27). dSPE with C18 alone or C18 and PSA has also been used to remove lipids, more frequently for samples of fat content 2–20% (2, 23–26). Higher amounts of sorbent in the dSPE (75 mg PSA compared to 25 mg PSA) were found to improve signal intensity and issues with retention time shifts (27), while others found that matrix issues due to fatty acids could not be reduced even by increasing the amount of PSA (18). In these cases the amount of C18 had to be tripled to compensate for reduced capacity on PSA when acetate buffer was used, and in some cases sample size was reduced to obtain acceptable recoveries (18).

Another approach to reducing issues with pesticides prone to hydrolysis is to avoid the use of water by using an acetonitrile extraction of the dry sample with sonication for 30 min with good recoveries for spiked samples, particularly for base-sensitive pesticides (2). However, recoveries were poor for acidic polar pesticides (40–70%) due to low polarity of acetonitrile that is unable to extract polar pesticides from basic matrixes (2). Better recoveries were also observed for acidic pesticides with addition of a wetting step and a phase separation step with $MgSO_4$ and NaCl (2). Care must be taken to ensure contact of the particles with acetonitrile, as certified reference materials of cereals did not give comparable results to spiked powder cereal samples. This was attributed to the spiked samples giving better extraction results as the pesticides were only on surface particles (2).

The modified QuEChERS method with addition of water to foods with higher lipid content including doughs has produced good reproducibility and recoveries and better removal of lipids than more time-consuming gel permeation chromatography or a freeze-out step (24). Feed samples are a difficult matrix, and matrix interferences in GC/MS/MS were still present with a modified QuEChERS method (wetting and dSPE; 26). When suppression of the MS signal or retention time shifts relative to matrix-matched standards were observed, an additional freeze-out step that precipitates co-extractables with low solubilities in acetonitrile significantly improved recoveries (26). A modified QuEChERS method with addition of water for wetting the dry flour sample in combination with dispersive liquid-liquid microextraction (DLLME) has been shown to provide improved recoveries and enrichment of more polar GC-amenable pesticides (36).

Other methods of sample preparation have included solid-phase microextraction with an ameyrn-imprinter polymer fiber or hollow-fiber liquid-phase microextraction, methanolic extraction with ultrasonication, classical liquid–liquid extractions (acetone, acetonitrile, methanolic, and ethyl acetate solvents), modified Luke method, acetonitrile-based DLLME, supercritical

fluid extraction, pressurized liquid extraction (PLE), microwave extraction, and disposable pipet extraction cleanup (30, 36–45). A number of the liquid–liquid extraction methods optimized partitioning between two solvents for the range of polarity of the pesticides under study and were designed more for GC analysis where the extract does not have water present (3). Comparison of a liquid–liquid extraction [wetted sample extracted with acetone–petroleum ether–dichloromethane (1 + 1 + 1, v/v/v)] with a buffered acetonitrile QuEChERS method (with wetting but without the cleanup with PSA) has shown that on average, recoveries with the modified QuEChERS method of a larger range of pesticides analyzed by positive mode LC/electrospray ionization (ESI)-MS/MS in soybean grain extract were better (40). However, the liquid–liquid extraction did better for the most polar pesticides that eluted first in LC/MS/MS, potentially due to acetonitrile extracting polar co-extracts more efficiently than acetone (40). Petroleum ether saturated with acetonitrile for a liquid–liquid extraction step followed by an SPE step with an OASIS MCX cartridge (Waters Corp., Milford, MA) has also been used for analysis of triazines by LC/MS/MS (18). PLE with cleanup on alumina sorbent at bottom of the extraction cell has also been used to obtain adequate recoveries with RSD <15%; however, for complex feed samples additional SPE or dSPE cleanup may still be required (44).

Nutraceuticals, Botanicals, and Herbs

A variety of nutraceuticals, botanicals, and herbs have been analyzed by GC/SIM-MS, GC/MS/MS, or LC/MS/MS methods, including Asian and American ginseng roots (*Panax ginseng and P. quinquefolius*, respectively), Radix ginseng, ginkgo (*Ginkgo biloba*) leaves, saw palmento (*Seronoa repens*) berries, astralagus (*Astragalis membranaceus*), bitter orange peel (*Citrus aurantium*), black cohash root (*Cimicifuga racemosa*), chamomile (*Matricaria chamonmilla*), cinnamon bark (*Cinnamom verum*), comfrey root (*Symphytum officinale*), dong quai (*Angelica sinensis*), Enchinea (*Echinacea purpurea*), fenugreek (*Trigonella foenum*), garlic (*Allium sativum*), ginger (*Zingiber*), hoodia (*Hoodia gordonii*), dogwood, *Herba Lophatheri*, *Semen Persicae, Flos Lonicerae,* green tea (*Camellia sinensis)*, dandelion, scutellaria, mangosteen, *Bajitian (Morinda officinalis), Pogostemon cablin* (oil and powder), and grape (4, 5, 15, 46–59). Nutraceutical and related raw plant material samples are dried, and this concentrates pesticides and co-extracts such that smaller sample size is generally required for chemical analysis to minimize interferences from co-extracts that can include pigments, alkaloids, sterols, fats, and biochemically active ingredients. Alkaloids are a common co-extract used in many nutraceutical products due to their anti-inflammatory, diuretic, analgesic, and cardiotoxic actions. Green tea co-extracts include catechins, polyphenols, and theanine (49). Other ingredients in the co-extracts and pesticides can vary significantly in their pKa values and have varying solubility in different extraction solvents. Gingko was observed to have the largest number of pesticides with matrix effects, which was attributed to presence of higher polyphenol, sterol, or chlorophyll contents in pigmented nutraceuticals leading to matrix suppression (46, 48). Production methods may also impact pesticide and co-extract levels, such as for red ginseng that is often observed to have lower pesticide levels than other ginseng varieties because of its production involving steaming or heating of raw ginseng (4).

As most of these nutraceutical and related plant products are dried to low moisture content and are either commercially available or made into powders, a wetting step is required for analysis. In all cases the dried powder samples (<10% water content) are wetted with water and vortexed prior to addition of acetonitrile [unbuffered or with addition of 1% (v/v) formic acid or 0.1 or 1% acetic acid] or other solvents (acetone and methanol with 1% formic acid; 4, 5, 15, 46–50). Even for cases when both powder and oil samples were available for chemical analysis, acetonitrile–water with 1% acetic acid (9 + 1, v/v) was used (15). Acetonitrile with formic acid was found to be the best solvent for extraction of pesticides with varying polarity from ginseng (4). As with other analyses, some pesticides are pH sensitive and had lower recoveries when no acid or buffering of acetonitrile was used for extraction (46). Some pesticides showed signal enhancement or suppression or shifts in retention times with matrix even within the same type of sample (ginseng varieties; 4, 47). The acetate buffered QuEChERS methods have been shown to provide acceptable recoveries for a wider range of LC-amenable pesticides, particularly pH-sensitive pesticides, than the citrate QuEChERS version (49). For analysis of more nonpolar GC-amenable pesticides, salt-out extraction of ginseng with acetonitrile or acetone–cyclohexane–ethyl acetate (2 + 1 + 1, v/v/v) and NaCl has been used with similar recoveries, with the latter providing faster reduction of extract volume during the drying step as well as better recoveries when GC/SIM-MS or GC/MS/MS were used (51).

Modified QuEChERS methods for nutraceuticals require a cleanup step following salt-out acetonitrile extraction. dSPE with PSA and C18 (50 mg each) and $MgSO_4$, or PSA and C18 (50 mg) each with 7.5 mg GCB has been used to improve recoveries for samples with pigments except when planar pesticides are present as they will adsorb to GCB (46). Higher amounts of PSA (210 mg) with GCB (70 mg) and $MgSO_4$ and NaCl have also been used depending on the sample matrix (48). PSA is used to remove organic acids, while GCB removes pigments such as chlorophyll and carotenoids, polyphenols, and sterols.

Cleanup with SPE cartridges containing 250 mg GCB (top layer) and 500 mg PSA (bottom layer) have also been used with salt-out ($MgSO_4$ and NaCl) acetonitrile extraction (47). The amounts of PSA, GCB, $MgSO_4$, and NaCl have also been optimized for various dried plant materials, powders, or oils (15, 48). In addition, it is preferred to do SPE rather than dSPE with a C18 SPE cartridge on top of a multilayered GCB/PSA SPE cartridge, as it was more effective in removing matrix containing more fat or waxes than a dispersive GCB/PSA QuEChERS method (48). dSPE with octylsilyl (C8) sorbent has also been used for ginseng extract for removal of fats, lipids, and nonspecific plant matrix followed by SPE with GCB/PSA cartridges, as dSPE with GCB/PSA was found to give low recoveries for hexachlorohexane and pentachlorobenzene (51). GCB at 10 mg/mL was needed for cleanup of some ginseng extracts, while for other ginseng samples containing more pigments this amount of GCB was not sufficient (48). DLLME has also been used for further cleanup and concentration of pesticides following the QuEChERS method with acetonitrile for ginseng extract (50). A mixture of sorbents with addition of Z-Sep+ to PSA, C18, and GCB provided colorless extracts

and more consistent chromatographic responses (53, 59). Z-Sep (mixture of C18 and silica coated with zirconium dioxide) and Z-Sep$^+$ (mixture of silica coated with zirconium dioxide and C18 siloxane as a carrier group) have been used for cleanup of nutraceuticals such as soya-based products, which are challenging due to the presence of pigments, saccharides, and fatty acids (53).

Not all nutraceuticals require cleanup prior to analysis, such as green tea extract which showed a similar number of pesticides with acceptable recoveries without a cleanup as with a cleanup step with PSA, GCB, and $MgSO_4$ (49). Extraction with (46) or without (4) cleanup has been used for ginseng. Early eluting polar pesticides had broad peaks in LC/MS/MS with peak shape improving when samples were diluted to 20% aqueous mobile phase rather than a higher percentage of organic modifiers (acetonitrile or methanol; 46). In some analyses where pesticide levels are low, the sample to water ratio is reduced, and then after cleanup the sample is concentrated to improve sensitivity (48).

A variety of other extraction methods for both LC- and GC-amenable pesticides have been used including soxhlet extraction, ultrasonic extraction, microwave assisted extraction, PLE, PLE assisted matrix SPE, pressurized hot water extraction (PHWE), or surfactant (Triton X-100 or sodium dodecyl sulfate) assisted PHWE (55–57). For GC-amenable pesticides, PLE assisted matrix SPE with acetone–hexane (1 + 1, v/v) solvent has been used, while others found that acetonitrile was the best solvent and both methods required a Florisil SPE cleanup (55, 57). Liquid–liquid extractions only were used for Chinese medicine health wines, with the best extraction solvent chosen as dichloromethane–acetone (1 + 1, v/v) with no cleanup steps required (58).

A modified QuEChERS procedure for extraction of pesticides from soya-based nutraceuticals showed that recoveries were acceptable for a much larger range of GC-amenable pesticides using salt-out extraction with ethyl acetate rather than acetonitrile, with the advantage that ethyl acetate does not require a drying step that can lead to loss of more volatile pesticides (53). Grape seed was also extracted using a modified QuEChERS procedure with ethyl acetate (59).

A "dilute and shoot" method for LC/MS/MS was found to provide similar results at higher pesticide levels (100 µg/kg) to acetate buffered QuEChERS method extracting 77 and 64% of pesticides, respectively, and both with acceptable recoveries (70–120%) (5). However, at lower pesticide levels the dilute and shoot did not perform as well, and a cleanup step with combination of PSA, GCB, C18, or Z-Sep$^+$ was necessary to obtain acceptable recoveries for a larger range of pesticides (5).

Baby Foods

Pesticide analysis methods have been developed for both nonfatty baby foods (fruit and vegetable based and cereal based) and for baby foods with higher fat contents such as those containing meat, as well as powdered infant formulae (60–68). Nonfatty baby foods are mixtures of fruit or vegetable pureé in combination with flour/starch and sugar. Options for extraction other than modified QuEChERS methods are more frequently used in the analysis of baby foods. Baby foods consisting of fruit juices can be extracted with ethyl acetate or acetone; however, pureé and cereal-based baby foods had significant amounts of co-extracts. In these nonfatty baby food products the high lipophilic compounds co-extracted resulted in signal enhancement or suppression for various GC-amenable OPs with lower recoveries for most OPs even when matrix-matched standards were used (60). Pressurized microwave-assisted extraction (PMAE) with methanol as the solvent has been used but required subsequent drying of the methanol extract and reconstitution in acetonitrile prior to LC/ESI-MS analysis of triazines, but it had the advantage that it did not require cleanup even for cereal-based baby foods (61). Other extraction solvents [acetone–hexane (1 + 1, v/v), dichloromethane–methanol, acetonitrile, and acetone] were also tested but provided lower recoveries for some or all triazines (61). Methods such as soxhlet extraction and liquid–liquid extraction had lower recoveries as compared to PMAE and were also more time-consuming (61). Ultrasonic extraction with acetonitrile followed by dilution of the sample extract with Millipore water and subsequent SPE extraction (Oasis HLB) has been used for analysis of dicarboximide pesticides in powdered infant formulas with LC/ESI$^-$ -MS/MS or GC/SIM-MS and provided adequate recoveries in milk or hypoallergenic formula but lower recoveries in soy-based powder formula (62). Water has also been added to fruit and vegetable baby foods with vortexing, and the filtered water extract was subsequently cleaned up with SPE (Oasis HLB) for analysis of degradates of OPs and pyrethroids using LC/MS/MS (63).

A large number of pesticides have been analyzed in fruit or vegetable-based baby foods using a QuEChERS extraction method with acetonitrile–acetic acid (99 + 1, v/v) and sodium acetate and $MgSO_4$, followed by dSPE with PSA and $MgSO_4$ cleanup (64, 65). A similar QuEChERS method has also been used without dSPE cleanup without large matrix effects when analysis was done using LC/quadrupole Orbitrap MS (6). The QuEChERS method has also been used with dSPE with <200 mg C18 for removal of planar, nonpolar co-extracts in baby foods (vegetable, lamb and vegetable, and baby yogurt dessert) with a range of fat contents (66). A QuEChERS extraction using acetonitrile with $MgSO_4$ and NaCl followed by dSPE with PSA, $MgSO_4$, and C18 showed that low fat baby foods had decreased recoveries of OPs with increasing amounts of C18, while baby foods with fat content ≥3% required higher amounts of C18 (66). QuEChERS extraction with acetonitrile followed by salt-out with NaCl and cleanup with SPE using multilayer GCB/PSA (ENVI-CARB-II/PSA) and addition of $NaSO_4$ to the top of the SPE cartridge has been used for extraction of OPs and pyrethroids from fruit, vegetable, meat, and yogurt baby foods (67).

Instrument Considerations

GC/MS/MS or LC/MS/MS is often preferred over GC/SIM-MS methods for pesticide analysis as they more easily meet U.S. Food and Drug Administration requirements for four points in identification of a pesticide. In LC/MS/MS or GC/MS/MS, two MRM transitions can be used, which is feasible for most analysis. In comparison, for GC/SIM-MS four ions would be required to meet this criterion, and it is frequently difficult to find four fragment ions with sufficient sensitivity. In most cases when GC/SIM-MS is used in electron impact (EI) or negative chemical ionization (NCI) modes, only three ions are available of sufficient sensitivity. Generally,

NCI produces fewer fragment ions in mass spectra than EI (21–23, 36). Also, for GC-amenable pesticides GC/MS/MS can provide better isolation of analyte response from isobaric interferences compared to GC/SIM-MS (17, 25–27, 37). Matrix suppression is commonly observed for extracts from corn (25); however, modified QuEChERS methods and GC/MS/MS provided results with acceptable recoveries for a range of cereals and dry animal feed samples (25). Use of more than one MRM transition for each analyte when sufficient sensitivity is available also provides improvement in confirmation because of higher selectivity of the analyte from co-extracted matrix. When GC/EI-SIM-MS with the criterion of selection of four ions for each pesticide was used for a ginseng extract, detection limits for pesticides were 7–9 times higher than with GC/EI-MS/MS (1–20 and 83–167 µg/kg for GC/SIM-MS and GC/MS/MS, respectively; 51).

Matrix interferences can increase the MS background noise or be visible in the chromatogram as large peaks or shift in the baseline over a large region in LC/MS/MS or GC/MS/MS. Using a minimum of two MRM transitions or response-ratios of MRM transitions (ratio ± 20% RSD determined from standards run on day of analysis) gives better confirmation of identification of positive findings and is now a common approach used in pesticide analysis with MS/MS methods (25, 26). Generally for GC/MS/MS, high mass ions (m/z >150–200), but frequently not the molecular ion, are chosen as the precursor ion with the collision energies optimized to give the highest S/N for product ions and greatest ion selectivity (21, 25, 26). Lower mass precursor ions generally suffer from more interferences, leading to false-positive results. Matrix interferences in corn and animal feed are the most challenging and may not be isolated even by GC/MS/MS (25). GC with a micro electron capture detector, flame photometric detector, or nitrogen-phospharus thermionic detector has also been used for analysis of cooked foodstuffs, cereal-based baby foods, bean products, and wheat flour and may be an alternative to MS detection (21, 38, 41–43).

With HPLC or ultra-HPLC (UHPLC)/MS/MS not all pesticides have two or more MRM transitions and sensitivity may vary, thus requiring use of different ion sources (ESI or atmospheric chemical ionization) or switching from the positive to negative mode. Switches in ion source or mode (positive to negative) may also be needed to reduce the impact of interferences (2). Dilution of sample extract if pesticide levels are high enough is often used to balance matrix effects and subsequent loss of MS sensitivity. Dilution such as with water rather than organic solvents is, however, not feasible if nonpolar co-extracts are present as they will precipitate before LC/MS/MS analysis. Some pesticides are also prone to greater degradation by hydrolysis in aqueous solutions as compared to pure organic solvents such as acetonitrile, including sulfonyl ureas, some OPs, metribuzin, quinoxyfen, or tepraloxydim (2).

For multiresidue analysis by GC/MS/MS or LC/MS/MS, multiple acquisition time windows over different retention time ranges will generally have about 20 MRM transitions/window. In GC/SIM-MS, this means that if three or four ions are selected for each pesticide there would be fewer pesticides analyzed in an MS acquisition window in order to obtain adequate sensitivity, as the dwell time is determined from the number of masses to be measured in the window and the scan rate. Different software packages generally have a maximum number of MRM transitions or ions that are permitted for an acquisition window. To minimize loss of S/N and peak shape, this is generally 10–20 MRM transitions/window (22). The number of acquisition windows would also increase for faster analysis such as UHPLC compared to HPLC/MS/MS due to shorter analysis times and more compounds eluted over a given time period. This may make selection of windows more challenging to ensure a sufficient time gap between peaks to allow for retention time shifts due to matrix effects and shifts in retention over time with column performance (2). The use of UHPLC with columns having particle diameters <2 µm or core-shell particles (2.6 µm particle diameter), lengths ≤100 mm, and 2–3 mm id allows for faster, efficient separations with narrow and sharp chromatographic peaks that provide resolution between isobaric pesticides or potentially co-eluted co-extracts (2, 16, 18, 19, 28). However, matrix-effects occur with these columns for a wide variety of cereal and flour samples and consequently, matrix-matched standards and isotopically labeled internal standards are still used (28, 39). C18 is the most commonly used stationary phase; however, other columns with mixed C18 and a strong cation-exchanger have been used to alter selectivity of a separation particularly for ionizable or ionic organic analytes (19).

Shorter, wider diameter columns for fast GC with higher temperature ramp rates led to poorer resolution and reduced response for some pesticides due to co-extracts, and generally standard GC column dimensions were favored (47). In some cases, increased thickness of stationary phase was used to increase sample capacity (25–27). Real samples were analyzed by GC/MS/MS with an injector equipped with a carbofrit liner and a guard column, but were not sufficient to prevent all nonvolatiles from reaching the analytical column (26). Backflushing methods have also been used to extend column life due to large amounts of co-extracts in injected samples (54). In GC/MS/MS analysis of dietary supplements, column backflushing has also been used to reduce or eliminate matrix co-extracts less volatile than the analytes from reaching the analytical column with the use of a 5 m length restrictive capillary (same stationary phase and diameter) and a purge valve prior to the analytical column (54). A postrun backflushing step was used along with a programmable temperature vaporization inlet with a dimpled liner and 5 µL injection with acetonitrile as the solvent to allow for 125 matrix injections without maintenance. This provided reduction in matrix on the column such as for dandelion root powder extract that contains high levels of sterols (54). Analyte protectants could also be used to improve GC analysis, but these were not frequently used in methods for these matrix types (69).

If the mass differences between the pesticide ion and interferences are large enough in the sample matrix, then high-resolution MS can be used to isolate the pesticide response (mass window can be reduced to 0.01 Da). GC/TOF-MS or LC/TOF-MS has also been used to allow for nontargeted pesticide residue analysis with fast acquisition of mass spectra (2, 3, 18, 24). With a low resolution mass spectrometer (triple quadrupole) such as in LC/MS/MS, there are generally fewer pesticides analyzed/run than with high resolution instruments where >200 compounds can be analyzed (5). Quadrupole Orbitrap MS was a feasible method for screening a large number of LC-amenable pesticides in baby foods but had lower reproducibility and greater uncertainty for quantification (6).

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