Journal of Chromatography A, 1483 (2017) 8–19

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Evaluation and prevention of the negative matrix effect of terpenoids on pesticides in apples quantification by gas chromatography–tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 14 September 2016
Received in revised form
16 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
Apples
Pesticides
Oleanolic acid
Matrix effect
GC/MS^2
HPTLC

ABSTRACT

The sample matrix can enhance the gas chromatography signal of pesticide residues relative to that obtained with the same concentration of pesticide in solvent. This paper is related to negative matrix effects observed in coupled gas chromatography-mass spectrometry ion trap (GC/MS^2) quantification of pesticides in concentrated extracts of apple peel prepared by the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) method. It is focused on the pesticides most frequently used on the apple varieties studied, throughout the crop cycle, right up to harvest, to combat pests and diseases and to improve fruit storage properties. Extracts from the fleshy receptacle (flesh), the epiderm (peel) and fruit of three apple varieties were studied by high-performance thin-layer chromatography hyphenated with UV–vis light detection (HPTLC/UV visible). The peel extracts had high concentrations of triterpenic acids (oleanolic and ursolic acids), reaching 25 mg kg^{-1}, whereas these compounds were not detected in the flesh extracts (<0.05 mg kg^{-1}). A significant relationship has been found between the levels of these molecules and negative matrix effects in GC/MS^2. The differences in the behavior of pesticides with respect to matrix effects can be accounted for by the physicochemical characteristics of the molecules (lone pairs, labile hydrogen, conjugation). The HPTLC/UV visible method developed here for the characterization of QuEChERS extracts acts as a complementary clean-up method, aimed to decrease the negative matrix effects of such extracts.

1. Introduction

Gas or liquid chromatography techniques coupled with mass spectrometry (MS or MS^n) are among the most powerful analytical tools currently available for monitoring pesticide residues in food. The use of mass spectrometry, particularly MS^n, has considerably improved the selectivity and sensitivity of the analysis. However, such methods may underestimate or overestimate pesticide levels in complex samples, such as food products, due to matrix effects. Such effects may result in significant differences in the signals obtained for chromatographic standards prepared in solvent and standards prepared in the matrix [1–8]. The chromatographic signal is increased by positive matrix effects and decreased by negative matrix effects. These effects may result from the adsorption of analytes and matrix components in the injector, the detector and/or the chromatography column [9].

Analysts have long focused on modifications to sample purification procedures as a means of compensating for the matrix effect. The QuEChERS method [10] has been successfully used and adapted for the extraction of pesticide residues from various types of food sample, including fruits, such as tomato, pear, apple, orange, lemon, guava, grape, avocado. . ., vegetables, such as cabbage, carrot, lettuce, cucumber, onion. . ., rice [11], cereal grains [12], liquids and beverages, such as fruit juice, olive oil, honey [13]. . ., and processed products, such as potato chips, and crackers [14–17]. Alternative methods have been developed, based on the addition of internal labeled standards [18], calibration in the matrix [19,20], the addition of analyte protectants (*e.g.* sorbitol, γ-lactone-gulonic acid) [20], or calibration correction factors [8]. Calibration correction factors are added to both the standards and the samples. They interact strongly with the active sites of the system (silanols), thereby minimizing matrix effects [18,19]. The gas chromatograph (loading of liners and precolumns) and the mass spectrometer (source cleaning) should undergo regular maintenance, to ensure that the sensitivity and reproducibility of the GC/MS method remain high [19].

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http://dx.doi.org/10.1016/j.chroma.2016.12.056
0021-9673/

The matrix compounds most likely to interfere with gas chromatography analysis are lipids (*e.g.* waxes, triglycerides, phospholipids), pigments (*e.g.* chlorophylls, carotenoids, melanoidins) and other molecules with a high molecular weight (*e.g.* resins) likely to dissolve in the solvents used to extract the analytes of interest [9]. A tailing off or fade-out of peaks of interest on chromatograms may be interpreted as a classic sign of a dirty detector. It must be noticed that tailing peaks can also occur when there is an interaction between the analyte and the stationary phase of the chromatographic column or because of unsuitable injection parameters (insert, injection speed, temperature, volumes...) [21]. Apples matrices consist of diverse components, including sugars, proteins, lipids [22–27], polyphenols [27–30] triterpenic compounds, paraffins, and alcohols [31–40], which may interfere with the analysis and contribute to matrix effects.

Positive matrix effects are stronger for pesticide molecules with particular functional groups: organophosphates (-P=O), carbamates (–O—CO—NH–), hydroxy compounds (–OH), amino compounds (–NH–), imidazoles, benzimidazoles (-N=) and urea derivatives (–NH—CO—NH–) [8,9]. Hydrophobic, non-polar compounds, such as persistent organochlorine contaminants, are less affected by positive matrix effects because they are less strongly adsorbed onto the liner surface. Organophosphates (*e.g.* chlorpyrifos, pirimiphos), organochlorides (*e.g.*, dicofol, captan), pyrethroids (*e.g.* fenvalerate, deltamethrin), azoles (*e.g.* tebuconazole, triadimefon), carbamates (*e.g.* carbaryl, pirimicarb), dinitroaniline derivatives (*e.g.* fluazinam, procymidone, trifluraline), amides (*e.g.* alachlor, butachlor), phenoxyacetic acid derivatives (*e.g.* 2,4-D-butylate, haloxyfop) and other compounds, such as piperonyl butoxide, chinomethionate, flutolanil, fluoroglycofen-ethyl, nitrofen, and hexazinone, are also typically sensitive to positive matrix effects [19]. Giacinti et al. [1] recently demonstrated negative matrix effects for flonicamid, chlorpyrifos, boscalid, fludioxonil, pirimicarb, and propargite in QuEChERS extracts of apple peel. They also demonstrated positive matrix effects for these compounds in flesh and fruit extracts. The analysis of pesticide residues by GC/MS^2 in apple peel results in higher target-analyte concentrations, at levels above the limits of detection (LOD), and a greater transfer of matrix analytes to extracts than analyses of the whole fruit.

The aim of this study was to investigate the composition of various QuEChERS extracts of peel/flesh/fruit, using an HPTLC method to determine the principal molecular markers of the apple matrix soluble in acetonitrile (sugars: fructose, glucose and sucrose, triterpenic acids, uvaol, paraffins C27-C29, phloridzin, primary fatty alcohols and polyphenols), *(i)* to identify the matrix compounds potentially responsible for the negative matrix effects in GC/MS^2, observed for flonicamid, chlorpyrifos, boscalid, fludioxonil, pirimicarb and propargite in peel extracts [1], and *(ii)* to propose a purification method for highly concentrated extracts for the limitation of these matrix effects.

2. Materials and methods

2.1. Target apple varieties

Three apple varieties (VARi) from among the most widely grown and popular in France were chosen for a previous study [1]. These varieties differ in terms of fruit color, composition, sensitivity to pests and ripening times. They were grown in various biotic and abiotic conditions and all trees were sprayed with commercial pesticide preparations according to the seasonal pest risk and the sensitivity of the variety concerned. The apples were collected from the orchard in August (VAR1), October (VAR2), or November (VAR3) and stored in a cold room at 4 °C until processing.

2.2. Selection of pesticides and matrix compounds

The matrix effects of six pesticides among the 11 selected by Giacinti et al. [1] were studied in GC/MS^2 here (Table 1). The matrix compounds likely to be present in the QuEChERS extracts of apples are also listed in Table 1.

2.3. Chemicals and materials

Chromasolv® for HPLC solvents were purchased from Sigma Aldrich (St Quentin Fallavier, France): ethyl acetate (≥97.7%), acetonitrile (≥99.9%), tetrahydrofuran THF (≥99.9%), hexane (≥97%) and isopropanol (99.9%). Chloroform HiPerSolv Chromanorm for HPLC and methanol id Reagent Ph. Eur. for HPLC-gradient grade were purchased from VWR (Strasbourg, France). Acetone Multisolvent® HPLC grade ACS ISO UV–vis Scharlau was purchased from Fischer (Illkirch, France).

Folin & Ciocalteu's phenol reagent 2N was purchased from Sigma (St Quentin Fallavier, France) and sodium carbonate Acros Organics was obtained from Fischer (Illkirch, France).

The Pestanal analytical standards and the matrix analytical standards (triterpenoids, primary fatty alcohols, paraffins, monosaccharides and polyphenols) were supplied by Sigma Aldrich (St Quentin Fallavier, France): boscalid (99.9%), captan (99.6%), chlorpyrifos (99.9%), dithianon (97.4%), flonicamid (91.9%), fludioxonil (99.9%), pirimicarb (98.5%), propargite (99.5%), pyraclostrobin (99.9%), thiacloprid (99.9%), thiamethoxam (99.7%), oleanolic acid (≥ 97%), ursolic acid (≥ 90%), uvaol (≥ 95%), 1-hexadecanol C16-OH ReagentPlus (99%), 1-octadecanol C18-OH ReagentPlus (99%), 1-eicosanol C20-OH (98%), 1-docosanol C22-OH (98%), 1-tetracosanol C24-OH (≥99%) and 1-hexacosanol C26-OH (≥97%), 1-octacosanol C28-OH (≥99%), 1-triacontanol C30-OH (≥98%), heptacosane C27 (≥98%), nonacosane C29 (≥98%), α-D-glucose (96%), D(−)-fructose (99%), sucrose (99.5%) and dihydrated phlorizin (≥98.5%).

The QuEChERS reagent (a mixture of $MgSO_4$, sodium chloride, disodium citrate and disodium hydrogen citrate; Q-Sep kit 26235), and a mixture of $MgSO_4$, primary secondary amine (PSA) and C18 (tubes 26221 + 26125), were obtained from Restek (Lisses, France).

2.4. Sample processing and preparation

The sampling procedure, extraction and purification by the QuECHERS method have been described in detail elsewhere [1]. In summary, the various samples (apple flesh, apple peel and whole apple) were ground and stored at −24 °C until extraction. Homogenized samples (10 g) were subjected to extraction in 10 mL of acetonitrile with the QuEChERS Restek Q-Sep™ salts kit. The entire supernatant (volumes ranged between 8.5-9.5 mL) was transferred to the Restek dSPE Q-Sep™ adsorbent kit (mix of one tube 26221–8 mL and two tubes 26125–1 mL each). Acetonitrile was removed by evaporation to dryness. The resulting dry extracts were then dissolved in 500 μL ethyl acetate for injection into the gas chromatograph. QuEChERS extracts were identified as follows: FRUITVAR1, 2 or 3; FLESHVAR1, 2 or 3 and PEELVAR1, 2 or 3, for the fruit, flesh and peel extracts of each apple variety, respectively.

2.5. Preparation of standards and calibration curves

2.5.1. Preparation of solvent-matched and matrix-matched pesticide standards for GC/MS^2 analysis

Pesticide standards were prepared as previously described [1]. Stocks were prepared at a concentration of about 100 ng μL^{-1} in ethyl acetate. Mixtures of standard stock solutions were diluted to give 80–8000 ng pesticide in 500 μL of ethyl acetate containing internal standards. Matrix-matched standards were obtained by spiking apple sample extracts from each variety.