

Original Research

THE NATURE OF THE MATRIX EFFECT IN GC/MS PESTICIDES ANALYSIS IN APPLES AND ITS RELATION TO EXTRACT DILUTION

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Abstract

The alteration in pesticides instrumental signal in complex samples analysis is defined as the matrix effect. The effect is a result of analytes and matrix components interaction within the injector, detector, or chromatography column and its level depends upon several factors such as the type of extraction solvent, clean-up, analyte concentration and chemical class, type and concentration of the matrix etc. The aim of this investigation was to assess the nature of the matrix effect in different matrices, by analysing different apple varieties and separately the peel and the flesh of a variety. Additionally, the possibility of the matrix effect minimization by dilution of sample extracts was investigated. Varietal differences in composition, as well as differences in the composition of peel and flesh, reflected in distinctive matrix effect for these matrices. The dilution seems to be promising approach for the matrix effect minimization. However, how much dilution is allowed depends on the desirable sensitivity of analysis.

Keywords: apple peel, apple flesh, solvent extraction, mass spectrometry.

INTRODUCTION

In complex samples analysis, alteration in pesticides instrumental signal is expected, since sample extracts usually contain great quantity of co-extractives which can influence analyte ionization and detector response. The alteration is defined as the “matrix effect” and its nature can be twofold: the matrix enhancement effects testify to the analyte signal enhancement compared to that in organic solvent; the matrix weakening effects testify to the opposite, that is analyte signal is much more suppressed than that in organic solvent. The effect is a result of analytes and matrix components interaction within the injector, detector, or chromatography column [1]. The level of the effect depends upon several factors such as the type of extraction solvent, clean-up, analyte concentration and chemical class, type and concentration of the matrix, chromatographic separation column and moreover, the structure of inlet, regarding the number and type of the activity points [2,3].

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Several procedures such as the modifications to sample clean-up [4], addition of internal labeled standards, calibration in the matrix or its combination [5], addition of analyte protectants [6] or sample dilution [7] are usually applied to minimize the matrix effect. To estimate the matrix effect two different procedures are usually applied. Single concentration level approach compares analyte signal in a certain matrix and in solvent, at individual concentration level. Multi-concentration level approach compares the slopes of calibration graphs for matrix matched standards against calibration standards in the solvent.

Apples are complex matrices consisting of many compounds including sugars (fructose, glucose, sucrose), organic acids, amino acids, phenolic compounds (chlorogenic acid, protocatechuic acid and hydroxycinnamic acids) and fatty acids from C₁₈ family [8,9]. This matrix is quite different among apple varieties and between peel and flesh within a variety. Main differences are expressed in acidity, sugar and pigment content, composition and content of phenolic compounds [10,11].

The aim of this investigation was to evaluate GC/MS analysis of several pesticides in apples by investigating the aspect of the matrix effect. The aim was to assess the nature of the matrix effect in different matrices, by analyzing different apple varieties and separately the peel and the flesh of a variety. Additionally, the possibility of the matrix effect minimization by dilution of sample extracts was investigated. Four pesticides, of which 3 are fungicides and 1 is insecticide, regularly used in apple crops protection were implemented into evaluation.

MATERIALS AND METHODS

Chemicals

Analytical pesticides standards were purchased from AccuStandard[®] (pyrimethanil and cyprodinil) and from Sigma-Aldrich[®] (trifloxystrobin and bifenthrin). Dibutyl adipate (DBA) was obtained from Sigma-Aldrich (St Louis, MO, USA). HPLC grade hexane was purchased from Fisher Scientific (USA).

Procedures

Analytical standards preparation

Stock solutions of 100 µg mL⁻¹ of individual standards were prepared in hexane and in methanol and were stored in a freezer. Stock standard mixtures of pesticides (working solutions) were prepared freshly each time in hexane by mixing appropriate quantities of the hexane stock solution and subsequent dilution, resulting in final pesticide concentrations: 1, 2.5, 5, 10, 25 and 50 µg mL⁻¹. Internal standard solution (ISTD) of 10 µg mL⁻¹ was prepared by dissolving DBA with hexane.

Sample preparation

Granny Smith, Demi Rouge and Golden Delicious apples were randomly chosen at the open-air market (JP 'Tržnica', city of Niš, Serbia). Apple fruits were free of visible damage and pests. Two hundred grams of sample taken from five individual units of selected apple varieties were peeled. Peel and flesh were separately chopped and homogenized in food chopper. Analytical sub portions of 20 g were transferred to a glass container. Afterwards, an extraction with 20 mL of hexane was performed. Obtained extracts were transferred to glass

cone tubes and centrifuged (10 min at 3,800 rpm). The upper hexane layer was separated from the solids–water dispersion and used for further experiments.

Instrumental analysis

The gas chromatographic analysis was performed on Agilent 6890 gas chromatograph equipped with 5973 Mass Selective Detector and 7683 autosampler and SGE 25QC2/BPX5 0.25 capillary column (25 m×0.22 mm×0.25 mm, non-polar). The mass spectra were recorded under an electron impact ionization (EI) voltage of 70 eV. The gas chromatograph was operated in split less injection mode. The oven temperature was programed from 90 °C to 280 °C (4 min) at 20°C/min rate; post run: 300 °C (2 min). Helium was the carrier gas with constant flow rate of 1.0 mLmin⁻¹. The MSD was used in the single ion-monitoring mode (SIM) at following *m/z*: 198 (pyrimethanil), 224 (cyprodinil), 116, 207, 131 (trifloxystrobin), 181, 166, 165 (bifenthrin) and 185 (DBA).

Matrix effect evaluation

The matrix effect (ME) was calculated by using the Eq. $ME (\%) = \frac{(S_1 - S_2)}{S_2} \times 100$, where *S*₁ is the slope of the calibration curve obtained by diluting of standards in organic extracts and *S*₂ is the slope of the curve obtained in organic solvent. Depending on the value of this percentage, matrix effects can be classified into different categories: no matrix effect (from -20 to +20 %), a weak matrix effect (from ±20 to ±50 %) and a strong matrix effect (>±50 %). Four solution sets containing the four pesticides at concentrations 0.10, 0.25, 0.50, 1.00, 2.50 and 5.00 µg mL⁻¹ each were prepared by mixing the reagents volumes as presented in Table 1.

Table 1 Composition of solution sets prepared in fruit matrix with different dilution factors

Set No.	ISTD vol., µL	Pesticide stock vol., µL	Apple extract vol. (AE _v), µL	Solvent vol., µL	Final vol. (F _v), µL	Dilution factor (DF=F _v /AE _v)
1	100	100	0	800	1000	/
2	100	100	800	0	1000	1.25
3	100	100	200	600	1000	5.00
4	100	100	100	700	1000	10.00

RESULTS AND DISCUSSION

Data acquisition for each apple variety was accomplished within one batch. The sequence of 42 injections was performed from lower to higher pesticides concentrations within single set, according to the following pattern: solvent-based, flesh-based and peel-based samples with decreasing dilution factor. Analytes responses were normalized to DBA as internal standard. ME was estimated in relation to the matrix type and matrix concentration.

The matrix effect vs. matrix type

The matrix effect was evaluated in six different matrices, that is, separately in peel and flesh of three apple varieties. As can be seen in figure 1 for most matrices there is no matrix effect, i.e., the matrix effect is in the range of ±20 %.

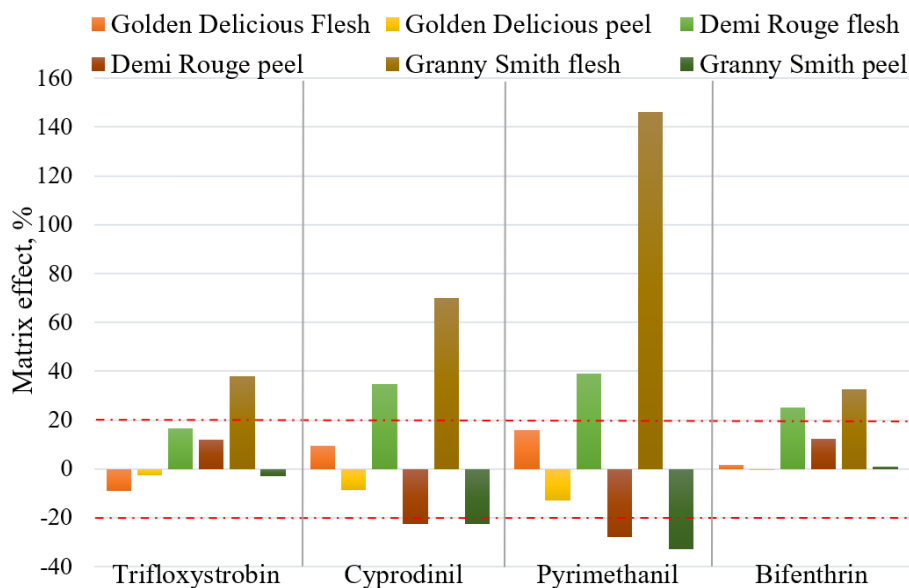


Figure 1 Matrix effect in peel and flesh extracts (DF=1.25) of tested apple varieties: dotted lines indicate range considered as no matrix effect

The matrix effect for all tested pesticides was mostly pronounced in Granny Smith extracts. This variety is known for much higher pigment content in both peel and flesh than other apple varieties such as Royal Gala, Golden Delicious [12] and much higher acid content. The positive matrix effect for all pesticides was observed in Granny Smith flesh extract, while in peel extract the effect was negative. Different nature of the ME in these matrices might be explained by the relative abundance of waxes present in these two. In chromatograms of peel extracts of tested varieties (not shown here), a peak of a compound eluting at 12.37 minutes can be observed. Mass spectra of the compounds contain mass fragments that suggest long-chain aliphatic structure, that is suggest the compound is from the group of waxes. This compound was most abundant in peel extract of Granny Smith, while in peel extracts of Golden Delicious and Demi Rouge variety was 5-times and 1.5-times less abundant, respectively. Comparison of chromatograms of peel and flesh extract of Granny Smith variety (not shown here) showed 42-times higher presence of this compound in peel that matches the fact that apple waxes prevail in peel.

The positive matrix effect observed in flesh extracts can be explained by phenomena called “matrix-induced chromatographic response enhancement effect”. The phenomena indicate that the co-extractives “compete” with the analytes for the injector active sites, causing transfer of a larger amount of analyte to the column. The inversion of the matrix effect in peel extract (with exception of bifenthrin) was possibly due to the evaporation and re-condensation in the injector of much larger amounts of waxes, which fixate analytes in wax matrix and/or blocks entrance to the column and analyte loading.

The matrix effect vs. matrix concentration

The matrix concentration is the mass of the extracted test portion divided by the volume of the extraction solvent. Here, the concentration of obtained extract was 1 gmL^{-1} and after the dilution decreased to 0.80 (DF=1.25) , 0.20 (DF=5) and $0.10 \text{ gmL}^{-1} \text{ (DF=10)}$.

Figure 2 shows the ME behaviour with dilution for tested pesticides in all matrices; additional discussion is made on dilution graphs for pesticides with the most pronounced ME (Figure 1).

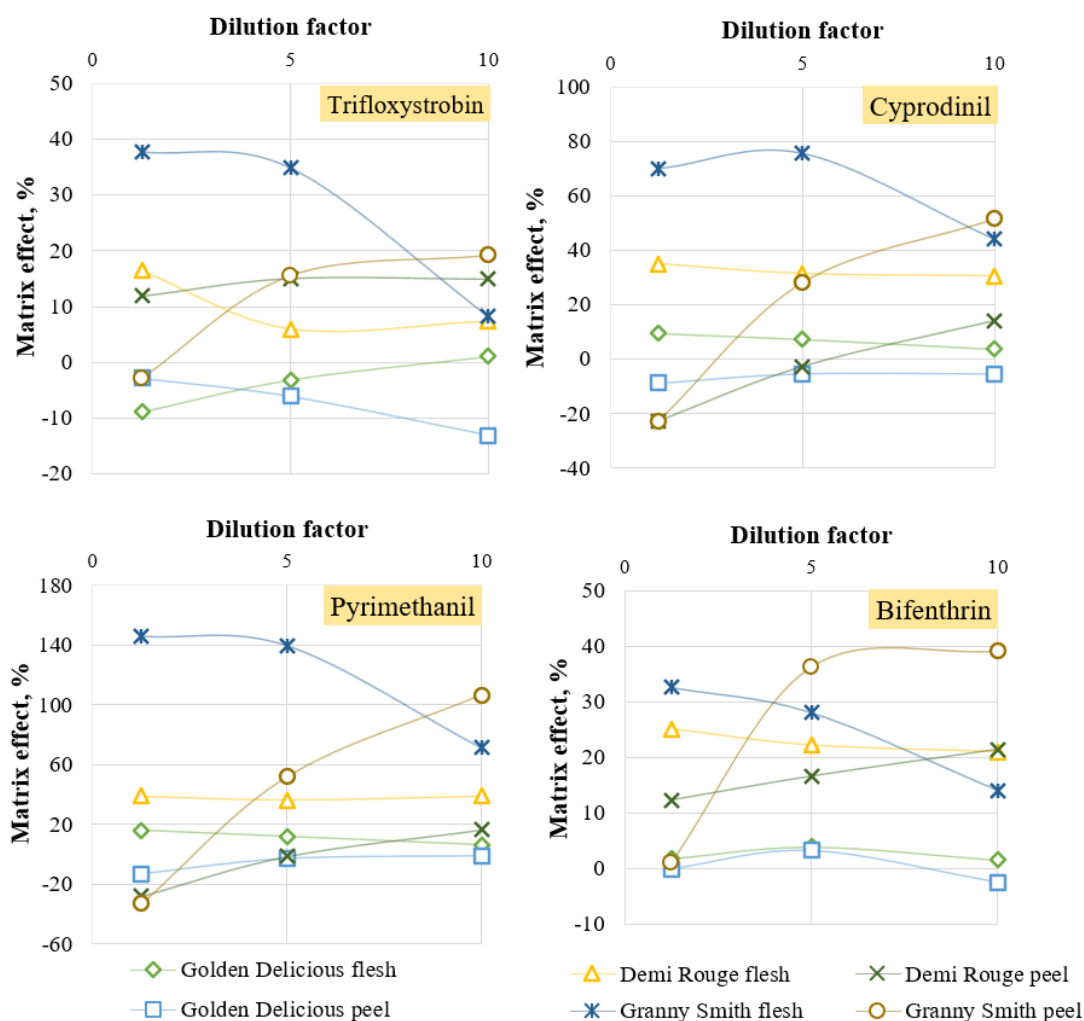


Figure 2 The matrix effect vs. extract dilution factor

The highest ME for trifloxystrobin (37.80 %) was observed in Granny Smith flesh extract and after 8-fold matrix dilution decreased to 8.20 % (4.6 times). For cyprodinil, the ME was most pronounced in Demi Rouge (34.90 %) and Granny Smith flesh extract (70.10 %). Final dilution resulted in 1.13- and 1.6-times lower ME, respectively. For pyrimethanil the ME in Demi Rouge flesh extract remained at about 40 % after the dilution, but in Golden Delicious peel and Granny Smith flesh extract decreased 14.3 and 2 times, respectively.

For the Granny Smith peel extract matrix effect – dilution graphs were different. The initial ME for all pesticides in this matrix, with exception of bifenthrin (0.98%), was negative, nevertheless with dilution moved towards positive values making an inversion. Inversion for trifloxystrobin ranged from -2.80 % (DF=1.25) to 19.20 % (DF=10). Increase in ME values from -22.50 to 51.50 % (cyprodinil) and from -33.00 to 106.40 % (pyrimethanil) made the inversion even higher. Negligible ME value for bifenthrin (0.98 %) increased with 8-fold matrix dilution up to 39.20 % (40 times). The manifestation of inversion with dilution is possibly related to reduction in the concentration of waxes.

CONCLUSION

In Golden Delicious variety, both in peel and flesh, the matrix effect for all tested analytes was negligible. Noticeable matrix effect for all analytes was observed for Demi Rouge and Granny Smith variety. The matrix effect in flesh matrices was positive. However, in peel matrices it was negative. In both cases, the matrix effect was more pronounced in Granny Smith variety.

Dilution of sample extracts in most cases led to minimization of the ME. In flesh matrices, the dilution resulted in less positive matrix effect values, in peel matrices it resulted in less negative values. However, in Granny Smith peel, the dilution resulted in inverted matrix effect. The highest difference was observed for pyrimethanil, where the matrix effect changed from – 33 % to 106 % after the dilution.

Matrix type has some influence on the expressed matrix effect. Varietal differences in composition, as well as differences in the composition of peel and flesh, reflected in distinctive matrix effect for these matrices. The dilution seems to be promising approach for the matrix effect minimization. However, how much dilution is allowed depends on the desirable sensitivity of analysis.

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