Evaluation of mixed injection solvents in gas chromatography as a measure to avoid solvent change during sample treatment

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1. Aim and scope

This document reports the effect of different injection solvents in gas chromatography (ethyl acetate, acetonitrile and a mixture of both solvents) on the pesticide analysis in tomato, orange and avocado matrices.

2. Short description

The vast majority of multiresidue extraction methodologies employ acetonitrile as the solvent. However, this solvent has not been typically considered as the optimum one for the injection in gas chromatography (GC), which results in the need of employing a solvent change step prior to the sample analysis. The solvent change might result in the loss of volatile analytes (and therefore an underestimation of their concentration in the samples) and it also entails a significant amount of time and laboratory work.

However, new chromatographic GC designs are currently capable of resisting the higher expansion volume of acetonitrile (compared to other organic solvents) and, therefore, it can be safely employed for pesticide residue analysis without detriment to the analytical instrument. The use of the extraction solvent for injection in GC (alone or in combination with other solvents) would avoid the need of an evaporation step prior to the analysis, thus increasing the recoveries of the most volatile analytes.

3. Experimental

3.1. Sample treatment

Tomato, orange and avocado samples were extracted using the QuEChERS method. For each matrix, one extraction of blank sample and one extraction of a sample previously spiked with a mixture of 193 GC-amenable compounds at a concentration of 10 µg/kg was performed. The general experimental procedure was as follows:

1. Weigh 10 g of sample in a 50-mL PTFE centrifuge tube.
2. Add 10 mL acetonitrile.
3. Shake the sample in an axial agitator (Agitax) for 6 minutes.
4. Add 4 g anhydrous magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogencitrate sesquihydrate and shake manually (3 sec).
5. Shake the sample in an axial agitator (Agitax) for 6 minutes.
6. Centrifuge the tubes at 4000 rpm for 5 min.
7. Transfer 5 mL of the supernatant to a 15-mL PFTE centrifuge tube containing
    a. Tomato and orange matrix: 750 mg anhydrous magnesium sulphate and 125 mg PSA (primary secondary amine).
    b. Avocado matrix: 750 mg anhydrous magnesium sulphate and 175 mg Z-Sep.
8. Vortex for 30 sec.
9. Centrifuge the tubes at 4000 rpm for 5 min.
10. Transfer the supernatant to a 4-mL vial and, only for tomato and orange, add 10 µL of a formic acid solution in acetonitrile (5 % volume) per mL of extract.

For the injection vial preparation, different procedures were followed according to the experiment. In all cases, a calibration curve from 2 to 300 µg/kg was employed for quantification. Additionally, 2 µL of lindane-D6 were added as an injection standard to the final vials.

- Injection in acetonitrile: 50 µL of each blank extract were evaporated under a gentle N_2 current and reconstituted with a standard solution in acetonitrile. The recovery samples were directly injected with no evaporation.

- Injection in ethyl acetate: 50 µL of each blank extract and each recovery sample were evaporated under a gentle N_2 current and reconstituted with a standard solution in ethyl acetate.
- Injection with mixed solvents (acetonitrile-ethyl acetate, 1:1): 50 µL of a standard solution in ethyl acetate were added to 50 µL of each blank extract. The recovery samples were diluted with ethyl acetate (50 µL ethyl acetate in 50 µL extract).

3.2. Analysis by GC-QqQ-MS/MS

All samples were analyzed by an Intuvo 9000 GC Instrument coupled to an 7010B GC/MS Triple Quad (Agilent Technologies). The analytical parameters are detailed below.

- Column: 2 Planar columns HP-5MS UI (15 m long × 0.25 mm i.d. × 0.25 µm film thickness)
- Injection mode: splitless, 1 µL (2 µL in the mixed solvent injection).
- Ultra-inert inlet liner with glass wool frit from Agilent
- Injector temperature: 70 °C (0.1 min), then up to 325 °C at 800 °C/min (hold for 5 min).
- Carrier gas: Helium at constant flow = 1.28 mL/min column 1, 1.48 mL/min column 2.
- Oven temperature: 60 °C for 0.5 min, up to 170 °C (80 °C/min) and up to 310 °C (20 °C/min).
- Ionization mode: electron impact ionization.
- Temperature of the transfer line: 280 °C.
- Temperature of ion source: 280 °C.
- Collision gas: nitrogen.
- Solvent delay: 2.6 minutes.

4. Results and discussion

4.1. Recoveries

The recoveries of 193 GC-amenable pesticide residues from a sample spiked at 10 µg/kg were evaluated for the three matrices included in the present study (tomato, orange and avocado). **Figure 1** shows, in each case, the number of compounds with a recovery lower than 80 % for the injection in ethyl acetate, acetonitrile and a mixture ethyl acetate-acetonitrile (1:1). No compounds showed a recovery higher than 120 %, –i.e., the remaining compounds had in all cases a recovery in the range of 80-120 %. As can be seen, the use of ethyl acetate as the only injection solvent resulted in a reduced recovery for 20 compounds in tomato and avocado whereas, in orange, the effect was negligible.

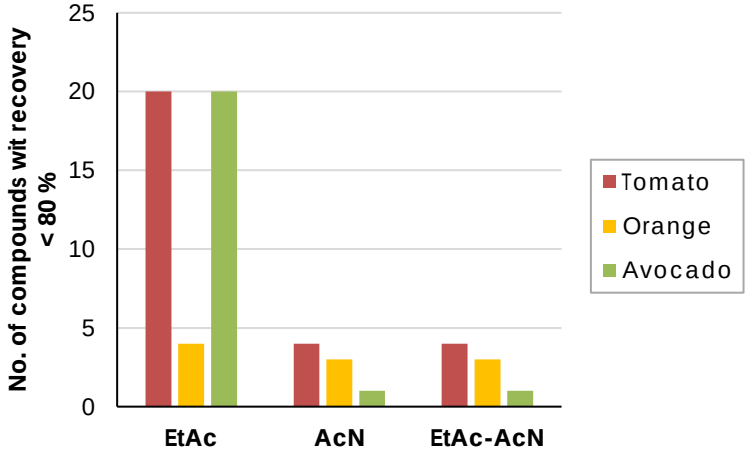


Figure 1. Number of compounds with recovery lower than 80 %, distributed according to the injection solvent, for three vegetable matrices (tomato, orang, avocado)

These results show that the evaporation step needed for the solvent change in the injection of ethyl acetate might lead to the partial loss of certain analytes (up to 10 % of compounds in the present study). However, the extent of this effect is difficult to foresee as can be seen in **Figure 1**, the compounds in orange matrix were not affected by the evaporation step, and therefore the matrix components (such as some essential oils) might have a role in preventing the loss of analytes. Additionally, the compounds which are more intensely affected by the evaporation step are not necessarily the most volatile ones (**Table 1**): in some case such as biphenyl, the high vapour pressure (1238 mPa) explains the low recoveries in tomato and avocado when the sample treatment includes an evaporation step. However, other compounds with affected recovery in both matrices (such as fenhexamid) can be considered as non-volatile and, in the majority of cases, analytes were affected differently by evaporation losses depending on the matrix. Therefore, mechanisms of analyte loss during sample evaporation are not clear and are difficult to anticipate. The use of direct acetonitrile injection or a dilution with another solvent might help enhance the results obtained for compounds with typically low recoveries in GC.
Three compounds showed a low recovery in all experiments in tomato and orange regardless of the injection solvent (chlorothalonil, dichlofluanid and tolylfluanid), due to strong interactions with the PSA salt employed in the clean-up of the extraction procedure. In avocado, this salt is replaced by Z-Sep, resulting in recoveries in the range 80-120 % for these three compounds.

Table 1. Recovery of compounds affected by analyte loss (%)

Tomato				Orange				Avocado			
	EtAc	AcN	Mix		EtAc	AcN	Mix		EtAc	AcN	Mix
2,4'-DDE	71	89	78	Chinomethionate	70	87	86	2,4'-DDE	65	78	79
2-Phenylphenol	73	87	96	Chlorothalonil	2	39	15	2,4'-DDT	71	88	87
Biphenyl	41	101	82	Dichlofluanid	9	37	17	4,4'-DDE	72	81	80
Bupirimate	74	97	96	Tolylfluanid	18	22	22	Biphenyl	55	94	101
Dimethipin	56	105	93					Butylate	70	89	93
Diphenylamine	73	109	79					Carbophenothion	71	81	105
Dodemorph	72	95	102					Chinomethionate	53	90	88
Fenamidone	63	98	96					Dieldrin	67	88	91
Fenarimol	68	100	93					Dodemorph	69	95	115
Fenhexamid	70	89	82					Fenazaquin	60	76	91
HCB	66	90	80					Fenhexamid	59	122	103
Ofurace	67	110	95					Flutriafol	66	114	104
Oxadixyl	59	103	93					Metalaxyl	65	104	107
Pirimicarb	62	99	97					Mevinphos	71	109	113
Propiconazole	61	82	88					Phenothrin	50	96	95
Pyrimethanil	72	102	84					Quinoxyfen	72	84	82
Chinomethionate	44	60	41					Tecnazene	73	90	94
Chlorothalonil	1	28	0					Thiobencarb	65	91	90
Dichlofluanid	23	29	8					Triallate	70	88	89
Tolylfluanid	44	44	25					HCB	50	56	62

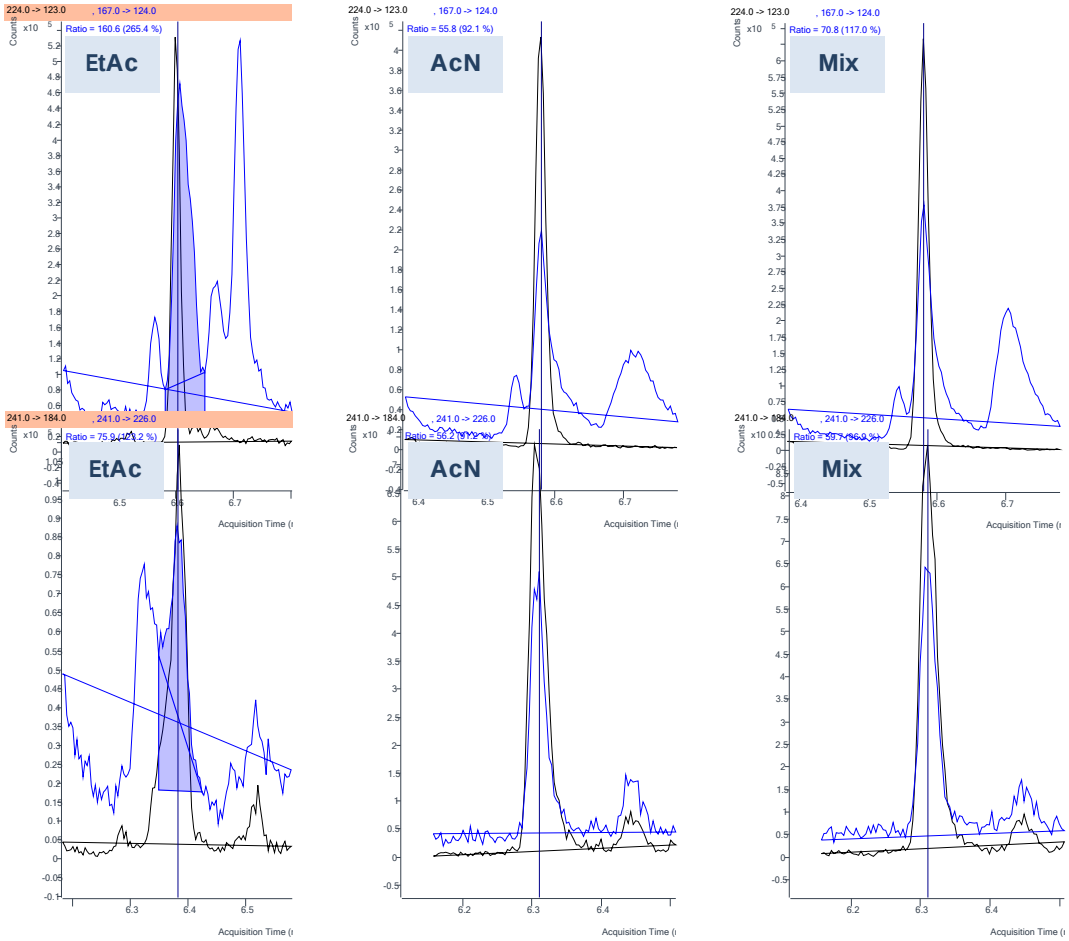
4.2. Interferences

In general, the use of a certain injection solvent did not result in any changes related to the presence of matrix components interfering with the analytes. However, in some specific

cases, the evaporation step (or the direct addition of ethyl acetate to the acetonitrile extract) lead to the presence of chromatographic signals that were not present in the vials containing only acetonitrile. These interferences could even lead to identification and/or quantitation issues, for example in the case of dichlofluanid (**Figure 2a**) or prometryn (**Figure 2b**) in avocado matrix.

The opposite happened in other occasions: the evaporation step removed matrix interferences that affected the identification/quantitation of certain analytes, such as acrinathrin in avocado (**Figure 2c**). In this case, the acetonitrile extract showed an intense interference in one of the acrinathrin transitions that made it impossible to identify acrinathrin at concentrations below 300 µg/kg. The addition of ethyl acetate diluted this interference, but still affected the correct identification of the analyte, whereas the evaporation step removed completely the interfering signal. The removal and appearance of interferences by different injection solvents was more intense in avocado than in any of the other samples due to the larger amount of matrix components

a) Dichlofluanid in avocado (50 µg/kg)



b) Prometryn in avocado (10 µg/kg)

c) Acrinathrin in avocado (50 µg/kg)

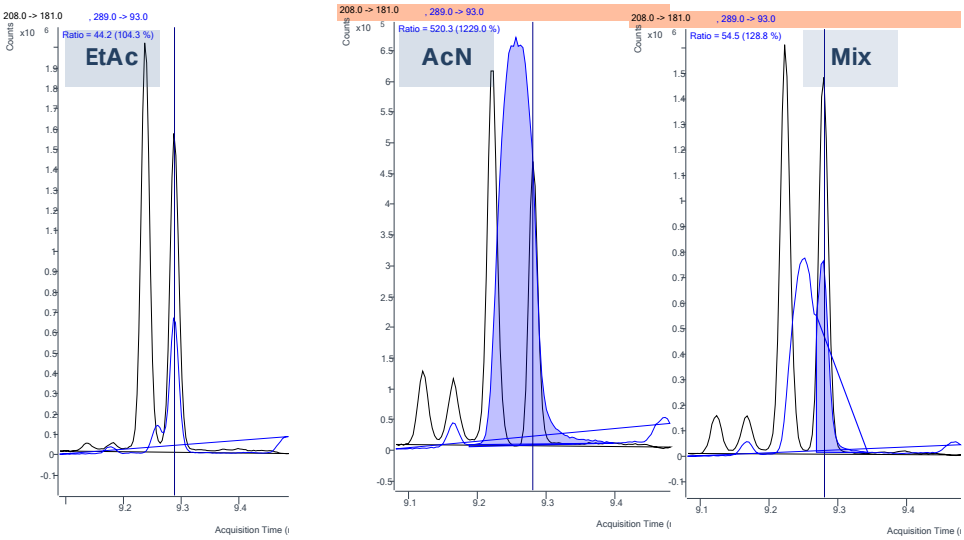


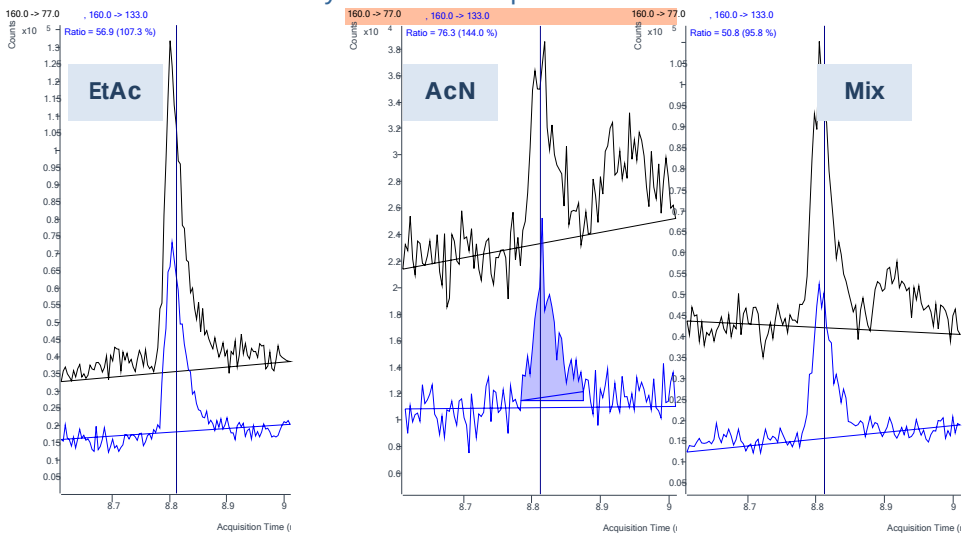
Figure 2. Effect of different injection solvent in matrix interferences of selected pesticides

4.3. Signal intensity

The experiments were performed on different days and with a different liner usage. Therefore, the relative areas of each injection should not be compared with other samples. Nevertheless, in the vast majority of cases, the possible effects of a change in the injection solvent did not affect the limits of detection of the analytes –i.e., the sensitivity of the compounds studied were similar regardless of the solvent employed.

In some cases, however, significant differences could be observed for a certain compound, at a given concentration and in the same matrix depending on the injection solvent employed. In these instances (less than 5 % of the analytes), the selection of a certain solvent or solvent mixture allowed to identify a compound affected by low sensitivity in other experiments, such as phosmet in orange (**Figure 3a**) or dodemorph in avocado (**Figure 3b**). The effect in these two examples was the opposite: whereas the presence of ethyl acetate (alone or in mixture) resulted in increased sensitivity for phosmet, the use of this solvent exclusively resulted in a loss of sensitivity for dodemorph.

a) Phosmet in orange (5 µg/kg)



b) Dodemorph in avocado (50 µg/kg)

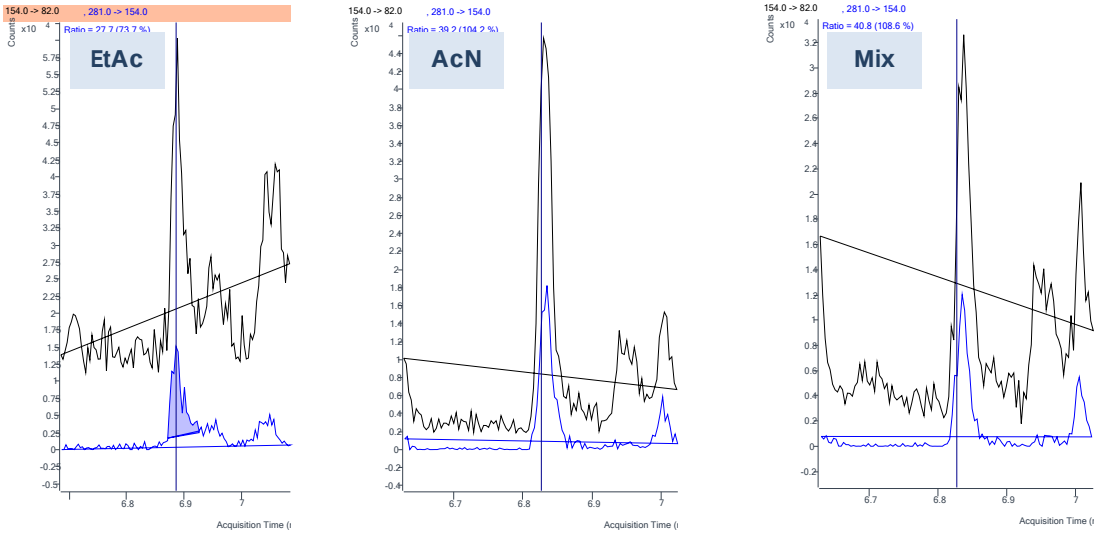


Figure 3. Effect of different injection solvent in the sensitivity of selected pesticides

The fact that the use of ethyl acetate as the only injection solvent resulted in some cases in an increase of the sensitivity (as happened with phosmet in orange) shows that the evaporation step might not be the only cause of sensitivity losses, and that certain solvent interactions with the sample components or the analytical instrument might also be related to this phenomenon. The sensitivity losses/enhancements do not seem to be affected by the matrix –i.e., the limits of detection of phosmet and dodemorph in the remaining matrices were similar regardless of the injection solvent employed).

Additionally, as can be seen on **Figure 3**, the use of the mixed injection solvents allowed in both cases to obtain acceptable sensitivity, only slightly lower than the one obtained with the optimum solvent. The same was observed in other compounds affected by different sensitivities according to the injection solvent: the signal intensity obtained with the mixture acetonitrile-ethyl acetate were comparable to those obtained with the optimum solvent in each case. Therefore, the addition of a different injection solvent to the extracts prior to analysis might help in some specific compounds affected by poor sensitivity in certain matrices.

5. Conclusions

The use of acetonitrile as the injection solvent in GC leads to important savings in terms of time and laboratory work, as it avoids the need of evaporating the samples during the solvent change. This may also result in higher recoveries of some compounds which might be affected during the process.

The use of a certain injection solvent might also lead to important differences in terms of sensitivity and presence of interferences in a low percentage of analytes. This effect is largely unpredictable, as it can affect the compounds only in some matrices and it is not always related to the evaporation step. However, even if the mechanisms of these effects are not known, the use of a different injection solvent might help in some specific cases where an analyte shows low sensitivity and/or coeluting interferences. This solution is easier to implement by routine laboratories than the search for alternative transitions (which are not always useful or available) or sample treatment methods, as it only implies testing a different injection solvent.

A similar approach involves using a mixture of solvents including the extraction solvent (acetonitrile) and the one typically employed for GC analysis (for example, ethyl acetate) at a constant proportion. With this strategy, very good results in terms of sensitivity and interferences are in most cases achieved, and it also avoid the evaporation step. An additional advantage of the use of mixed solvents of the injection is that the blank extracts

do not need to be evaporated for matrix-matched calibration analyses: the blanks are directly diluted with the standard solutions in the desired solvent, thus avoiding any possible matrix effects derived from the loss of matrix components during the evaporation step. The time and work savings are also higher than those obtained with the acetonitrile as the only solvent (any extract needs to be evaporated). With this method, the injection solvent should be increased so as to compensate the dilution effect (for example, inject 2 µL instead of 1 µL for a mixture of solvents 1:1). Acetonitrile should not be employed for dilution in this case: the proportion of water impurities in this solvent is higher than in other organic solvents with lower polarity, and the injection of larger volumes might affect the instrument performance.

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