

Task for a laboratory
Determination of concentration of imazalil and thiabendazole in tangerines by liquid chromatography mass spectrometry.

The quality of the results should comply with the requirements in the EU directives 93/58/EEC and 00/42/EEC/ on pesticide residues analysis

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PART I. Description of the analytical procedure

The objective of this analysis is post-registration control and monitoring of imazalil and thiabendazole (polar pesticides) based on Maximum Residue Limits (MRLs) set by the EU Directives 93/58/EEC and 00/42/EEC.

Sample preparation procedure is modified AOAC official method 985.22. Analysis was carried out on an LC-MS system using a self-developed chromatographic method.

1. Scope

A modified AOAC 985.22 sample preparation procedure was used, to suite LC-MS-MS analysis. The analysis was carried out using liquid chromatographic separation and atmospheric pressure electrospray ionisation with tandem mass spectrometric detection (AP-ESI-LC-MS-MS).

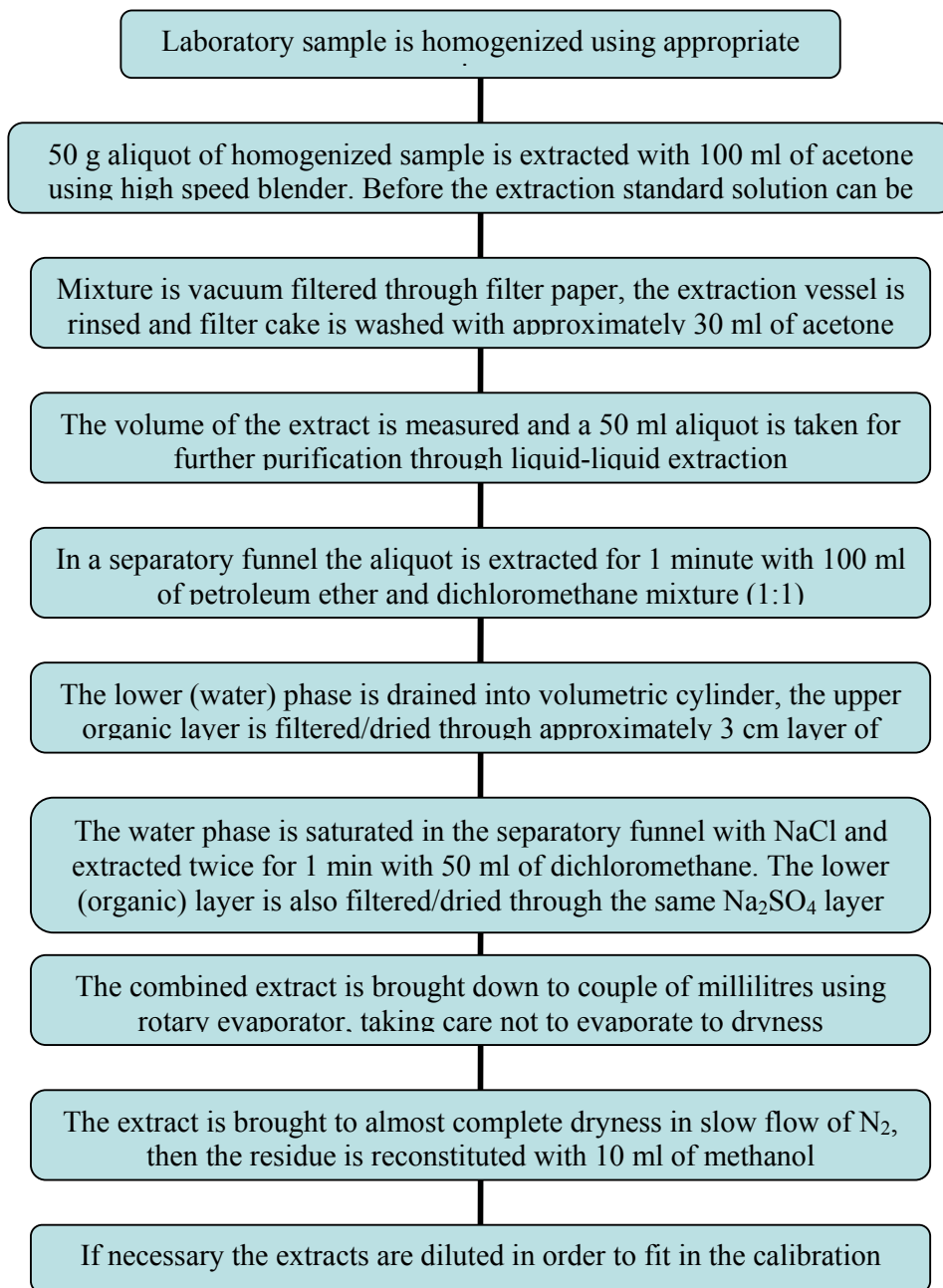
Sample preparation procedure is suitable for berries, fruits and vegetables containing less than 2 % of fat and more than 70 % of water (water can be added if its content is insufficient). All in all 14 residues of polar pesticides are analysed in this analytical procedure. In this example, only two of them will be discussed in detail: imazalil and thiabendazole.

2. Principle

An aliquot of homogenized sample is extracted with acetone and filtered. A portion of the extract is subjected to liquid-liquid clean-up step consisting of one extraction with petroleum ether (40 – 60° C)-dichloromethane mixture and two extractions with dichloromethane from saturated NaCl solution. Organic extracts are dried using anhydrous sodium sulphate. Then the solvent is exchanged to methanol through evaporation and dissolving. The obtained extract is analysed using LC-MS.

In the LC-MS system the samples are separated chromatographically using acetonitrile (B) and buffer solution (1mM ammonium acetate, 0.1 % formic acid) (A) as eluent. The gradient program was as follows: B 20 → 100 % 15 min, B 100 % 17 min at 0.8 ml/min. Analysed substances were then ionized through the ESI procedure and analysed with the ion-trap MS using fragmentation of quasimolecular ions ($[M+H]^+$). Calibration graphs are compiled using peak areas of certain characteristic fragment ions on different concentrations.

The result is calculated in mg of pesticide residue per kg of sample or ppm.



3. Interferences

ESI procedure is dependent on ionization efficiencies of the species. The ionisation efficiencies can be affected by co-eluting polar matrix components. Thus sample preparation and in most part chromatographic separation should be able to cope with these circumstances. For this reason retention times should be reasonably large compared to the dead volume of the column. In addition suitable buffer solution should be used.

The best ways to correct these effects are using matrix matched calibration, standard addition or labelled internal standards. However these means will make the analysis procedure significantly more complex and are not used in the current procedure.

4. Reagents

1000 mg/kg individual pesticide standard solutions

Prepare pesticide standard solutions by dissolving 10 mg of substance in 10 g of acetone (1000 mg/kg) in 15 ml vials.

20 mg/kg combined pesticide standard solution

Weigh 0.2 g of each individual pesticide standard solution into 15 ml vial and fill it up with methanol (9.6 g in the case of two components)

Calibration solutions

Using the 20 mg/kg standard solution and other dilutions the calibration solutions can be prepared in methanol. Suitable number of solutions should be prepared in the range of 5 – 0.003 mg/kg.

Solvents/eluent:

Gradient grade methanol, ultra pure water (Milli-Q or bottle), ammonium acetate and formic acid (suitable for LC-MS buffer), petrol ether, dichloromethane and acetone (for residue analysis or GC/HPLC grade if suitability checked)

Other:

NaCl, MgSO₄ pure for pesticide analysis (e.g. heated before use)

5. Sampling and pre-treatment

Sampling shall be carried out in accordance with European Commission Directive 2002/63/EC. While getting a laboratory/analytical sample one has to obtain homogenous and representative sample, also a great care has to be taken in order to avoid cross-contamination before or during or after sample preparation. Standard solutions should be kept separate from samples.

6. Calculation

The residue content C in the sample is found according to the following equation.

C_c is found from the calibration graph.

$$C = \frac{C_c * V_{10} * \rho * V_e}{V_{50} * m}$$

C	concentration of extractable pesticide in sample (mg of pesticide per kg of sample) [mg/kg]
C_c	concentration of extractable pesticide in analysed extract [mg/kg]
V_{10}	the volume of final extract in methanol [ml]
ρ	density of methanol (extract) [g/ml]
V_e	the full volume of acetone extract [ml]
V_{50}	the volume of acetone extract to be purified [ml]
m	mass of homogenised sample to be extracted [g]

7. Results

Calculations are performed using calibration graph and the model equation given above. Obtained results are compared against MRLs set by EU Council – 5 mg/kg for both pesticides. The samples at or over MRL must be reanalysed and/or otherwise confirmed.

PART II. The customer's requirement concerning quality of the measurement result

The laboratory should provide at least the following LODs for pesticide residues:

- ♪ Imazalil 0.02 mg/kg (for citrus) (93/58/EEC)
- ♪ Thiabendazole 0.05 mg/kg (for citrus) (00/42/EC)

Extract from the EU Quality Control Procedures for Pesticide Residues Analysis, SANCO/10232/2006

58. The method must be tested to assess for sensitivity, mean recovery (as a measure of trueness or bias) and precision. This effectively means that spiked recovery experiments to check the accuracy of the method should be undertaken. A minimum of 5 replicates is required

Mean recovery range should be within 70 – 110 %. In that case no recovery correction is performed.

Exceptionally, where recovery is low but consistent (i.e. demonstrating good precision) and the basis for this is well established (e.g. due to pesticide distribution in partition), a mean recovery below 70% may be acceptable. However, a more accurate method should be used, if practicable.

In the case of low recovery one has to take it into account when making decisions at or above MRL.

78. EI-MS or MS/MS, performed with acquisition of spectra, may provide good evidence of identity and quantity in many cases. In other cases, as with mass spectra produced by other processes (e.g. CI, API) that can be too simple for absolute confirmation of identity, further evidence may be required. If the isotope ratio of the ion(s), or the chromatographic profile of isomers of the analyte, is highly characteristic it may provide sufficient evidence. Otherwise, the evidence may be sought using:

- (i) a different chromatographic separation system;
- (ii) a different ionisation technique;
- (iii) MS/MS;
- (iv) medium/high resolution MS; or
- (v) inducing “in-source” fragmentation in LC-MS.

Table 3 Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS ⁿ , LC-MS, LC-MS ⁿ (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10%	± 50 %	± 50 %

PART III. Validation of the measurement procedure – relevant equations and measurement data

Equations

$$R = \frac{C_{\text{exp}}}{C_{\text{theor}}} * 100\%$$

$$STDEV = \sqrt{\frac{n \sum x^2 - (\sum x)^2}{n(n-1)}}$$

$$AVERAGE = \frac{\sum x}{n}$$

$$RSD = \frac{STDEV}{AVERAGE} * 100\%$$

R	recovery of the method [%]
C_{exp}	experimentally measured concentration of the pesticide residue in the sample, in the recovery studies the pesticide is spiked into the sample homogenate [mg/kg]
C_{theor}	theoretically calculated concentration of the pesticide residues in the spiked sample [mg/kg]
n	the number of data points in the set
x	Individual data points (in our case x denotes R) in the set
STDEV	standard deviation [%]
AVERAGE	average value of the data set [%]
RSD	relative standard deviation [%]

Measurement data

Imazalil			Thiabendazole			Imazalil	Thiabendazole	
C_{exp} (mg/kg)	C_{theor} (mg/kg)	R (%)	C_{exp} (mg/kg)	C_{theor} (mg/kg)	R (%)	Peak area	Peak area	
0.06427	0.05597		0.03120	0.04244		3996669	300802	
0.07516	0.05871		0.03281	0.04452		3459066	281164	
0.04812	0.05821		0.03181	0.04413		3838651	230775	
0.10238	0.07342		0.04095	0.05567		3727188	274366	
0.04201	0.06088		0.03400	0.04616		3414893	296724	
0.05741	0.06241		0.03331	0.04732		3553740	258916	
AVERAGE recovery			AVERAGE recovery					AVERAGE concentration
STDEV of recovery			STDEV of recovery					STDEV of concentration
RSD of recovery ($u_{\text{rel_rec}}$)			RSD of recovery ($u_{\text{rel_rec}}$)					RSD of concentration ($u_{\text{rel_meth}}$)

@* The recovery determinations were carried out two per day on three consecutive days.

PART IV. Measurement uncertainty of the result – relevant equations and measurement data

Equations

$$u_c = \sqrt{u_{\text{sys}}^2 + u_{\text{rnd}}^2}$$

$$u_{\text{rnd}} = \frac{\sqrt{u_{\text{rel_rec}}^2 + u_{\text{rel_meth}}^2}}{100\%} * c$$

$$d = c - c_{\text{ref}}$$

$$u_{\text{ref}} = \frac{s}{\sqrt{n_1}}$$

$$u_{\text{dev}} = \sqrt{\frac{d^2}{n}}$$

$$u_{\text{sys}} = \sqrt{u_{\text{ref}}^2 + u_{\text{dev}}^2}$$

u_c	standard uncertainty of concentration of pesticide [mg/kg]
u_{sys}	systematic component of uncertainty [mg/kg]
u_{rnd}	random component of uncertainty [mg/kg]
$u_{\text{rel_rec}}$	relative uncertainty of recovery [mg/kg]
$u_{\text{rel_meth}}$	relative uncertainty of analysis method [mg/kg]
C	pesticide concentration in standard sample as obtained with the measurement procedure [mg/kg]
d	difference in concentration between our laboratory and reference value (laboratory bias) [mg/kg]
c_{ref}	reference concentration of pesticide in the reference sample [mg/kg]
S	the standard deviation of the results of the participants of the interlaboratory comparison [mg/kg]
n_1	the number of laboratories who took part in interlaboratory comparison (ILC)
N	number of completed ILCs

Measurement data

	Imazalil	Thiabendazol e	Comments
$u_{\text{rel_rec}}$	27 %	2 %	The relative standard deviation of recovery calculated from parallel measurement results (two measurements per day on three consecutive days)
$u_{\text{rel_meth}}$	10 %	6 %	The relative standard deviation of results obtained for the same solution from repeated injections of the same solution
c	1.3350 mg/kg	3.5230 mg/kg	
c_{ref}	1.2975 mg/kg	3.2863 mg/kg	consensus value of interlaboratory comparison measurement
s	0.0530 mg/kg	0.5571 mg/kg	
n_1	2	3	
n	1	1	