

Determination of Polar Pesticides by Liquid Chromatography Mass Spectrometry

Scope of the presentation

- The Customer's Requirements
- The Analytical Procedure
 - Sample preparation
 - LC-MS analysis
- Model Equation

Span. Clementinen

Kl.1

1kg

gewachst, konserviert mit
Orthophenylphenol

Thiabendazol

ur imzalil

Schale nicht zum
Verzehr geeignet

1,99

Taste Nr.19

Pesticides are used for example to prolong the shelf life of fruit and vegetables (they act against e.g. molds). Besides the useful action they are potentially hazardous and must be monitored.



The Customer's Requirements

Post-registration control and monitoring of pesticides based on MRLs set by the EU Directives 93/58/EEC and 00/42/EEC for **imazalil** and **thiabendazole** respectively:

- LOD \leq 0.02 mg/kg (imazalil), LOD \leq 0.05 mg/kg (thiabendazole)
- Recovery between 70 – 110 %
- Identity confirmed by MS/MS experiments

LC-MS as an Analytical Tool

Unites two powerful methods:

LC (liquid chromatograph) **separates** the analytes from each other

MS (mass spectrometer) **detects, identifies** and **quantifies** the analytes



The Analytical Procedure

- Sample preparation procedure is a modification of the AOAC official method 985.22
 - Modifications were made to cut down sample size and solvent consumption
 - Changes were made in the solvent of final extract to suite LC-MS system
- Analysis is carried out on an LC-MS system using a self-developed chromatographic method

Partial Validation Required!

Sample Preparation

- 50 g of homogenized sample is extracted with 100 ml of acetone using high speed blender
- Mixture is filtered and the volume of extract is measured
- 50 ml of extract is extracted with 100 ml dichloromethane petroleum ether mixture (1:1), organic layer is filtered through a layer of sodium sulphate (for drying purpose)
- Water phase is saturated with NaCl and extracted twice with 50 ml of dichloromethane
- Organic extracts are dried
- Solvent is evaporated to almost dryness and the sample is dissolved in 10 - 20 ml of methanol
- Sample is filtered through a syringe filter and analysed using LC-MS system

Complex Sample Preparation!

The LC-MS Analysis Procedure

- In the LC-MS system the samples are separated chromatographically
 - Eluent: acetonitrile (B) and buffer solution (1mM ammonium acetate, 0.1 % formic acid) (A) as eluent
 - The gradient program : B 20 → 100 % 15 min, B 100 % 17 min at 0.8 ml/min
 - Analysed substances were then ionized using the ESI procedure and analysed with the ion-trap MS using fragmentation of quasimolecular ions ($[M+H]^+$)
- Using the 20 mg/kg standard solution and other dilutions the calibration solutions are prepared in methanol in the concentration range of 5 – 0.003 mg/kg
- Calibration graphs are compiled using peak areas of certain characteristic fragment ions on different concentrations

Model Equation

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

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] (found from the calibration graph)
V_{10}	the volume of final extract in methanol [ml]
ρ	density of methanol [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]