Assessment of Matrix Effects in Gas Chromatography Electron Capture Pesticide-Residue Analysis

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Summary
The analysis of pesticide residues in vegetable samples leads in most cases to different results when solvent or matrix matched calibration is used for quantitation. Matrix effects in GC-ECD analysis of pesticides in vegetable samples have been assessed by comparing calibration curves prepared in solvent and in blank matrix extracts. Eight different vegetables have been considered among the most common commodities in routine pesticide residue laboratories.

Solvent and matrix matched calibrations have been statistically compared using analysis of covariance. As an alternative to the use of matrix-matched calibration for quantifying pesticide residues, a correction function has been proposed in each case, which correlates both kinds of calibration curve. The correction functions allow, quantifying using a simple solvent calibration similar recovery rates than those obtained when matrix-matching is used. The stability of such calibrations and correction functions over a six-month period and the contribution of the correction function to the uncertainty of quantitation of pesticides has been established and is discussed.

Introduction
Chemical measurements of various types play a rapidly expanding role in modern society and increasingly form the basis of important decisions. One of the aims of certification and accreditation by the European Commission is to obtain the mutual recognition of testing and certificates to decrease cost and bureaucracy. The analytical laboratories supply analytical information and are concerned by the requirements of quality in analytical chemistry. Specific practical approaches for assuring accuracy in analytical chemistry include sampling, uncertainty calculations, calibrations, traceability, validation, quality control and proficiency testing.

An important area for protection of health is control of pesticide residues (PR) in fruits and vegetables. The maximum residue level (MRL) for each pesticide and matrix is regulated by the European Commission. Regulation means setting limits and verifying compliance with these limits entails chemical measurements. The difficulties of PR analysis in fruits and vegetables are well known and include among others:

- the need to use multiresidue methods (MRM) to cover a wide range of analytes with different physico-chemical properties,
- effective for analytes at concentration levels near to quantitation levels (LOQ)
- in many matrices (fruits and vegetables) with different water and fat content and biochemical composition

Several analytical methods have been published using GC techniques with different detectors [1 – 3], the main difference among these methods being the solvent used in the extraction step.

Official pesticide-residue laboratories have published different guides [4 – 6] for analysing pesticide residues in fruit and vegetables, stating in most cases that calibration solutions must be prepared in a blank matrix to improve accuracy of the calibration step, but at the moment, the matrix, considered as part of the analytical system, is often neglected and we have little idea about its magnitude [7].

Co-extractives may modify the analytical resolution by increasing the level of random errors and/or introducing a systematic error in analytical results, either constant, affecting the blank, or proportional, affecting the analytical sensitivity. Calibration is regarded as an essential part of method validation [8 – 11], but systematic errors in calibration are seldom
considered during calibration validation, in fact, there is a largely unrecognised need to evaluate the uncertainty associated with matrix effects.

Recent related papers in different fields of pesticide analysis include the matrix effect in the calibration step by preparing calibration solutions with extracts from blank samples (matrix-matched calibrations) instead of using a solvent [12–16]. This is considered an effective way for avoiding errors arising from matrix effects in the quantitation of analytes and should be used unless demonstrated to be unnecessary [16]. However, this procedure does not provide the magnitude of the effect of co-extraction and greatly increases the cost and time of analysis.

A new approach can be described. In many cases, corrections or correction factors follow a defined pattern within a range of measurements, given by an instrument, which can be mathematically modelled by a correction or calibration function and fitted to a correction curve. On the basis of the characterisation of such a matrix effect, data should be obtained from indirect calibrations carried out using two types of calibration standards: i) chemical standards prepared from pure analyte dissolved in pure solvent and ii) reference materials prepared incorporating the matrix co-extractives in the standard solution. A statistical study of the differences between regression parameters of both calibrations will provide correction factors with their associated uncertainty. These values will be validated by data obtained from recovery studies.

In summary, this approach will allow a reliable quantitation of pesticides in samples with important matrix effects, such as fruit and vegetable samples, using calibration curves using pure solvent. This has advantages for both economic and practical aspects of analysis, i.e. decreasing the cost as much as the analysis time.

This study considers seven analytes (achrinathrin, bromopropylate, endosulfan α, endosulfan β, endosulfan sulphate, lindane and tetradifon) and eight vegetable matrices (tomato, pepper, green bean, aubergine, courgette, cucumber, melon and watermelon). Estimation of the effect of the pepper matrix in the determination of lindane have been described in more detail as an example of the whole study.

**Experimental**

The analytical methodology presented in this work is being used as a routine method in a pesticides residues laboratory (Centro Universitario Analitico Municipal, CUAM) where more than 10000 samples are analysed per year. It achieved accreditation ensuring the compliance with ISO17025 requirements.

**Chemicals**

N-hexane, cyclohexane and dichloromethane (residue analysis grade, Panreac, Barcelona, Spain) were used for dissolving and extracting samples. Anhydrous sodium sulphate for residue analysis was from Panreac. All pesticide standard reference materials were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The following pesticides were tested: lindane, endosulfan α, endosulfan β, endosulfan sulphate, bromopropylate, tetradifon and achrinathrin.

**Commodities**

Commodities considered were tomato, pepper, green bean, aubergine, courgette, cucumber, melon and watermelon. All were obtained from experimental greenhouses and none was previously treated with any pesticide in order to provide blank extracts.

**Instrumentation**

Two gas chromatographs were used: a Perkin-Elmer model 8500 and a Hewlett-Packard model 5890 both equipped with an electron capture detector (ECD $^{69}\text{Ni}$).

GC-ECD operating conditions were: injector temperature, 250°C; detector temperature, 350°C; initial oven temperature, 180°C for 5 min, raised at 3°C min$^{-1}$ to 250°C, and then held at 250°C for 2 min. The carrier gas was nitrogen at 10 mL min$^{-1}$. A fused silica semicapillary (HP-1) column containing 100% methylpolysiloxane as stationary phase, 25 m, 0.33 mm i.d. and 1.0 µm film, was used for the separation in the Perkin-Elmer model and a fused silica capillary (HP-1) column containing 100% dimethylpolysiloxane as stationary phase, 60 m, 0.25 mm and 0.22 µm film was used for separations in the Hewlett-Packard model.

**Analytical Procedures**

**Extraction Procedure**

The extraction method used was similar to that by Martinez-Vidal et al [12], which consists of mixing 50 g chopped sample with anhydrous sodium sulphate and dichloromethane, the mixture is homogenised for 1 min in a polytron and filtered. Solvent is removed under vacuum at 40°C in a rotary evaporator until almost dry and then just to the point of dryness with a slight N2 stream, then dissolved in 20 mL n-hexane. 1 µL of sample extract is injected into the GCs.

**Calibration Curves**

A stock solution of each pesticide was prepared in n-hexane to obtain the primary calibration solutions (200 mg L$^{-1}$) from which the secondary standard solution of lower concentration (ca 10 mg L$^{-1}$), containing a mixture of all pesticides was prepared by dilution with n-hexane. They were stored at 4°C.

In order to determine the matrix influence and whether it is possible to obtain a correction function for each pesticide, two different types of calibration curve were studied:

- Calibration curves prepared using a solvent (solvent calibration, SC): four standard solutions were prepared as a calibration set, at 0.25, 0.50, 0.75 and 1 mg L$^{-1}$ for all pesticides, except achrinathrin, which was prepared at 0.025, 0.05, 0.075 and 0.1 mg L$^{-1}$. These ranges were chosen on the basis of the maximum residue levels in vegetables allowed by the European regulations for such pesticides in the commodities studied. Calibration solutions were prepared by diluting with pure n-hexane, 50, 100, 150 and 200 µL of the secondary standard solution to 2 mL after adding 40 µL of a 10 mg L$^{-1}$ solution of dieldrin as internal standard. 1 µL samples were injected into the GC-ECD.
- Calibration-set solutions prepared in a vegetable matrix (MC): these solutions were prepared as above but using blank extract of each commodity, instead of pure solvent, for making up to the final 2 mL. These extracts were obtained by applying the extraction method above, to uncontaminated commodities.