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Short communication

Optimization of a sample preparation method for multiresidue analysis of pesticides in tobacco by single and multi-dimensional gas chromatography-mass spectrometry

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ABSTRACT

A selective and sensitive multiresidue analysis method, comprising 4 7pesticides, was developed and validated in tobacco matrix. The optimized sample preparation procedure in combination with gas chromatography mass spectrometry in selected-ion-monitoring (GC-MS/SIM) mode offered limits of detection (LOD) and quantification (LOQ) in the range of 3–5 and 7.5–15 ng/g, respectively, with recoveries between 70 and 119% at 50–100 ng/g fortifications. In comparison to the modified QuEChERS (Quick-Easy-Cheap-Effective-Rugged-Safe method: 2 g tobacco + 10 ml water + 10 ml acetonitrile, 30 min vortexing, followed by dispersive solid phase extraction cleanup), the method performed better in minimizing matrix co-extractives e.g. nicotine and megastigmatrienone. Ambiguity in analysis due to co-elution of target analytes (e.g. transfluthrin-heptachlor) and with matrix co-extractives (e.g. δ-HCH-neophytadiene, 2,4-DDE-linolenic acid) could be resolved by selective multi-dimensional (MD)GC heart-cuts. The method holds promise in routine analysis owing to noticeable efficiency of 27 samples/person/day.

1. Introduction

India is the world's second largest producer of tobacco (*Nicotiana tabacum* L.) with $901.95 million/year worth of export [1,2]. Cultivation of tobacco receives frequent application of pesticides, the residues of which might sustain processing treatments and cause health hazards [3–5]. The need for a multiresidue analysis method for pesticides in tobacco is pertinent to support the Indian tobacco industry to comply with the Guidance Residue Levels (GRL)[6]. Considering the complex nature of its matrix, in most literature, only 2-7.5 g of tobacco has been considered for extraction [7–9] with selective determination by GC [10], two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS) [11], GC-MS/MS [9,12], high performance liquid chromatography [13] etc. However, with these methods, we have recorded high matrix effect (ME) and false positives/negatives for several pesticides. In the present study, we therefore, aimed to develop an effective sample preparation method to minimize co-extractives and also attempted to resolve matrix interferences for target pesticides by GC-MS/SIM and MDGC heart-cuts.

2. Experimental

2.1. Selection of pesticides and tobacco matrix

A total of 47 GC amenable compounds out of the GRL list (23 organochlorines, 8 organophosphates, 16 pyrethroids) were considered [6]. Sample preparation was optimized and validated in KLS (Karnataka Light Soil) tobacco (highest exported type), and further evaluated in three other tobacco matrixes viz. NLS (Northern Loamy Soil), SBL (Southern Black Soil) and SLS (Southern Light Soil) (Supplementary Table 1).

2.2. Reagents and materials

Certified pesticide reference standards (>98% pure) were purchased from Ehrenstorfer GmbH (Augsburg, Germany). The

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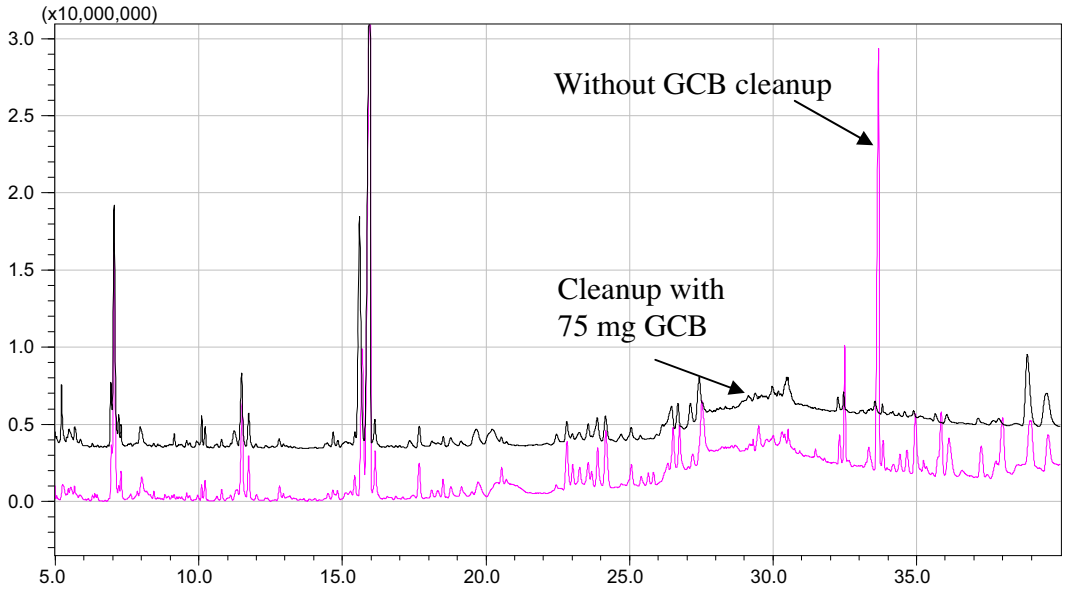

Fig. 1. Overlaid full-scan chromatogram of control matrix with and without GCB cleanup (75 mg) showing effect of cleanup on removal of matrix co-extractives.

solvents used were of pesticide residue analysis grade (Sigma–Aldrich, Bangalore, India). The dispersive solid phase extraction (d-SPE) sorbents viz. primary secondary amine (PSA), C18 and graphitized carbon black (GCB) were purchased from United Chemical Technology (Bristol, PA, USA). The other reagents were of analytical reagent grade. A homogenizer (Silent Crusher M, Heidolph, Saffron Walden, UK) was used for proper mixing of the sample with solvent during extraction.

2.3. Preparation of standard solutions

The stock solutions (w/w) of the individual pesticide standards were prepared by dissolving 10 mg of each analyte in 9 g ethyl acetate (EtOAc, 10 mL = 9 g). An intermediate mixture of 10 mg/L was prepared by mixing appropriate quantities of the individual stock solutions followed by requisite volume make-up, from which the calibration standards (5-250 ng/mL) were prepared by serial dilution.

2.4. Standardization of sample preparation technique

2.4.1. Pre-treatment

To obtain homogeneity, the dry tobacco samples (25 g) were soaked in water (225 mL, containing 0.5% acetic acid) for 30 min and subsequently homogenized (2 min) to form a fine paste with smooth appearance without any visual granules. Homogeneity test was carried out at 100 ng/g ($n = 6$). For this, 100 g of tobacco samples were spiked at 100 ng/g. The pretreatment was done as follows:(a) six samples (2 g) drawn from 100 g spiked sample(b) to 100 g sample, 900 mL water was added and soaked for 30 min. Further, the sample was homogenized in a grinder and 10 g sample was drawn for extraction.

The samples were extracted using the procedure described in Section 2.4.5.

2.4.2. Sample size optimization

Tobacco homogenates, 10 g (1 g tobacco + 9 mL of water) and 20 g (2 g tobacco + 18 mL of water), fortified with the pesticide mixture (100 ng/g), were extracted in separate batches ($n = 6$) with 10 mL EtOAc, followed by d-SPE cleanup using 150 mg PSA + 150 mg C18 + 75 mg GCB + 300 mg $MgSO_4$ for 10 g and proportionately double amounts for 20 g. The recoveries were statistically compared.

2.4.3. Sample:solvent ratio

To optimize the sample-solvent ratio, 20 g of the fortified tobacco homogenate (at 100 ng/g) was extracted with varying amounts (5 and 10 mL) of ethyl acetate in separate batches each in six replicates. The cleanup in each case was carried out with d-SPE sorbents in proportionate amounts as mentioned below:

- Solvent volume 5 mL: 300 mg PSA + 300 mg C18 + 150 mg GCB + 600 mg MgSO4
- Solvent volume 10 mL: 150 mg PSA + 150 mg C18 + 75 mg GCB + 300 mg MgSO4

The quantification of residues in the recovery samples was carried out using matrix-matched standards prepared separately with the strategies selective for 5 and 10 mL solvent volumes to ensure comparability of results.

2.4.4. Optimization of GCB for cleanup

Effect of variable quantities of GCB (0, 75 and 150 mg) was investigated in combination with PSA (150 mg), C18 (150 mg) and $MgSO_4$ (300 mg). Since GCB tends to adsorb planar pesticides like chlorothalonil [14], the effect of toluene addition (200, 500 and 1000 μl) on its recovery (at 100 ng/g) was also evaluated. In all cases, quantification was done using corresponding matrix-matched calibrations.

2.4.5. Optimized sample preparation method

Samples (20 g homogenate) were extracted with EtOAc (10 mL, +10 g Na_2SO_4) by homogenization (15000 rpm, 2 min), followed by centrifugation (5000 rpm, 5 min) for phase separation. An aliquot of 3 mL EtOAc extract was drawn, mixed with toluene (1000 μl), vortexed (30 s), and cleaned by d-SPE (150 mg PSA + 150 mg C18 + 75 mg GCB + 300 mg $MgSO_4$). The supernatant was filtered through PTFE membrane (0.22 μm, Chromatopack, Mumbai) before injection into GC-MS.

The performance of the above method was compared with the modified QuEChERS method [15] in terms of recovery and cleanup efficiency.

2.5. GC-MS

A QP-2010 Plus GC-MS (single quadrupole, Shimadzu Corporation, Kyoto, Japan) with VF-5MS (30 m × 0.25 mm, 0.25 μm)