Simultaneous determination of 223 pesticides in tobacco by GC with simultaneous electron capture and nitrogen-phosphorous detection and mass spectrometric confirmation

DOI: 10.1515/chem-2015-0129
received December 11, 2014; accepted July 8, 2015.

Abstract: LSE (liquid-solid extraction), MSPD (matrix solid phase dispersion) and QuEChERS (quick, easy, cheap, effective, rugged and safe) extractions followed by GC-µECD/NPD to determine 223 pesticide residues in tobacco simultaneously were developed and compared. The identities of ten model pesticides were confirmed by GC-MS/MS. The type and amount of dispersant (Florisil, silica gel and alumina), sample mass, cleanup adsorbent, and the eluent (hexane, acetone and acetonitrile) were optimized. Linearity, recovery, LOQ, LOD, and matrix effect were compared. Most recoveries were 71−120% (RSD < 18%). LOD and LOQ were much lower than the CORESTA GRLs. The best method was QuEChERS: acetonitrile extraction and dispersive solid-phase extraction using primary-secondary amine and graphitized carbon.

Keywords: tobacco, pesticide residues, gas chromatography

1 Introduction

Poland produces about 40,000 tons of tobacco from 17,000 acres annually, approximately 0.4% of global production. Two varieties predominate – Virginia bright tobacco and dark varieties of Burley.

Most people are aware that tobacco (Nicotiana tabacum L.) consumption damages health, including increased lung cancer and cardiovascular disease risk. However, people may be less aware of the pesticides used to grow it [1,2]. A large number are used against fungi, pests, and weeds. Their traces remain; there is growing concern over exposure to their pyrolyzed residues [3,4]. The Agrochemical Advisory Committee of the Cooperation Centre for Scientific Research on Tobacco (CORESTA) has published a list of Guidance Residue Levels (GRLs) for 120 pesticides [5] in tobacco and tobacco products.

The complex tobacco matrix varies with curing method, growing conditions, fertilizers and pesticides. Approximately 4200 components have been found in tobacco – not including additives like flavors. Ultra trace pesticide determinations in plants is often complicated because fats, carbohydrates [6], chlorophylls [7], lipids, sterols, glycosides, triglycerides and tarry substances interfere with chromatographic detection. Cleanup is required before analysis.

Liquid-solid extraction (LSE) followed by multistep operations has been used [8-10]. As this consumes time and a large amount of glassware and organic solvents, reduced-solvent extractions were developed. Among these are pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE) [11] followed by solid-phase extraction (SPE) cleanup; stir bar sorptive extraction (SBSE) [12]; and hybrid field-assisted solid-liquid-solid dispersive extraction (HP-SLSDE) [13] followed by dispersive liquid-liquid microextraction (DLLME) [14]. ASE requires special and expensive equipment and maintenance [15] but is the most robust and efficient. Several miniaturized methods have been introduced, e.g. matrix solid phase dispersion MSPD [16] and QuEChERS (quick, easy, cheap, effective, rugged, and safe) [17-19] which eliminate most of the complications of classical extraction.

There have been no reports of simultaneous determinations of a large number of pesticides of many types (carbamates, organochlorines, organophosphates,
pyrethroids, strobilurins, triazines, triazoles, etc.) [20] in tobacco.

The scope of this study was to optimize, evaluate, and compare LSE, MSPD and QuEChERS for simultaneous determination of over 220 pesticides in tobacco using gas chromatography with dual electron capture and nitrogen-phosphorous detection (GC-μECD/NPD). The goal was a simple method minimizing solvent consumption, cost, and analysis time. Gas chromatography with triple quadrupole mass spectrometry (GC/MS/MS) was an investigative tool.

2 Procedure

2.1 Chemicals, reagents and materials

Acetone, acetonitrile and hexane for pesticide determination were provided by J.T. Baker (Deventer, Netherlands), Florisil (60–100 mesh) was supplied by POCH (Gliwice, Poland), anhydrous sodium sulfate, Celite and octadecylsilica gel C\textsubscript{18} (200–400 mesh) were purchased from Fluka (Seelze-Hannover, Germany). Silica gel (230–400 mesh) and neutral alumina (0.063–0.200 mm) were obtained from Merck (Darmstadt, Germany). C\textsubscript{18} SPE cartridges (500 mg/3 mL) were supplied by J.T. Baker (Deventer, Netherlands). QuEChERS sorbent kits and salt pouches were purchased from Agilent Technologies (Santa Clara, CA, USA). The sorbents used were: PSA, C\textsubscript{18}, bulk Cerograph and salt pouches: magnesium sulfate, sodium chloride, sodium citrate, and citric acid disodium salt.

Neutral alumina was activated at 130°C and silica gel at 600°C. Deactivated sorbents were prepared by adding 5% distilled water.

Virginia type tobacco from southeastern Poland was used.

2.2 Standard solutions

The 223 pesticide standards (95–99.8% pure) were purchased from the Dr. Ehrenstorfer Laboratory (Augsburg, Germany). Triphenyl phosphate was obtained from Sigma-Aldrich (Steinheim, Germany). Stock solutions were prepared in acetone and stored at 4°C for further dilution. Standard multi-compound working solutions (0.01–2.50 mg kg\textsuperscript{-1}) were prepared by dilution in 9:1 (v/v) n-hexane/acetone. The stock and working solutions were stored at -20°C in completely filled vials closed with Parafilm until analysis.

2.3 Sample preparation

Tobacco leaves were air dried, milled with a knife, ground to 40–60 mesh, homogenized, and stored in plastic bags at 4°C out of direct sunlight.

2.3.1 Procedure I – LSE

5 g of ground tobacco and 50 mL acetonitrile in an Erlenmeyer flask were shaken in a rotary shaker at 2500 rpm for 30 min, then filtered with 5 g Celite and 5 g anhydrous sodium sulfate. The sample was replaced in the flask with 20 mL acetonitrile and shaken for 30 min. The extracts were combined and evaporated at 40°C in a rotary evaporator to near dryness. The residue was redissolved in 2 mL acetonitrile.

Extract cleanup
An SPE C\textsubscript{18} (500 mg/3 mL or 1000 mg/6 mL) column was rinsed with 10 mL acetonitrile; 1 mL of tobacco extract was loaded and eluted with 5 mL acetonitrile. The eluent was collected and evaporated at 40°C in a vacuum rotary evaporator to near dryness. The residue was diluted to 2 mL with 9:1 n-hexane/acetone.

2.3.2 Procedure II – MSPD

Two grams of the ground tobacco sample were manually homogenized with 4 g solid support (Florisil) in a mortar and pestle (2 min), quantitatively transferred to a glass column packed with anhydrous sodium sulfate (5 g) and eluted by gravity with 25 mL acetonitrile at 1–2 drops s\textsuperscript{-1}. The extract was evaporated at about 40°C in a rotary evaporator to near dryness and redissolved in 2 mL of acetonitrile.

Extract clean-up
Cleanup was as above for LSE.

2.3.3 Procedure III - QuEChERS

Two grams of ground tobacco and 10 mL of water in a 50 mL polypropylene centrifuge tube (Agilent, Santa Clara, CA, USA) were hand shaken for 1 min and allowed to stand for 10 min. 100 µL of the fortification standard was then added to fortified samples. 20 mL of acetonitrile and 50 µL of 100 µg mL\textsuperscript{-1} triphenyl phosphate (GC-MS/MS
internal standard) were added and the samples vortexed for 7 min in a digital Vortex-Mixer (Velp Scientifica, Usmate, Italy). A salt mixture (4 g MgSO$_4$, 1 g NaCl, 1 g trisodium citrate dihydrate (Na$_3$C$_6$H$_5$O$_7$·2H$_2$O), and 0.5 g disodium hydrogen citrate sesquihydrate (Na$_2$HC$_6$H$_5$O$_7$·1.5H$_2$O) was added. The tubes were immediately shaken for 1 min and vortexed for 5 min at 4500 rpm. The top acetonitrile layer was collected and aliquots were taken for subsequent cleanup.

**Figure 1:** Extraction and cleanup procedures.

**Extract cleanup**

6 mL of the extract was transferred to d-SPE3 tubes containing 150 mg MgSO$_4$, 25 mg PSA and 2.5 mg GCB. The tubes were gently but vigorously shaken for 0.5 min and centrifuged for 10 min in a Rotina 420R centrifuge at 4500 rpm (Hettich, Tuttingen, Germany). 2 mL of extract was transferred to a flask and evaporated to near dryness. The residue was dissolved in 2 mL 9:1 n-hexane/acetone. Extraction procedures are summarized in Fig. 1.
**2.4 Instrument**

**2.4.1 GC-µECD/NPD conditions**

Pesticides were separated and determined using an Agilent (Waldborn, Germany) 7890 A gas chromatograph with an Agilent HP-5 column [(5%-phenyl)-methylpolysiloxane; 30 m × 0.32 mm ID and film thickness 0.25 µm] equipped with microelectron capture (µEC) and nitrogen-phosphorus (NP) detectors, and with a Chemstation chromatography manager data acquisition and processing system (Hewlett-Packard, version A.10.2). Operating conditions were:

- Injector temperature 210°C; helium carrier (99.9998%) at 3.0 mL min⁻¹; for detectors: temperature 300°C (ECD and NPD); make-up gas nitrogen (99.9998%) at 57 mL min⁻¹ (ECD) and 8 mL min⁻¹ (NPD), hydrogen (99.9998%) 3.0 mL min⁻¹, air (99.9998%) 60 mL min⁻¹; for the oven:
  - Initial temperature 120°C, increased to 190°C at 16°C min⁻¹,
  - then to 230°C at 8°C min⁻¹, and finally to 285°C at 18°C min⁻¹, and held for 10 min.

2 µL of the final sample extract was injected at 210°C in splitless mode (purge time 2 min). Total analysis time was 22.5 minutes.

Compound identification was by comparison of the retention time with that of the standard (± 0.005 min for positive match). Quantitation was by height comparison with the standard peak.

The method allows determination using two selective detectors functioning simultaneously. A “Y” piece at the end of the column divided the flux into two branches of equal flow (to the NPD and to the ECD), allowing pesticides of different nature to be quantitated in the same run; 157 pesticides were detected by the ECD and 155 by NPD, although both provided a discernible signal for 89 of them.

**2.4.2 GC-MS/MS confirmation**

Identities were confirmed using GC-MS/MS. An Agilent 7890A GC was equipped with an Agilent 7693 autosampler and coupled to an Agilent 7000B triple quadrupole mass spectrometer operated in electron ionization mode (EI – 70 eV). Splitless injection of a 2 µL sample was separated by an HP-5 MS capillary column. Temperature program:

- 70°C for 2 min, 25°C min⁻¹ up to 150°C, 3°C min⁻¹ up to 200°C, 8°C min⁻¹ up to 280°C, then held for 10 min. The total running time was 41.88 min. The temperatures of the transfer line, ion source, 1st and 2nd quadrupole were 280°C, 300°C, 180°C and 180°C, respectively. Pesticides were identified by comparing retention times and three ions (one precursor and two products).

**2.5 Method validation**

Validation followed the EURACHEM guide using pesticide-free tobacco [21]. The matrix effect, linearity, limits of detection/quantitation (LOD/LOQ), recovery and precision were examined. Calibration curves were produced from matrix-matched solutions. The lowest concentration in the calibration curve was at the CORESTA GRL or much lower. Analytes without GRL were tested at the smallest GRL of other regulated pesticides, 0.01 mg kg⁻¹. Calibration standards were prepared by spiking a blank tobacco matrix to produce final concentrations of 0.01–0.05 mg kg⁻¹, 0.10–0.5 mg kg⁻¹, and 0.5–2.5 mg kg⁻¹. Linearity was determined from the coefficient of determination (R²).

Recovery data was obtained at three concentrations. After homogenization, matrix blanks were spiked with the pesticide standard mixture and equilibrated for 2 h, then prepared and analyzed as were the samples. Precision was expressed as the relative standard deviation (RSD). Accuracy was measured by comparing the measured and known values. LOQs were defined as described in SANCO/12495/2011 [22]. LOD = 3 S/N and LOQ = 10 S/N.

**2.6 Matrix effect**

A change in the measurement of concentration or mass due to undetected sample components is called the matrix effect (%ME) [23].

Matrix-matched calibration standards were prepared by adding standard solutions to blank tobacco extracts. Calibration curves for the solvent and the matrix-matched standard were compared at 0.01–0.05 mg kg⁻¹, 0.05–0.10 mg kg⁻¹, 0.10–0.50 mg kg⁻¹, 0.2–1.0 mg kg⁻¹, and 0.5–2.5 mg kg⁻¹. Matrix effects were calculated by:

\[
\text{ME} (%) = \left[\frac{\text{slope in matrix}}{\text{slope in solvent}} - 1\right] \times 100.
\]

Negative matrix effects signify signal suppression, positive values enhancement.

**3 Results and discussion**

**3.1 Optimized conditions**

Sample mass, solvent volume and polarity, cleanup sorbents and techniques were optimized to get the “cleanest” matrix. The extraction and purification of
pesticide residues from tobacco by LSE, MSPD and QuEChERS are summarized in Fig. 1.

All three methods were based on acetonitrile extraction/clean-up. The high vapor volume and polarity of acetonitrile make it a less than optimal solvent for gas chromatography. It was exchanged by sample evaporation to near dryness and redissolution in n-hexane/acetone.

3.1.1 Procedure I – LSE

LSE is a traditional sample preparation technique described in many reviews and official methods [24,25]. The pesticides included in this study are non-polar, somewhat polar and polar, which partly explains the analysis difficulties. The main LSE variables: purification sorbent, elution solvent and volume, extraction time and sample mass were optimized (Fig. 2). Hexane, acetone and acetonitrile were tested as single extraction solvents.

Non-polar hexane gave recoveries below 40% for most of the compounds, but acetone gave unsatisfactory recovery for 25% of them. Double extraction with acetonitrile (50 mL and 20 mL) gave the best results. Increasing extraction time to 2 hours and volume to 100 mL gave little improvement but increased costs. The sample mass (5 g) was excellent.

Matrix interferences made cleanup necessary. As the SPE sorbents silica gel and C_{18} with an anhydrous sodium sulfate layer in a plastic column have mixed polarity, these were tried. Different activation methods were tested for

Figure 2: LSE optimization: effect of (a) extraction solvent, (b) cleanup adsorbent on recoveries.
their effect on purification efficiency [26]. The efficacy of C$_{18}$ was highest for all pesticides studied; most recoveries exceeded 70%.

As was the case for LSE, double acetonitrile extraction gave best efficiency. Therefore this combination of sorbent and solvent was chosen for further work.

3.1.2 Procedure II – MSPD

Kadenczki, Arpad, and Gardi [27] developed an MSPD technique for fruits and vegetables using Florisil activated at 600ºC as the dispersing sorbent, and dichloromethane/acetone for elution. Our MSPD procedure was optimized for four variables: dispersing phase (activated or deactivated: Florisil, silica gel and alumina), cleanup adsorbent (silica gel or C$_{18}$), matrix/sorbent ratio, and extraction selectivity (different solvents and volumes) (Fig. 3).

Deactivated Florisil, silica gel, or alumina as the dispersive phase gave recoveries below 40% for most of the compounds. Optimum extraction was with Florisil activated at 600ºC. In general, the smaller the matrix particle size the more rapid and complete the extraction.

The second step was to optimize the sample mass to sorbent ratio. The ratios 1:1, 1:2, 1:4 and were examined; 1:2 was best.

Studies of hexane, acetone and acetonitrile showed that the solvating powers of acetone and acetonitrile are practically identical, so the solvent does not limit compound elution; 25 mL acetonitrile gave effective elution.

The final MSPD extract contained a large amount of co-extracts. Those with retention times close to those of the target prevent low detection limits. Silica gel and C$_{18}$ SPE cleanup cartridges were tested. Silica gel gave recoveries below 40% for six compounds (acetamiprid, cypermethrin, dimoxystrobin, iprodione, metalaxyl, pendimethalin). C$_{18}$ SPE cartridges minimized interference and gave the best recoveries.

In sum, a 2 g sample was blended with 4 g inorganic normal phase and activated Florisil; 25 mL of acetonitrile was optimal. Simultaneous extraction using a glass column with anhydrous sodium sulfate and purification using C$_{18}$ SPE cartridges represented the best compromise.

3.1.3 Procedure III - QuEChERS

The original QuEChERS method extracts pesticide residues from fruits and vegetables with a high water content. It employs acetonitrile extraction followed by MgSO$_4$ alone or in combination with other salts to remove water, then a dispersive solid-phase extraction (d-SPE) cleanup with primary-secondary amine (PSA) [28].

A comparison was made among acetone, acetonitrile and hexane extractants. Acetonitrile extracts contained less interference than acetone or hexane extracts. Extraction recoveries with hexane and acetone were similar for most pesticides (49%–143%) but lower than with acetonitrile (69%–118%). Acetonitrile was chosen as eluent, giving satisfactory and stable recovery.

After optimized solvent extraction the quicker d-SPE cleanup was examined (Fig. 4). The sorbents were:

- (d-SPE1) 20 mg anhydrous MgSO$_4$ + 10 mg PSA;
- (d-SPE2) 20 mg anhydrous MgSO$_4$ + 25 mg PSA + 25 mg C$_{18}$;
- (d-SPE3) 20 mg anhydrous MgSO$_4$ + 25 mg PSA + 2.5 mg GCB;
- (d-SPE4) 20 mg anhydrous MgSO$_4$ + 30 mg PSA + 25 mg C$_{18}$ + 2.5 mg GCB

Anhydrous MgSO$_4$ absorbs water, GCB removes most of the visible pigment and PSA removes fatty acids by ion exchange. Although high recoveries occurred when only MgSO$_4$ was used, PSA combined with GCB was used (d-SPE3).

3.2 Comparison of different extraction methods using GC-µECD/NPD

All three methods successfully extract and isolate over 200 pesticides from tobacco at 0.005–0.04 mg kg$^{-1}$. However, it is important to optimize the procedure to save time, reduce solvent consumption, limit costs, reduce instrument maintenance, and to improve performance. Extraction solvent optimization improved efficiency and minimized potential interferences.

Linearity was satisfactory over the range 0.01–2.5 mg kg$^{-1}$ with correlation coefficients $\geq 0.997$. The recoveries, RSD values, limits of quantitation (LOQ) and limits of detection (LOD) are shown in Table S1 in the Supplementary Data. LOQs and LODs in the three methods were similar, and ranged from 0.001 to 0.0012 and from 0.005 to 0.04 mg kg$^{-1}$. The LOQs were below CORESTA guidance levels.

Mean recoveries at three spiking levels varied from 70 to 120% for MSPD, with the exceptions of 29 analytes below 70% and p,p′ DDT, procyanine above 120%. For LSE recoveries were 70–113%, with the exception of 39 analytes with mean recoveries < 70% and acetamiprid > 120%. However, other validation parameters were satisfactory (RSDs < 20%) [22]. As seen in Table S1, the LSE and...
Figure 3: MSPD optimization: effect of (a) dispersing sorbent, (b) extraction solvent, (c) cleanup adsorbent on recoveries.
MSPD methods gave RSDs from 1−19%. Carbosulfan and thiabendazole (insecticides) were not isolated by MSPD extraction. Cyanazine (herbicide), amitraz, carbosulfan, flonicamid, thiabendazole and thiacloprid (insecticides) were not extracted by LSE and fuberidazole (fungicide) was not isolated by either method (Table S1). The MSPD and LSE extraction procedures met the multi-residue method requirements, isolating 221 or 215 pesticides out of 223 analyzed with good validation parameters.

QuEChERS proved a good alternative to LSE and MSPD. The materials cost per sample was around 6 €; less than the 8-10 € LSE and MSPD method costs (Table 1). The four-fold reduction of solvent volume is important. A chemist could prepare around 30 samples used in the QuEChERS method and 20 for the LSE or MSPD methods in an 8-h workday.

**Figure 4:** QuEChERS optimization: effect of (a) extraction solvent, (b) cleanup adsorbent on recoveries.
3.3 Matrix effect

This effect was evaluated by comparing the slopes of calibration curves obtained with solvent-based standards with those using matrix-matched standards. An increased response represents a sensitivity enhancement due to the matrix. The %ME can be negative or positive and the effect can be classified as [29]: none; medium; or strong. No matrix effect is detected when the %ME values are within the accepted reproducibility (± 20%). %MEs are listed in Table S1 and shown in Figure 5. Matrix effects for the vast majority (77%) exhibited enhancement rather than suppression. In GC an enhancement is typical as matrix components block column active sites allowing more pesticide to reach the detector [30]. GC-MS/MS gave smaller matrix effects. One reason is improved selectivity, avoiding interferences and background noise present in GC-µECD/NPD. A smaller injection also decreases matrix introduction.

Of over 200 analyzed pesticides, 141 in the QuEChERS extract, 68 in MSPD, and 95 in the LSE extract exhibited soft matrix effects (< ± 20%). Effects in this range can be treated as negligible. The higher number of minimally affected pesticides suggests that QuEChERS extracts are cleanest. Nevertheless, when the GRL concentration is approached or an unapproved pesticide detected, matrix-matching may improve quantitation. QuEChERS gave the fewest (60 compounds) with medium matrix effects (20% < enhancement/suppression < 50%), while MSPD gave 85 pesticides and LSE 68 in this group. Acrinathrin, azinphos-methyl, bromuconazole, captafol, captan, carbaryl, carbofuran, fipronil, fluoxastrobin, flutolanil, iprodione, isocarbophos, lenacil, paraoxon methyl, tau-fluvalinate and tritosulfuron gave strong matrix effects (> ± 50%) in all three methods.

For the four pesticides acetamiprid, boscalid, chlorpyrifos, and pendimethalin the matrix effects were similar for all methods. Azoxystrobin, dimoxystrobin and metalaxyl showed negligible matrix effects by QuEChERS (< ± 20%) with GC-µECD/NPD.

To check the amount of co-extractives, blank matrices were studied. The GC-µECD/NPD dual detection...
chromatogram of a matrix (Fig. 6) shows the most co-extractives in the LSE extract. QuEChERS gave lower baseline noise and drift compared to other extracts because acetonitrile extracts relatively small amounts of non-polar fatty acids and waxes and the strongly adsorbent GCB was the cleanup.

3.4 Applications to real sample analysis

The results of the method applied to 1085 tobacco samples from southeastern Poland are in Table 2. 53.7% (583) of the samples were without residues. Pendi-methalin (67.5%), cypermethrin (17.7%), boscalid (16.4%) and metalaxyl (12.2%) were the most frequently detected.

Table 2: Pesticide residues detected in real tobacco samples (2012 – 467; 2013 – 618 samples).

| Active substance | Number of samples with residues (percentage of samples with residues) | Range of detected residues (mg kg⁻¹) | LOQ* (mg kg⁻¹) | GRLs** (mg kg⁻¹) |
|------------------|---------------------------------------------------------------|--------------------------------|---------------|-----------------|
|                  | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 |
| Acetamiprid      | 3 (0.7) | – | 0.02–0.30¹ | – | 0.01 | 2.5 |
| Azoxy-strobin    | 1 (0.2) | 3 (0.5) | 3.85² | 0.03–3.55² | 0.01 | – |
| Benalaxyl        | 3 (0.7) | – | 0.07–0.20 | – | 0.01 | 2.0 |
| Boscalid         | 67 (14.3) | 13 (2.1) | 0.01–2.39¹ | 0.02–0.86¹ | 0.008 | – |
| Chlorpyrifos     | 18 (3.9) | 12 (1.9) | 0.01–0.10 | 0.02–0.12 | 0.005 | 0.5 |
| Cypermethrin     | 41 (8.8) | 55 (8.9) | 0.01–0.08 | 0.01–2.33² | 0.01 | 1.0 |
| Dimethoxystrobin | 31 (6.6) | 2 (0.3) | 0.02–1.16¹ | 0.05–0.65¹ | 0.01 | – |
| Metalaxyl        | 51 (10.9) | 8 (1.3) | 0.03–0.30 | 0.06–0.31 | 0.01 | 2.0 |
| Iprodione        | 3 (0.7) | – | 0.07–0.10¹ | – | 0.01 | 0.25 |
| Pendimethalin    | 239 (51.2) | 101 (16.3) | 0.02–0.30 | 0.01–2.20¹² | 0.01 | 5.0 |

*LOQ-Limit of quantification
**GRLs-Guidance Residue Levels developed by the CORESTA Agro-Chemical Advisory Committee
¹The substance not recommended for tobacco protection
²The residue of substance exceeded GRLs

Figure 6: Dual detection chromatogram of tobacco matrix using LSE (red), MSPD (blue) and QuEChERS (green) by GC (a) µECD, (b) NPD.
The highest concentrations were found for azoxystrobin (3.55 mg kg\(^{-1}\)), cypermethrin (2.33 mg kg\(^{-1}\)) and pendimethalin (5.20 mg kg\(^{-1}\)) – all above recommended values.

Samples (502, 46.3%) containing one, two and up to 6 residues were noted. Ten pesticides were found most frequently in combination with one or more other residues. A single residue was detected in 35.0% of the samples, and two in 71%. In 3.0% and 0.7%, three and four residues were found, respectively. Five compounds were found in 0.5% with the highest total concentration of 0.34 mg kg\(^{-1}\). In 0.6% of the samples, six compounds were detected with the highest total concentration of 0.19 mg kg\(^{-1}\): boscalid (0.01 mg kg\(^{-1}\)), chlorpyrifos (0.02 mg kg\(^{-1}\)), cypermethrin (0.04 mg kg\(^{-1}\)), dimoxystrobin (0.02 mg kg\(^{-1}\)), metalaxyl (0.05 mg kg\(^{-1}\)) and pendimethalin (0.05 mg kg\(^{-1}\)).

The list of registered plant protection products in Poland includes about 20 items approved for tobacco, based on the active ingredients cypermethrin, clomazone, imidacloprid, mancozeb, metalaxyl, and propamocarb [32]. Some samples contained pesticides not legally registered for tobacco (e.g. acetamiprid, azoxystrobin, boscalid, dimoxystrobin, iprodione, pendimethalin) from preparations registered for other crops.

Pesticides were confirmed by GC-MS/MS in MRM mode. This is important when identification is supported by only a single ECD or NPD detector. For example, benalaxyl and metalaxyl gave a signal on only the NPD but were confirmed by MS/MS. Typical chromatograms of a real QuEChERS sample extract by GC µECD, NPD and MS/MS are shown Fig. 7.

### 4 Conclusions

QuEChERS has significant advantages over classical LSE and MSPD. QuEChERS not only greatly reduces solvent consumption, reducing cost and time, but recoveries were better and all analytes were extracted. QuEChERS was also more sensitive due to its cleaner extracts than those of LSE and MSPD. It avoids blending, filtration and repeated solvent transvers; 30 QuEChERS samples can be prepared in an 8-h workday, but only 20 LSE or MSPD samples.

QuEChERS meets the requirements for pesticide residue determinations in tobacco. It allows quantitation by GC-µECD/NPD and confirmation by GC/MS/MS. The linearity, sensitivity and reproducibility were quite good. The LODs were much lower than the CORESTA GRLs. The procedures could be used in commercial analysis for monitoring pesticide residues on tobacco.

**Acknowledgments:** This work was supported by the Ministry of Science and Higher Education (Ministerstwo Nauki i Szkolnictwa Wyższego), project ID: SBI-05. The authors are very grateful to Mrs. Teresa Janowicz for research assistance.
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Supplemental Material: The online version of this article (DOI: 10.1515/chem-2015-0129) offers supplementary material.