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Multiresidue pesticide analysis in nutraceuticals from green tea extracts by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry

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ABSTRACT

A new analytical method was developed and validated for simultaneous analysis of 423 pesticides, isomers, and pesticide metabolites in nutraceutical products obtained from green tea (*Camellia sinensis*) extract. Response surface methodology was employed to optimize a generic extraction method. The automated extraction procedure was achieved in a simple disposable pipet extraction. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry was used for the separation and detection of all the analytes. The method was validated by taking into consideration the guidelines specified in European SANCO/12571/2013 Guideline 2013 and Commission Decision 2002/657/EC. The extraction recoveries were in a range of 81.6–113.0%, with coefficient of variation <6.4%. The limits of decision for the analytes are in the range 0.04–4.15 μg kg^{-1}. The detection capabilities for the analytes are in the range 0.07–6.92 μg kg^{-1}. The 423 compounds behave dynamic in the range 0.1–200 μg kg^{-1} concentration, with correlation coefficient >0.99. This validated method has been successfully applied on screening of pesticide residues in one hundred and twenty-four different commercial nutraceutical products from green tea extract, and methamidophos, resmethrin, propoxur, tridemorph, ethiofencarb, flamprop isopropyl, furalaxyl, bifenthrin and fenpropathrin were detected in a few samples tested in this study.

1. Introduction

Currently, nutraceuticals are becoming a part of the daily diet because the incidence of lifestyle diseases worldwide, the life expectancy and inadequate nutrition due to current habits. Green tea [*Camellia sinensis* (L.) Kuntze] is one of the most popular and extensively used dietary supplements in the United States. Green tea extracts, containing tea solids including amino acids, caffeine and several polyphenols with antioxidant properties [1]. The United States Department of Health and Human Services explains that green tea is produce from the non-fermented leaves of the plant of *C. sinensis* [2,3]. The extracts of green tea can be presented in tablets or capsules as nutraceuticals. The nutraceuticals from green tea extracts can be used to prevent and treat some diseases, contributing to mental alertness, stress reliever, skin protection from sun damage, and lowering cholesterol levels [4–6].

Considering that nutraceutical products come from an herbal concentrate and this could be treated with pesticides in order to prevent insects and fungus attacks, there is evidence that some botanical ingredients used in nutraceuticals may be contaminated with pesticide residues [7,8]. Maximum residue levels (MRLs) for pesticide in every food and animal feed have been defined by different international organizations such as the Food and Agriculture Organization of the United Nations (FAO), the European Commission and the United States Environment Protection Agency (US EPA). For instance, the Regulation EC/396/2005, which includes MRLs, only concerns raw material but it does not include nutraceutical products [9–12]. The presence of pesticides, isomers, and pesticide metabolites in the nutraceuticals indicates that monitoring methods should be established in order to assure the safety of these products, considering that future legislation could establish tolerances and regulations.

In this sense, validated methods are needed for analyzing pesticides, isomers, and pesticide metabolites in nutraceuticals, but the complexity of these matrices presents difficulties in their

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analysis. There are few articles describing the analysis of nutraceuticals, and up to our knowledge, there is only one focused on green tea extract [13,14]. Several procedures have been developed for the determination of pesticide residues in green tea during harvest, post-harvest, and final consumer products [15–19]. Nutraceuticals from green tea extracts are dried and concentrated, which creates a greater challenge to the analysis because several interferences from the matrix can be coextracted with the target compounds, and sensitive instrumentation is needed for detecting trace levels of pesticides, isomers, and pesticide metabolites. Quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction is the most successful extraction procedure for determination of multiresidue pesticides in green tea-related matrices. Normally, most of these methods are focused on specific groups of residues, not being suitable for wide-scope multiresidue analysis [20–24]. To be able to analyze multiresidue pesticide with a wide variety of physicochemical properties simultaneously, non-selective, generic sample-preparation procedures are applied. The most frequently reported generic sample-preparation methods are "dilute and shoot" and QuEChERS methods [25–27]. A clear drawback of these strategies is the occurrence of abundant matrix effects, which compromise method selectivity, detection limits, maintenance frequency and quantitative aspects [28,29]. Nevertheless, the lack of selectivity in generic sample preparation can be compensated by selectivity in instrumental analysis. Several review papers clearly report that comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–TOFMS) offers substantial advantages over conventional one-dimensional GC for the analysis of trace compounds in complex matrices: increased peak capacity, generation of unique structured chromatograms, enhanced resolution, and an ability to reduce the interferences related to the matrix [30–32].

The focus of the present research is development and validation of efficient multiresidue methods for the analysis of pesticides, isomers, and pesticide metabolites in the nutraceuticals from green tea extracts using automated QuEChERS followed by GC × GC–TOFMS analysis. Response surface methodology was applied to study the behavior of the main generic extraction variables. The 423 pesticides, isomers, and pesticide metabolites were chosen based on EPA guidelines and their presence and persistence in the nutraceuticals from green tea extracts. Once the method was validated, it was used to analyze commercial nutraceutical products for incurred pesticide residues.

2. Experimental

2.1. Chemicals and reagents

Pesticide, isomer, and pesticide metabolite analytical standards were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), Sigma-Aldrich (Steinheim, Germany), Fluka (Buchs, Switzerland), LGC Standards (Teddington, UK) and Witega (Berlin, Germany). Stock solutions of individual pesticide standards were prepared, depending on the specific solubility properties, by dissolving 250–1000 mg of pesticide in 25 mL of toluene, acetone (ACE), methanol (MeOH) or acetonitrile (MeCN) for calibration and fortification standards, and stored at −20 °C in the dark. A multi-compound working standard solution (100 mg L^{-1} concentration of each pesticide) was prepared by dilution of the stock solutions with acetone and stored under refrigeration at 4 °C. Pesticide-grade toluene, ACE, MeOH and MeCN were sourced from J.T. Baker (Deventer, Holland). A [D_8]4,4-dichloro-2,2-bis(*p*-chlorophenyl) ethylene (*p*,*p*′-DDE-d_8) solution (10 mg L^{-1}) was also prepared as internal standard (IS) in the same way as the stock standard solutions. Anhydrous magnesium sulfate ($MgSO_4$), acetic acid and sodium acetate were of analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany). Empty 1 mL disposable pipet extraction (DPX) tips were purchased from Gerstel (Columbia, MD, USA). Ultrapure Water (resistivity, 18.2 MΩ) was purified on a Milli-Q Plus apparatus (Millipore, Brussels, Belgium).

2.2. Instrumentation

The GC × GC–TOFMS experiment were carried out on a Pegasus 4D instrument (Leco, St. Joseph, MI, USA), consisting of Agilent Technologies 7890A GC (Palo Alto, CA, USA), a dual-stage, four-jet cryogenic (liquid N_2) modulator and a Pegasus III time-of-flight mass spectrometer (Leco, St. Joseph, MI, USA). All automated sample preparation steps for the pesticide residues determination were performed using a dual-head MultiPurpose Sampler (MPS XL) equipped with a CF-100 dual position centrifuge, ^{m}VORX vortex, and DPX Option (Gerstel, Columbia, MD, USA). Liquid nitrogen used for cold pulses was automatically filled into a Dewar using a liquid leveler, which accessed to a 240 L Taylor-Wharton liquid nitrogen storage containers (Hamilton, OH, USA).

2.3. Analytical procedure

2.3.1. Sample preparation

A total of one hundred and twenty-four different green tea (*C. sinensis*) nutraceutical products (twenty-five tablets and ninety-nine capsules presentation) were obtained from different retail commercial outlets. Those samples found to contain no response at the retention times of reference compounds or metabolite were selected for use as negative controls. Green tea extract tablets or capsules were homogenized with an IKA Grinder Tube Mill (Staufenberg, Germany) for 60 s and stored at 4 °C prior to analysis. Samples for studying recoveries were spiked with standard solutions of the target compounds and left for 1 h before performing the extraction procedure.

For the extraction, 2.5 g of each sample was weighed in a 10 mL vial. Gerstel MPS XL configured for automated sample preparation. 5 mL volume of a MeCN/water solution (84/16, v/v) with 1% acetic acid was added as an extraction solvent and the tube was tightly capped and vigorously mixed for 1 min using the ^{m}VORX vortex at maximum speed. 1.00 g of anhydrous magnesium sulfate and 0.30 g of sodium acetate were added to the DPX tip (5 mL), to induce phase separation. 2 mL of sample was then aspirated into the DPX tip three times from the bottom followed by an equilibration time of 30 s. The DPX tip acts as a filter removing the salt particulate matter from the solution. After that, the solution was immediately vortex for 1 min, and then centrifuged for 5 min at 2264 × *g*. An aliquot of the final upper layer (990 μL) was transferred into a Mini-UniPrep vial with 10 μL of 10 mg L^{-1} IS for GC × GC–TOFMS analysis.

2.3.2. Experimental design for response surface methodology (RSM)

The variables involved in generic extraction were evaluated using response surface methodology (RSM), choosing the best fitting models. The optimal composition of the three variables (water content in the mixture solvent, extraction solvent volume and pH) was determined by using a central composite design (CCD) approach. In this work, the full CCD consisted of (1) a complete two-factorial design; (2) two axial points on the axis of each design variable at a distance of $\alpha = 2.000$ from the design center, and (3) n_0, center point ($n_0 > 1$). The actual variable was coded to facilitate multiple regression analysis. Hence, a total number of design points of $N = 2^k + 2k + n_0$ was used. The complete design consisted of 15 combinations including five degrees of freedom for calculation of errors with seven replicates of the center point in the experiments. Table 1 indicates the coded and CCD-processed variables