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QuEChERS-based method for the determination of carbamate residues in aromatic herbs by UHPLC-MS/MS

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ABSTRACT

A new reliable, fast and highly sensitive method based on ultra-high performance liquid chromatography tandem mass spectrometry has been developed and validated for the determination of 28 carbamates in aromatic herbs. A modified QuEChERS-based method was optimized for the extraction of carbamate residues from a wide variety of fresh herbal products. The proposed method allowed recoveries higher than 72%, achieving quantification limits of 2 μg kg^{-1}, therefore below maximum residue limits established for this type of samples. The combination of QuEChERS with UHPLC-MS/MS introduces a high-throughput methodology for the monitoring of these residues in this type of matrices scarcely explored. The analysis of the real samples revealed that several samples sold in the European Union and in the North West region of Cameroon contain pesticides in concentrations below the maximum residue limits.

1. Introduction

Aromatic vegetable products used for flavouring foods and drinks are known as 'spices' or condiments. These products are characterized by pungency, strong aroma, and sweet or bitter taste. Spices may derive from many parts of the plant such as bark, buds, flowers, fruits, leaves, rhizomes, roots, seeds, stigmas and styles or the entire plant (FAO, 2005). Herbs are subset of spices and refer to plants with aromatic leaves used to impart flavour and odour of foods, with sometimes, the addition of colour. Herbs have multi-purpose uses, including culinary (e.g., parsley and thyme), culinary and aromatic (e.g., basil, celery, rosemary and mint) and medicinal (peppermint and anise) (FAO, 2005; Kathe, Honnef, & Heym, 2003). Due to the increased demand, the world markets for spices and herbs are under constant expansion (FAO, 2005). For instance, the European Union (EU) market is the second largest one for seasonings, with spices and herbs consumption estimated at 520,000 tonnes in 2013 with a value of € 1.8 billion. Also worldwide seasoning and spices market was valued at US $12 billion in 2012 and is expected to grow at a compound annual growth rate of 4.8% to reach an estimated value of US $16.6 billion in 2019 (The 3rd CARIFORUM-EU Business Forum, 2015).

The growing demand of spices and herbs requires increased agricultural production. Sustained and extensive agricultural practices involve the use of chemicals including fertilizers and pesticides (Bryden, McKnight, & Westneat, 2005; FAO, 2000). Pesticide residues however may be transferred from plants to animals or humans through bioaccumulation in the food chain, direct contamination during farming activities, or ingestion of contaminated food (Manfo et al., 2012). Carbamates (CRBs) are one of the pesticide group widely used as insecticides or fungicides (Santaladchaiyakit, Srijaranai, & Burakham, 2012). They

Abbreviations: 3-CF, 3-hydroxy carbofuran; MeCN, acetonitrile; ALD, aldicarb; ALDSFN, aldicarb sulfoxide; ASL, asulam; BFU, benfuracarb; BTH, benthiocarb; CRB, carbamate; CAR, carbaryl; CBZ-BY, carbendazim-benomyl; CY, cymoxanil; DETH, diethofencarb; dSPE, dispersive solid phase extraction; ESI, electrospray ionization; EtOAc, ethylacetate; ETH, ethiofencarb; FEN, fenobucarb; FNX, fenoxycarb; FURA, furathiocarb; GBC, graphitized carbon black; ISO, isoprocarb; LOQ, limit of quantification; ME, matrix effect; MRL, maximum residue limit; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MTH, methiocarb; MTHSFN, methiocarb sulfone; MTHSFX, methiocarb sulfoxide; MTY, methomyl; MRM, multiple reaction monitoring; NP, napropamid; OX, oxamyl; PIR, pirimicarb; PIRDES, pirimicarb desmethyl; PR, promecarb; PRM, propamocarb; PSA, primary secondary amine; PX, propoxur; PY, pyraclostrobin; QqQ, triple quadrupole; QuEChERS, quick, easy, cheap, rugged, effective and safe; RT, retention time; S/N, signal to noise ratio; SPE, solid phase extraction; TH, thiodicarb; UHPL, ultra-high performance liquid chromatography; WHO, World Health Organization.

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are derived from carbamic acid, act as acetylcholinesterase inhibitors and prevent acetylcholine to build up. They can produce severe cholinergic toxicity following cutaneous exposure, inhalation, or ingestion, causing some effects in human such as headaches, vomiting, abdominal cramps, uncontrolled urination or defecation, and even a comatose state (Bjørling-Poulsen, Andersen, & Grandjean, 2008; Thompson, 2002). CRB residues have been found in plants and food commodities such as honey, banana, eggplant, strawberry, cauliflower, potatoes, cabbage, cucumber, carrot and sweet pepper (Santaladchaiyakit et al., 2012). However, little has been done in relation to the monitoring of pesticide residues in aromatic herbs, including CRB, despite their large cultivation and consumption by humans. Anyway, their control has been regulated by the European Union (EU) and maximum residue limits (MRLs) of pesticides have been established for this type of samples (Regulation of the European Parliament and of the Council, 2005).

The analysis of pesticide residues in herbal products is usually carried out using mass spectrometry (MS) or tandem mass spectrometry (MS/MS) detection coupled to gas chromatography (GC) or high performance liquid chromatography (HPLC) (Esturk, Yakar, & Ayhan, 2014; Sadowska-Rociek, Surma, & Cieślik, 2013). On the other hand, ultra-high performance chromatography (UHPLC) can be an interesting alternative for the determination of these residues in this type of samples because of the high resolution, reduced analysis time, less solvent consumption and increased sensitivity (Chen, Cao, & Liu, 2011; Moreno-González, Huertas-Pérez, Gámiz-Gracia, & García-Campaña, 2015; Zhang et al., 2010). Herbal products are composed of a mixture of phytochemical compounds including antioxidants, essential oils, vitamins, phytosterols, pigments and many other plant-derived nutrients (Parthasarathy, Chempakam, & Zachariah, 2008), which make them complex matrices in chromatographic analysis. Although techniques such as liquid–liquid extraction with various solvents followed by solid-phase extraction (SPE) have been traditionally used for determination of these contaminant residues in herbs (Fenoll, Hellin, Flores, Sotomayor, & Nicolas, 2008; Hajjo, Afifi, & Battah, 2007; Tuzimski, 2011), the quick, easy, cheap, effective, rugged and safe (QuEChERS) method is the procedure that has properly addressed the matrix complexity in the case of herbs. QuEChERS is a two steps method composed of an extraction step based on partitioning via salting-out involving the equilibrium between an aqueous and an organic layer, and the dispersive SPE (dSPE) step that involves further clean-up using combinations of $MgSO_4$ with different sorbents, such as C_{18}, graphitized carbon black (GCB) or primary-secondary amines (PSA), to remove interfering substances. It is effective for cleaning-up complex samples such as vegetables, fruits and herbal products and also enables the use of smaller volume of organic solvent (Arienzo, Cataldo, & Ferrara, 2013; Chen et al., 2011; Koesukwiwata, Lehotay, Miaoc, & Leepipatpiboon, 2010; Moreno-González et al., 2015; Zhang et al., 2010). Due to all its advantages, the QuEChERS method has been increasingly used and adopted as recommended method for determination of pesticide residues in fruits and vegetables, including CRBs (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; European Committee for Standardization, 2008).

The goal of this work has been to develop a fast, simple, selective, and efficient UHPLC-MS/MS method for the simultaneous determination of 28 CRB pesticides in fresh herbal products. QuEChERS methodology was used for sample treatment, and the clean-up step was optimized in order to minimize matrix effect and maximize recovery. The developed method was further applied for determination of CRB residues in a great variety of aromatic herbs from Granada (Spain) and Santa and Bamenda (Cameroon).

2. Materials and methods

2.1. Chemicals and reagents

The solvents acetonitrile (MeCN), methanol (MeOH) and ethylacetate (EtOAc) were from VWR Prolabo (Leuven, Belgium), and formic acid from Fisher Scientific (Geel, Belgium). Acetic acid ($\geqslant$99.8%) was purchased from Sigma Aldrich (St Louis, USA). NaCl ($\geqslant$99%) and anhydrous $MgSO_4$ were obtained from Panreac ITW Companies (Darmstadt, Germany). Bulk C_{18}, PSA and GCB were supplied by Agilent Technologies (Waldbron, Germany) while alumina was obtained from Supelco (Bellefonte, USA). All these chemicals were of analytical reagent grade and the solvents were LC–MS grade.

Ultrapure water (18.2 MΩ cm^{-1}, Milli-Q plus system, Millipore, Bedford, MA, USA) was used throughout the work.

The CRB standards used in the study were propamocarb (PRM), asulam (ASL), aldicarb sulfoxide (ALDSFX), oxamyl (OX), carbendazim (CBZ), benomyl (BY), primicarb desmethyl (PIRDES), methiocarb sulfoxide (MTHSFX), 3-hydroxy carbofuran (3-CF), methiocarbsulfone (MTHSFN), cymoxanil (CY), aldicarb (ALD), primicarb (PIR), propoxur (PX), carbaryl (CAR), ethiofencarb (ETH), thiodicarb (TH), isoprocarb (ISO), fenobucarb (FEN), diethofencarb (DETH), methiocarb (MTH), promecarb (PR) and napropamid (NP), fenoxycarb (FNX), pyraclostrobin (PY), benthiocarb (BTH), benfuracarb (BFU), furathiocarb (FURA), all purchased from Fluka Analytical (St Louis, USA). Individual stock standard solutions of each compound were prepared by dissolving accurately weighted amounts in MeOH and were stored in the dark at 4 °C. In these conditions, they were stable for at least 4 months. Working standard solutions containing all the CRBs were freshly prepared by proper dilution of the stock standard solutions with MeOH.

Nylon syringe filters, 0.22 μm × 13 mm (Bonna-Agela Technologies Inc., Wilmington, USA) were used for filtration of sample extracts prior to the injection into the UHPLC system.

2.2. Instrumentation

UHPLC-MS/MS determination of CRBs was performed in an Agilent 1290 Infinity LC system using a C_{18} column (Zorbax Eclipse plus RRHD 50 × 2.1 mm, 1.8 μm) supplied by Agilent Technologies. The mass spectrometer measurements were performed on a QqQ mass spectrometer API 3200 (ABSciex, Toronto, ON, Canada) with electrospray ionization (ESI). The instrumental data were collected using the Analyst® Software version 1.5 with Schedule MRM™ Algorithm (ABSciex).

A centrifuge (Universal 320 model from Hettich; Leipzig, Germany), a nitrogen evaporator (System EVA-EC from VLM GmbH, Bielefeld, Germany), a mechanical shaker (model 384 from Vibromatic, Noblesville, USA), and a vortex (Genie 2 model from Scientific Industries; Bohemia, NY, USA) were also used.

2.3. Samples

Fresh herb samples were from Granada (Spain), Santa and Bamenda (Cameroon). Basil (*Ocimum basilicum*), spearmint (*Mentha spicata*), wild mint (*Mentha longifolia*), parsley (*Petroselinum crispum*), Chinese parsley (*Coriandrum sativum*), chives (*Allium schoenoprasum*), thyme (*Thymus vulgaris*) and salvia (*Salvia divinorum*) were obtained from normal or/and ecological local markets in Granada. Samples of commonly used parsley, celery (*Apium graveolens*), leek (*Allium ampeloprasum*), welsh onion (*Allium fistulosum*) and sweet pepper (*Capsicum annuum*), were collected in local