Rapid analysis of tristyrylphenol ethoxylates in cucumber-field system using supercritical fluid chromatography–tandem mass spectrometry

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ABSTRACT

Tristyrylphenol ethoxylates (TSPxEOs), nonionic surfactant adjuvants, are widely used to replace alkylphenols in many agricultural formulations. Herein, a rapid and sensitive method based on a modified quick, easy, cheap, effective, rugged, and safe "QuEChERS" and supercritical fluid chromatography–tandem mass spectrometry (SFC–MS/MS) method was developed to quantify TSPxEOs in cucumber, leaves, and soil. Following optimization, the tested oligomers were fully separated within 8 min. The matrix-matched calibration standards showed satisfactory linearity with coefficients of determination (R^2) ≥ 0.991. The limits of quantification (LOQs: 0.001–0.048 µg kg^{-1}) were excellent for TSPxEOs (x = 6–29). The average recoveries at three (low, medium, and high) fortification levels were ranged between 62.5% and 112.9% with relative standard deviations (RSDs) ≤ 20.0% for TSPxEOs (x = 6–29) in various matrices. The method was successfully applied to determine TSPxEOs residual levels in field-incurred samples. Conclusively, the developed method is versatile and could be used for monitoring TSPxEOs in food and environmental samples.

1. Introduction

Tristyrylphenol ethoxylates (TSPxEOs, where x is the number of ethoxylate units) are a class of nonionic surfactants commonly used as adjuvants in agrochemical formulations (Glaubitz & Schmidt, 2015). With the phase-out of nonylphenol-based surfactants, TSPxEOs are the most widely used category in agrochemical applications produced in large volume. It is therefore, expected that the residual levels of parent compounds and/or their degradation products (metabolites) are reaching the environment (soil and natural water); posing toxicity to aquatic organisms. For instance, the 48 h-50% effective concentration (EC$_{50}$) values of TSPxEOs were found to be 16.40 to 36.13 mg L^{-1} in *Daphnia magna* (Li, Li, Chen, Li, & Liu, 2013) and the 120 h – 50% lethal concentration (LC$_{50}$) values in zebrafish embryo were 84.46 mg L^{-1}

(Liu et al., 2014). Moreover, juvenile zebrafish might develop teratogenic effects while exposing to TSPxEOs (Liu et al., 2014). It was reported that styrenated phenols (intermediates in the production and degradation of TSPxEOs) might create estrogenic activities (Ogawa et al., 2006) or acute toxicity for *Oryzias latipes* (fish) and growth inhibition for *Pseudokirchneriella subcapitata* (algae) as well (Furuhama, Hasunuma, & Aoki, 2015a, 2015b).

Concerns about the potential effects of TSPxEOs on food safety and ecosystems necessitated development of analytical methods for their separation, identification, and quantification in food commodities and environment samples. TSPxEOs are chemical mixtures of oligomers with different numbers of ethoxylate units (Fig. S1 in Supplementary materials) (Glaubitz & Schmidt, 2015); properties, toxicities, and environmental behavior of each oligomer may be related to structural

details. So far, literature review identified a very limited number of articles for analysis of TSPxEOs as following: Glaubitz and Schmidt (2015) characterized TSPxEOs from different suppliers using reversed-phase liquid chromatography-time-of-flight mass spectrometry (RPLC-TOF-MS) and multivariate data analysis. Nevertheless, the whole range of ethoxylate oligomers were coeluted in a single peak, leading to competitive ionization, isobaric interferences, and quantification errors. Gargano, Duffin, Navarro, and Schoenmakers (2016) developed a combination of hydrophilic interaction liquid chromatography (HILIC) and RPLC with a diode-array detector (DAD) for two-dimensional separation and determination of TSPxEOs. However, it was challenging to apply the method for trace analysis in some complicated matrices (such as vegetables, fruits, and corps), owing to the inability to eliminate certain endogenous interferences and the lack of high specificity and sensitivity by DAD detector. Zhang et al. (2017) evaluated a pseudo reversed-phase liquid chromatography–tandem mass spectrometry (RPLC–MS/MS) for the analysis of TSPxEOs (x = 5–18). However, the major pitfall was the poor stability of the chromatographic performance, owing to binding the silica column to water (Shao, Hu, & Yang, 2002).

Supercritical fluid chromatography (SFC) is a promising alternative to liquid chromatography (LC) or gas chromatography (GC); based on high efficiency, high capacity and rapid chromatographic separation with better resolution, short analysis time, and low solvent consumption (Tan et al., 2017; Zhou, Du, & Zhang, 2014). Tandem mass spectrometry (MS/MS) can specifically identify and exactly quantify the analytes of interest at lower concentrations using multiple reaction monitoring (MRM) mode (Ross & Liao, 2015). SFC–MS/MS has been applied for analysis of pesticides and their metabolites (Chen et al., 2014, 2015; Cheng et al., 2017; Liu et al., 2015, 2016; Pan et al., 2016; Tao et al., 2014, 2018), organic contaminants (Bieber, Greco, Grosse, & Letzel, 2017; Jiang et al., 2017; Jumaah et al., 2017; Mullin et al., 2015; Yeung, Stadey, & Mabury, 2017), and pharmaceutics (Camacho-Muñoz, Kasprzyk-Hordern, & Thomas, 2016; Gonzalez-Marino, Thomas, & Reid, 2018) in food and environmental samples. The extraction of TSPxEOs from samples is also pivotal before chromatographic analysis (Olkowska, Polkowska, & Namiesnik, 2011). The quick, easy, cheap, effective, rugged, and safe "QuEChERS" approach had been proposed for the analysis of pesticides in foods in 2003 by Anastassiades, Lehotay, Štajnbaher, and Schenck (2003), and currently being a robust and attractive procedure for analysis of different organic compounds in various matrices, because of flexibility, simplicity, and rapidity (Bruzzoniti et al., 2014; González-Curbelo et al., 2015). Several studies (Chen & Mullin, 2013, 2014, 2015; Jiang et al., 2017) have confirmed that this method can be used for extraction of alkylphenol ethoxylates (APEOs) and trisiloxane surfactants from leafy vegetables and beehive samples. Consequently, the aim of the present study was to develop a rapid and sensitive analytical method for simultaneous identification and quantification of TSPxEOs oligomers (x = 6–29) in cucumber, leaves, and soil using a modified QuEChERS and SFC-MS/MS. To the best of the author's knowledge, this is the first report on the simultaneous analysis of TSPxEOs oligomers in foods and environmental samples.

2. Materials and methods

2.1. Chemicals and materials

Due to the lack of commercially available reference materials, TSPxEOs standard was prepared through purification of agricultural emulsifier 601# (AE-601, generously gifted by Jiangsu Zhongshan Chemical Co., Ltd., Nanjing, Jiangsu, China) using preparative liquid chromatography. Purification and characterization details of TSPxEOs standards are shown in Supplementary Text S1 and Figs. S2–S4. Stock solution ($1 \, g \, L^{-1}$) of TSPxEOs reference standards were prepared in methanol and stored at $-20 \, °C$ in a dark amber vial. Carbon dioxide

(CO_2) (for SFC), argon, and nitrogen gases (for mass spectrometry) were of high purity (99.999%) and produced by Beiwenqiti (Beijing, China). Ammonium acetate (NH_4Ac, LC-MS grade) was obtained from Sigma Aldrich (Steinheim, Germany). LC-MS grade acetonitrile (ACN) and methanol (MeOH) were supplied by Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water ($\geq 18.2 \, M\Omega \, cm$) produced by a Milli-Q purification system (Millipore, Bedford, MA, USA) was used in all experiments. Multi-walled carbon nanotubes (MWCNTs) with external diameters < 8 nm was acquired by Nanjing XFNANO Materials Tech (Nanjing, China). Primary secondary amine (PSA, 50 μm) and octadecyl-modified silica (C18, 50 μm) were procured from SUPELCO (Bellefonte, PA, USA). Analytical grade anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were bought from Sinopharm Chemical Reagent (Shanghai, China).

2.2. Sample preparation

Blank samples (cucumber, leaves, and soil) collected from a local organic farm were used for method validation. Samples were cut into small pieces and comminuted with liquid nitrogen, while soil samples were air-dried, crushed, and sifted through a 2-mm sieve. All samples were packed in seal aluminum foil bags, labelled, and then stored at $-20 \, °C$ pending analysis.

Sample preparation was conducted using QuEChERS method (Anastassiades et al., 2003) with some modifications. Ten grams of thawed samples (cucumber, cucumber leaves, and soil) were weighed into 50 mL polypropylene centrifuge tubes (Eppendorf, Hamburg, Germany). For recovery studies, the blank samples were spiked with 100 μL working standard solutions, vortex-mixed (Vortex-Genie 2, Scientific industries, Bohemia, NY, USA) for 30 s and left to stand for 1 h at room temperature before extraction. Afterward, 10 mL ultrapure water (for leaves and soil) and 10 mL ACN were added. The samples were vigorously vortexed for 1 min (5 min for soil) at full speed, and then were ultrasonically extracted using a KQ-500DB ultrasonic water bath (300 W, 40 kHz, Kunshan Ultrasonic Instrument Co., Ltd, Jiangsu, China) for 10 min. Subsequently, 4 g anhydrous $MgSO_4$ and 1 g NaCl were added to each tube. Following vigorous vortexing at full speed for 1 min, the samples were centrifuged at 6000 rpm (4025 g *RCF*) for 5 min using a Sorvall ST 16R centrifuge (Thermo Fisher Scientific, Osterode, Germany). Thereafter, 1.0 mL supernatant was transferred into a 2.0 mL centrifuge tube containing 150 mg anhydrous $MgSO_4$ and sorbent mixtures (5 mg MWCNTs for cucumber; 10 mg MWCNTs, 50 mg PSA, and 50 mg C18 for cucumber leaves; 25 mg PSA and 25 mg C18 for soil). After vortex-mixing for 30 s, the tubes were centrifuged at 10000 rpm (8944 g *RCF*) for 5 min. The resulting supernatant was filtered into a vial (Agilent, USA) through a 0.22-μm pore size polypropylene syringe filter (13 mm diameter, Bonna-Agela Technologies Inc., Tianjin, China) for SFC-MS/MS analysis.

2.3. SFC–MS/MS analysis

The SFC–MS/MS analyses were conducted on a Nexera UC chromatographic system (Shimadzu, Kyoto, Japan), consisting of a LC-30AD$_{SF}$ CO_2 pump, a LC-20AD$_{XR}$ modifier pump, a LC-20AD$_{XR}$ make-up solvent pump, a SFC-30A back pressure regulator, a DGU-20A$_{5R}$ degasser, a SIL-30AC sample manager, a CTO-20AC column manager, and a CBM-20A system controller, coupled with a LCMS-8050 triple quadrupole mass spectrometer equipped with a heated electrospray (ESI) interface (Shimadzu, Kyoto, Japan). The LabSolutions software (ver. 5.82 SP1, Shimadzu) was used for instrument control, data acquisition, and analysis. The SFC separation conditions were as follows: column, Acquity UPC^2 BEH column (3.0 mm i.d. × 100 mm, 1.7 μm, Waters, Dublin, Ireland) with a VanGuard pre-column (2.1 mm i.d. × 5 mm, 1.7 μm); injection volume, 1 μL; column temperature, 40 °C; back pressure, 14 MPa; mobile phase, CO_2 (A) with modifier MeOH(B); the gradient ($1.5 \, mL \, min^{-1}$), 0–1 min, 5–15% B; 1–5 min, 15–35% B;