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Analytical Methods

Consequences of the matrix effect on recovery of dinotefuran and its metabolites in green tea during tandem mass spectrometry analysis

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ARTICLE INFO

ABSTRACT

Determining the residues of dinotefuran and its metabolites (MNG, UF, and DN) is highly problematic because of their polar characteristics. Additionally, tea contains many compounds that can interfere with residue analysis. Thus, the aim of the present study was to refine the extraction method that assures good recoveries for dinotefuran and its metabolites and removes most of the matrix components in green tea using liquid chromatography–tandem mass spectrometry (LC/MS/MS). We attempted to increase the extraction efficiency of the QuEChERS method by selecting the appropriate solvents among ethyl acetate, acetone, isopropanol, 25% methanol in acetonitrile, and methanol. We found that methanol was the best extraction solvent for dinotefuran and its polar metabolites in dry green tea samples; however, due to a limitation of an appropriate partitioning salt, acetonitrile was used as the extraction solvent. Matrix enhancement and suppression effects were observed for all analytes, which made the recovery rates variable. DN recovery was <70% when compared with matrix-matched calibration, whereas it was within the acceptable range (70–120%) when compared with solvent calibration. The opposite was observed for MNG and dinotefuran due to a matrix suppression effect. UF recovery was consistent in both matrix-matched and solvent calibrations despite having little suppressive effect. The method was successfully applied and dinotefuran and its metabolite residues were found in field-incurred green tea samples. The current findings suggest that using methanol as an appropriate QuEChERS solvent for problematic polar pesticides and investigating a suitable partitioning salt would considerably strengthen the practical impact of such data.

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1. Introduction

Green tea (*Camellia sinensis*) has spread across Asia (including the Republic of Korea and Japan) and several countries in North Africa and the Middle East after originating in China (Graham, 1992; Kim, Jombart, Valentin, & Kim, 2013). Several studies have reported that green tea extract has antioxidant, antibacterial, antiviral, anticarcinogenic, and antimutagenic functions (Jian, Xie, Lee, & Binns, 2004; Lu, Lee, Mau, & Lin, 2010; Setiawan et al., 2001; Wu,

Yu, Tseng, Hankin, & Pike, 2003). Additionally, green tea is esteemed as a mental stimulant that promotes stamina (Shen, Han, & Ryu, 2014). Thus, quality control and safety of green tea have received a great deal of attention (Naldi et al., 2014). Pesticide application during tea cultivation and/or storage represents a common practice for pest and plant disease control (Peter, 2006). However, residues of active ingredients and/or metabolites may occur in the final product under certain circumstances. Maximum residue limits have been established for many pesticides by national and international organizations because of the potential health risk for consumers resulting from acute and/or chronic dietary exposure (Cajka et al., 2012).

Because of selectivity, neonicotinoid insecticides show good activity against pest insects resistant to other classes of insecticides with comparatively lower risk to the environment, mammals, and other non target organisms (Jeschke & Nauen, 2008; Nauen,

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Ebbinghaus-Kintscher, Salgado, & Kaussmann, 2003). Dinotefuran is a third generation neonicotinoid insecticide, which is widely used to control various harmful pest species, including plant bugs, plant hoppers, green rice leafhoppers (Watanabe, Baba, & Miyake, 2011). Neonicotinoids are used extensively in green tea cultivation because of their excellent properties against the green leafhopper, *Empoasca flavescens fabricius*. Ford and Casida (2008) showed that neonicotinoids are converted to numerous metabolites in plants. Dinotefuran is metabolized in plants as are other neonicotinoids, and the reported major metabolites are MNG, UF, and DN (Watanabe et al., 2011). The toxicity of these metabolites and the parent compound are approximately the same; however, the metabolites are more mobile and persistent than that of the parent compound. Analyses of the metabolites are highly challenging, as they are less sensitive to conventional detectors or are unstable during the extraction and cleanup procedures. Most of the existing analytical methods were established for determining the parent compound, dinotefuran, with very few effective methods for metabolite analysis. In our earlier study (Rahman et al., 2013), we tested the feasibility of a high performance liquid chromatography–UV detector method (HPLC–UVD) for analyzing dinotefuran and its metabolites in melon. Simultaneous extraction and sequential purification through solid-phase extraction (SPE) cartridges were carried out for different compounds using separate detection due to the differences in wavelength. The SPE cartridge cleanup procedure and separate detection make the analysis more time consuming. Green tea is a complex matrix consisting of large amounts of polyphenols and pigments, which can be easily co-extracted, making the analysis difficult due to interference by a broad spectrum of co-extractants (Lozano et al., 2012; Nkhili et al., 2009).

The QuEChERS (quick, easy, cheap, effective, rugged, and safe) sample preparation method was introduced in 2003 to improve analytical quality and laboratory efficiency when determining multiple pesticide residues in fruits and vegetables using a dispersive-SPE technique (Anastassiades, Lehotay, Stajnbaher, & Schenk, 2003). The method was originally developed based upon mass spectrometry detection coupled with gas chromatography or liquid chromatography (LC) according to the analyte polarity and amenability. The limitation of this method was the use of either acetonitrile (ACN) or ethyl acetate (EtOAc) as the extraction solvent, which are sometimes unsuitable for extracting polar pesticides. Kamel (2010) refined a methodology for detecting neonicotinoids along with dinotefuran and its two metabolites (UF and DN) in honey and honey bees following the QuEChERS extraction method, SPE cartridges for purification, and LC–tandem mass spectrometry (LC/MS/MS) analysis. In that study, DN provided unstable recovery and MNG was not analysed.

LC/MS/MS techniques to determine neonicotinoids in tea and other agricultural samples have been investigated (Liu et al., 2010; Xie et al., 2009, 2011); however, the analyses suffered from analyte ionisation suppression or enhancement via co-eluting compounds in the electrospray ionisation (ESI) source, known as the matrix effect. The matrix effect refers to the change in ionisation efficiency in the presence of other compounds. The exact mechanism of matrix suppression or enhancement is unknown; however, it is assumed that matrix components influence the effectiveness of the ionisation process at the atmospheric pressure ionisation interface (causing a mutual positive or negative effect in the number of ions formed from the target analyte). Another hypothesis given in literature refers to the radius of droplets of gas-phase ions emitted (Thompson & Inbarne, 1979). If samples contain non-volatile matrix components, droplets are prevented from reaching their critical radius and surface field; hence, ionisation efficiency decreases, which, in turn, reduces the analyte signal (Thompson & Inbarne, 1979). Matrix components may also influence the effectivity of ion formation during the ionisation process by altering the surface tension of electrospray droplets and by building adduct ions or ion pairs with the analytes (Qu, Wang, & Luo, 2001). The matrix effect strongly depends on the chemical nature of the analyte. Notably, the ionisation efficiency of polar compounds is more influenced by co-eluting compounds than ionisation of less polar compounds (Taylor, 2005). Some instrumental parameters, e.g., ionisation source (APCI vs. ESI) (King, Bonfiglio, Fernandez-Metzler, Miller-Stein, & Olah, 2000), ionisation mode (Thurman, Ferrer, & Barcelo, 2001), and flow rate (Kloepfer, Quintana, & Reemtsma, 2005) also have an impact on the extent of matrix effect.

Recovery is affected by the matrix enhancement or suppression effect when compared with matrix free solvent calibration during method validation. Therefore, SANCO recommended using matrix-matched calibration for consistent recovery (SANCO, 2009). A silent practice is currently ongoing by matching recovery sometimes with solvent and sometimes with matrix-matched calibration according to the demand of the method (enhancement or suppression) to maintain recovery within the internationally acceptable range (70–120%). Matrix-matched calibration always provides the exact recovery resulting from the method. Notably, solvent calibration is not being discarded. If the method can be validated by solvent calibration in the acceptable range, the residues will be quantified in the same acceptable way.

Determining the residues of dinotefuran and its metabolites (MNG, UF, and DN) is highly problematic because of their polar characteristics. Additionally, tea contains many compounds that can interfere with residue analysis. Therefore, the aim of this study was to improve the recovery of dinotefuran and its metabolites in green tea after a slight modification of QuEChERS using LC/MS/MS and to investigate the consequences of the matrix effect on recovery in dry green tea and a green tea infusion.

2. Experimental

2.1. Reagents and chemicals

Standard dinotefuran ((RS)-1-methyl-2-nitro-3-(tetrahydro-3-furylmethyl)guanidine; 98%) and its metabolites; MNG (1-methyl-2-nitroguanidine; 99.5%), UF (1-methyl-3-(tetrahydro-3-furylmethyl)urea; 99.7%), and DN (1-methyl-3-(tetrahydro-3-furylmethyl)guanidine; 99.5%) were purchased from Mitsui Chemicals Agro, Inc. (Tokyo, Japan). HPLC grade ACN and methanol (MeOH) were obtained from Burdick and Jackson (SK Chemical, Ulsan, Republic of Korea). Analytical-grade anhydrous magnesium sulphate ($MgSO_4$), sodium acetate (NaOAc), formic acid, acetic acid, and triethylamine (TEA) were obtained from Junsei Chemicals Co., Ltd. (Tokyo, Japan). Primary secondary amine (PSA), and C_{18} dispersive sorbents were supplied by Agilent Technologies (Santa Clara, CA, USA). Water was distilled through a Milli-Q apparatus (ULTiMA DUO 200 (COMBI), Balmann Tech, Daegu, Republic of Korea) prior to use.

Stock standard solution ($100\ mg\ L^{-1}$) of dinotefuran and its metabolites (MNG, UF, and DN) were prepared individually in methanol. An intermediate standard solution was prepared after mixing all of the stock standard solutions and diluting with 1% MeOH in water. The working standard solution was prepared following serial dilution of the intermediate standard solution with 1% MeOH in water. The concentration ranges for dinotefuran, MNG, UF, and DN were 0.01–6 mg/kg, 0.05–5 mg/kg, 0.004–1.4 mg/kg, and 0.01–3 mg/kg in green tea and 0.01–8 mg/kg, 0.05–5 mg/kg, 0.004–1.4 mg/kg, and 0.01–3 mg/kg in tea infusion, respectively.

2.2. Field experimental design

Field trial was conducted in a green tea field located at Bosung area, Jeonnam Province, Republic of Korea. Four plots (each plot