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Original Research Article

Trace enantioselective determination of triazole fungicides in honey by a sensitive and efficient method

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ABSTRACT

This work presents, for the first time, the simultaneous enantioselective determination of triazole fungicides (paclobutrazol, myclobutanil, diniconazole, and epoxiconazole) in honey samples by chiral liquid chromatography-tandem mass spectrometry (LC–MS/MS). An efficient procedure, solid-phase extraction (SPE) coupled with dispersive liquid-liquid microextraction (DLLME), was used for the extraction, purification, and preconcentration of target analytes. In order to obtain high extraction efficiency, several experimental parameters including the type of SPE cartridge, the pH of sample, the type and volume of elution solvent and extraction solvent, and salt concentration were optimized. Upon optimal conditions, good sensitivity was achieved with limits of detection ranging from 0.005 to 0.028 ng/g and limits of quantification ranging from 0.017 to 0.093 ng/g. Recoveries for eight enantiomers were satisfactory lying between 87.1% and 108.6% in four different honey samples. The intra- and inter-day relative standard deviations were all below 9.1%. This convenient, sensitive, and reliable method was applied to the enantioselective determination of triazole fungicides in 11 honey samples. As a result, the concentrations and enantiomeric fractions of paclobutrazol were determined successfully.

1. Introduction

Triazole fungicides, the most popular class of agricultural fungicides, are widely applied to fruits, vegetables, and cereals due to excellent antifungal activity (Kahle et al., 2008). However, because of the high chemical stability and low biodegradability, triazole fungicides may persist longer in environment (Bromilow et al., 1999; Lewis et al., 2006). Meanwhile, due to the lipophilic nature, they tend to accumulate in living organisms, and even can be transferred among various compartments of ecosystem. Studies have shown triazole fungicides may disturb endocrine activities in animals and human beings via inhibiting enzymes involved in the biosynthesis of steroid hormones (Taxvig et al., 2007). Furthermore, it was found that triazole fungicides have potential reproductive toxicity and hepatic toxicity (Rockett et al., 2006; Tully et al., 2006). Thus, monitoring the concentrations of triazole fungicides in food matrix is a crucial step in risk control.

It is worth noting that a large portion of triazole fungicides are chiral and generally contain one or two stereogenic centers. Although the enantiomers of a chiral compound have identical physical and chemical properties, in some cases they show different biological activity. For example, the S-enantiomer of diniconazole exhibits stronger plant growth regulating activity than the R-enantiomer, while the latter presents higher fungicidal activity (Furuta and Doi, 1994). Four optical isomers of paclobutrazol also show great differences in biological activity (Burden et al., 1987). Besides, the enantiomers may differ significantly in toxicity (Cheng et al., 2013). Similarly, their degradation (Liang et al., 2012; Zhang et al., 2011), transformation (Garrison et al., 2011; Kenneke et al., 2010), and bioaccumulation (Yu et al., 2012) are usually enantioselective. Enantioselectivity plays an important role in the environmental fate and ecological risk of a chiral compound. Consequently, it is of great significance to establish enantioselective analytical method for chiral triazole fungicides in food matrix to provide more accurate and reliable data for understanding their enantiomeric discrimination in these media, thereby facilitating their risk assessments and regulatory decisions.

Honey is consumed worldwide as a wholesome natural product due to its nutrition value. Pesticide residues, occurred in crop fields near the hive, can compromise the quality of honey and may further threaten human health. Because it can not only remain in the edible parts of crops, but also be transferred to honey through food chain transmission

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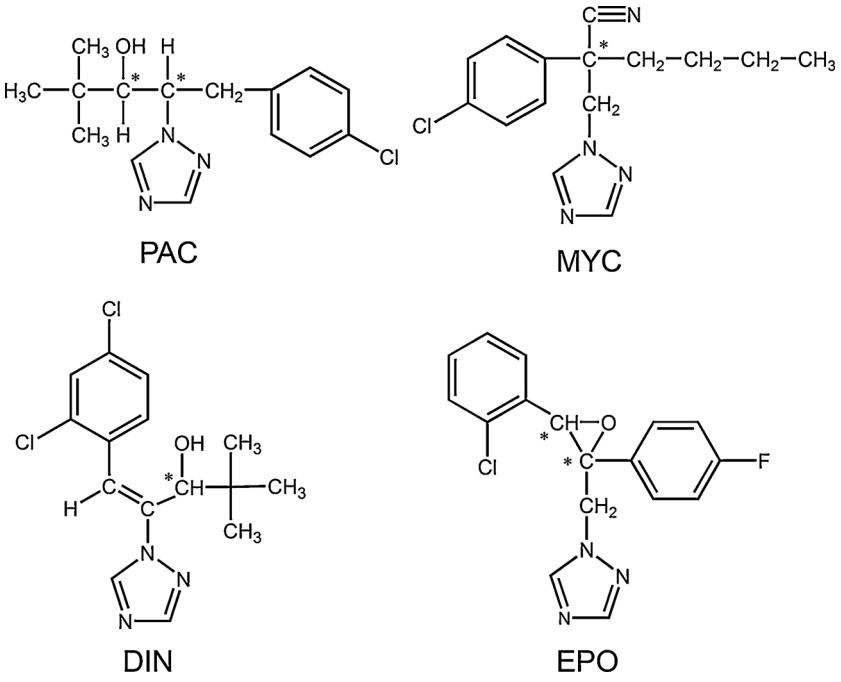

Fig. 1. Chemical structures of four chiral triazole fungicides.

(Tette et al., 2016). Several studies demonstrated the presence of triazole fungicides in honey (Farajzadeh et al., 2014a, b; Miao et al., 2016), highlighting a possible risk for consumers. The EU regulation has specified the maximum residue limits for some triazole fungicides in honey and other bee products (royal jelly and pollen), which were 0.05 mg/kg for both diniconazole (Reg. (EU) No. 1317/2013) and epoxiconazole (Reg. (EU) No. 978/2011). To date, only limited literatures have reported the enantiomeric analysis of one or two triazole fungicides in food matrices such as vegetables, fruits, and cereals (Dong et al., 2012a, b; Li et al., 2012a; Zhang et al., 2014). However, there is no study on the enantioselective determination of chiral triazole fungicides in honey.

As far as we know, gas chromatography (GC) is commonly used for the achiral determination of triazole fungicides (Farajzadeh et al., 2014b; Xue et al., 2016; Wang et al., 2016b; Bordagaray et al., 2011). But as for the chiral separation and detection of triazole fungicides, LC–MS/MS is more applicable than GC (Dong et al., 2012a, b; Li et al., 2012b). For one thing, the rapid development of chiral stationary phase of liquid chromatography has greatly facilitated the enantiomeric separation of chiral compounds; for another thing, the sensitivity and specificity of tandem MS detection allows for the simultaneous quantification of a series of chiral compounds enantiomers. Nevertheless, studies have shown that honey is one of the most complex food matrices and contains monosaccharides, pigments, organic acids, flavonoids, essential oils, amino acids and so on (Blasco et al., 2011). Therefore, a powerful sample pretreatment procedure for the purification and preconcentration of the trace analytes is required prior to instrumental analysis. Solid-phase extraction (SPE) is a universal sample-preparation technique based on the selective retention of the target analytes on sorbents filled in cartridge followed by the selective elution with organic solvent. The merits of simplicity of operation, good reproducibility, and high enrichment factor make this technique popular in trace analysis (Ares et al., 2015; Paiga and Delerue-Matos, 2013). Dispersive liquid-liquid microextraction (DLLME) was proposed by Assadi et al on the basis of ternary solvent system (Berijani et al., 2006). In this process, an appropriate mixture of extraction solvent and dispersive solvent was rapidly injected into sample solution to form a cloudy solution, thereby the analytes were extracted. The feasibility of DLLME in the analysis of trace residues has been proved by several researches (Chen et al., 2016; Wang et al., 2016a; Zhang et al., 2016). Nevertheless, due to the diversity and complexity of food matrix, the application of single SPE or DLLME technique for the analysis of trace organics in complex samples becomes very limited. Recently, the combination of SPE and DLLME has gained much more attention and has been applied to the analysis of pesticides in different matrix (Campillo et al., 2013; Shamsipur et al., 2016; Zhou et al., 2012). This combination has taken full advantages of SPE and DLLME, showing a strong clean-up ability and favorable enrichment performance. It seems that SPE-DLLME is a promising pretreatment method for the enrichment of the selected triazole fungicides in complex honey matrix. However, its potential application for triazole fungicides has not been exploited.

The aim of this study is to establish a chiral analytical method which can not only enrich the trace amount of pesticides, but also decrease the matrix effect. For this purpose, an efficient and sensitive method, SPE-DLLME coupled with chiral LC–MS/MS, was developed, optimized, and validated. Ultimately, the proposed method was successfully applied to the enantioselective determination of triazole fungicides in honey samples of different floral origins.

2. Materials and methods

2.1. Chemicals and reagents

Racemic paclobutrazol (PAC), myclobutanil (MYC), diniconazole (DIN), epoxiconazole (EPO) were obtained from Rainbow Chemical Co., Ltd (Shandong, China). The chemical structures of target triazole fungicides are presented in Fig. 1. It should be noted that PAC and EPO are marketed as one enantiomeric pair although they have two chiral centers. To be specific, PAC is a mixture of 2*R*, 3*R* and 2*S*, 3*S*-enantiomers, and EPO is a mixture of 2*R*, 3*S*- and 2*S*, 3*R*-enantiomers. Stock solutions of analytes were prepared in pure acetonitrile at a concentration of 1 mg/mL. Working solutions was prepared by diluting the stock solution with mobile phase. All solutions were stored in amber glass vials at −20 °C. Acetonitrile of chromatographic grade was obtained from Fisher Scientific (Pittsburgh, PA, USA). Ultrapure water was prepared by a Milli-Q Water system (Millipore, Bedford, MA). Methanol, acetone, dichloromethane, and chloroform of