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Rapid screening and quantification of residual pesticides and illegal adulterants in red wine by direct analysis in real time mass spectrometry

Tianyang Guo [a], Pingping Fang [a], Juanjuan Jiang [a], Feng Zhang [b], Wei Yong [b], Jiahui Liu [a], Yiyang Dong [a,*]

[a] Beijing Key Laboratory of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, PR China
[b] Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100176, PR China

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ABSTRACT

A rapid method to screen and quantify multi-class analytic targets in red wine has been developed by direct analysis in real time (DART) coupled with triple quadruple tandem mass spectrometry (QqQ-MS). A modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) procedure was used for increasing analytical speed and reducing matrix effect, and the multiple reaction monitoring (MRM) in DART-MS/MS ensured accurate analysis. One bottle of wine containing 50 pesticides and 12 adulterants, i.e., preservatives, antioxidant, sweeteners, and azo dyes, could be totally determined less than 12 min. This method exhibited proper linearity ($R^2 \geq 0.99$) in the range of 1–1000 ng/mL for pesticides and 10–5000 ng/mL for adulterants. The limits of detection (LODs) were obtained in a 0.5–50 ng/mL range for pesticides and 5–50 ng/mL range for adulterants, and the limits of quantification (LOQs) were in a 1–100 ng/mL range for pesticides and 10–250 ng/mL range for adulterants. Three spiked levels for each analyte in wine were evaluated, and the recoveries were in a scope of 75–120%. The results demonstrated DART-MS/MS was a rapid and simple method, and could be applied to rapid analyze residual pesticides and illegal adulterants in a large quantities of red wine.

1. Introduction

Pesticides (e.g., fungicides, insecticides, acaricides, and herbicides) could be used to protect crops from exogenous organisms, while excessive levels may have serious adverse effects on human health and disturb the equilibrium of the ecosystem [1,2]. However, the phenomenon of pesticide residues in wine was barely satisfied. Pesticide action network (PAN) Europe has reported 100% of conventional wines made in eight countries contained pesticides. The average number of pesticides per bottle was four, and the number in some wines even extended to ten. Almost half of all wines, including three wines at more than €200 per bottle, contained pesticides as being carcinogenic, mutagenic, reprotoxic or disruptive to the endocrine system [3]. To our knowledge, there was no standard about the maximum residue limits (MRLs) of pesticides in wine, whether National Food Safety Standards in China [4] or the General Standard in Codex Alimentarius [5], but that in grapes [4,5] and grape juice [5]. In consideration of the transitivity of pesticides in fermentation and the potential risk of pesticides, an accurate and low-level detection method for pesticides in wine was necessary.

Food additives can be added into food in the food processing with a reasonable content to guarantee quality traits [6], while overrange usage of food additives was regarded as an intended food contamination behavior, limited by the National Food Safety Standards in China [7] and the General Standard for Additives in Codex Alimentarius [8]. For example, acesulfame, dehydroacetic acid and gallic acid-propyl ester are prohibited from adding into red wine [7,8]. Illegal adulterants such as industry dyes were also prohibited. However, illegal adulteration in red wine often occurred in China. Therefore, developing a fast, simple analytical method for detecting residual pesticides and illegal adulterants was required.

Direct Analysis in Real Time (DART) was developed by Cody's group in 2005 [9] as well as desorption electrospray ionization (DESI) by Cooks's group in 2004 [10]. In the past decade, DART coupled with mass spectrometry (DART-MS) has been gaining momentum in the area of food quality and safety.

Screening of strobilurin fungicides by DART-TOF MS was first established in milled wheat grains in 2008, which had lower limits

* Corresponding author.
E-mail addresses: yydong@mail.buct.edu.cn, yiyangdong@gmail.com (Y. Dong).

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of quantification (LOQs) result than the MRLs in European Union (EU) [11]. Combined with surface swabbing techniques, multiple pesticides in fruits and vegetables were detected at low level by DART-orbitrap-MS or DART-Q-orbitrap-MS [12–16]. Subsequently, xenobiotic fungicides, antioxidants, and sugars in fruits peel could be directed identified by DART-LIT-orbitrap-MS without preparation process [17]. A confirmation of highly hazardous insecticides in commercial agrochemicals by DART-quadrupole-MS [18] and in fatty foods by DART-TOF-MS [19] were also investigated.

Various food additives, i.e., preservatives, sweeteners, and acidulants, were fast analyzed in soft drink by DART-TOF-MS [20]. A strong central nervous system depressant, gamma-hydroxybutyric acid (GHB), has been identified by DART-TOF-MS in fifty drinks, including non-alcoholic beverages (i.e., soda and juice) and alcoholic beverages (i.e., beer, wine, and liquor) [21]. Melamine (MEL) and dicyandiamide (DCD) as illegal food adulterants added into protein-contained milk powders and pet foods were determined by DART-TOF-MS [22]. Some interference (due to extremely close m/z) of certain substances, such as protonated 5-hydroxymethylfurfural (5-HMF) in positive mode and deprotonated cyanuric acid (CYA) in negative mode were eliminated [23,24].

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) was an efficient preparation method proposed by Anastassiades and Lehotay in 2003 [25], and successful applications were reported in these years [26,27]. It was based on acetonitrile extraction combined with salting out, and absorbents purification by dispersive solid-phase extraction (dSPE). Some modifications of QuEChERS were reported, such as pH, extraction solvent, salt, buffer system, and absorbent [28].

In this paper, we reported an analyzing strategy for rapid determination of residual pesticides and illegal adulterants in red wine. To our knowledge, direct analysis in real time (DART) combined with triple quadruple tandem mass spectrometry (QqQ-MS) was firstly used to detect pesticides and adulterants. The simple preparation procedure of modified QuEChERS was evaluated, and the powerful screening and quantified abilities of DART-MS/MS under the multiple reaction monitoring (MRM) mode were also assessed. The method was developed with sufficient promptness, compared with conventional method, such as ESI-LC–MS.

2. Experimental

2.1. Reagents and materials

Acetonitrile, ethyl acetate, and acetone (HPLC grade) were purchased from Thermo-Fisher Scientific (Waltham, MA, USA). Sodium chloride, anhydrous magnesium sulfate, sodium citrate, disodium hydrogen phosphate, acetic acid, hydrochloric acid, ammonium hydroxide and sodium hydroxide were purchased from Sinopharm (China). Primary secondary amine (PSA) and C_{18} were purchased from Agilent Technologies (Harbor City, USA). Red wines were purchased from COFCO Group (Beijing, China). The standards of pesticides and adulterant were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Ultra high purity helium (99.999%, v/v) used as DART carrier gas was purchased from Beijing Greenoxy Tiangang Technology Development Co., Ltd. (Beijing, China). High purity nitrogen (99.999%, v/v) used as MS collision gas and DART carrier gas, and high purity liquid nitrogen (99.999%, v/v) used as drying gas was purchased from Beijing Haipu Beifen Gas Industry Co., Ltd. (Beijing, China). The module of 12 dip-it (i.e. glass tube and the cap holder) for holding a small amount of liquid, and metal mesh (0.3 mm × 0.3 mm) with transmission module (TM, a moving sampling stage) for holding a large amount of liquid were purchased from Xing-an Bolting Cloth Factory (Shanghai, China).

2.2. Analytes selection

Although the registration status and MRLs of the pesticides and additive were supervised by the ministry of agriculture, there were still many phenomena, such as illegally using unregistered or high-hazard pesticides, and over-scope or over-dose using adulterants. In order to solve practical problems, fifty multi-class pesticides used in grape growth, and twelve adulterants prohibited in red wine production were selected, and their basic information were summarized in Table 1.

2.3. Sample preparation

Individual stock standard solutions were prepared at a concentration of 1000.0 μg/mL dissolved by acetone. These solutions were stored at 4 °C in the dark. Standard mixture at a concentration of 5.0 μg/mL in each class for parameter optimization was prepared with dilution of the stock solutions in acetonitrile. Working standard mixture solution at a concentration of 10.0 μg/mL for pesticides and 50 μg/mL for adulterants was prepared by appropriate dilution of the stock solutions with acetonitrile. Calibration standards diluted with extracting solution at concentrations in the range of 1.0–1000.0 ng/mL for pesticides and 5.0–5000.0 ng/mL for adulterants were also prepared for the calibration curves. Calibration curves of peak area vs. concentration (μg/mL) were used to calibrate the DART-MS and DART-MS/MS system, and spike samples in recovery experiments.

Red wine was extracted by modified QuEChERS method. The process of QuEChERS was following: 10 mL red wine was load into 50 mL centrifuge tube, and shaken with10 mL acetonitrile under the under vortex oscillator for ca. 30 s. The mixture was added with salts (sodium chloride 1 g, anhydrous magnesium sulfate 4 g, sodium citrate 1 g, disodium hydrogen phosphate 0.5 g), and vibrated for ca.1 min. Then the mixture was centrifuged under 8000 rpm 5 min. One milliliter of supernatant was transferred into 25 mg PSA-loaded 2 mL centrifuge tube, vibrated for ca. 10 s and centrifuged under 8000 rpm 5 min. Then the supernate was filtrated through 0.22 μm organic membrane and loaded into 1.5 mL centrifuge tube.

2.4. DART-MS/MS detection

The analyses were performed using an Agilent 6490 triple quadrupole tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) coupled with direct analysis in real time(DART) ion source (IonSense Inc. Saugus, MA, USA, model number SVP100). Mass spectrometer was tuned every two weeks under the ESI ion source with tuning mix solution supplied by Agilent (5301 Stevens Creek Blvd, Santa Clara, CA, USA). When jointed with DART ion source, the mass spectrometer interface was changed to a VAPUR Interface. A vacuum pump (Welch-Ilmvac; Gardner Denver, Inc., Niles, IL, USA) was connected to the VAPUR interface for the suction of the redundant discharge gas before flowing into the mass spectrometer inlet. Data acquisition and data processing were accomplished with MassHunter B.07.00 Software (Agilent Technologies, Santa Clara, CA, USA).

MS was performed in both positive and negative ion modes. The instrument operational parameters in positive ion mode were as follows: polarity, positive; scan range, m/z 50–600; gas temperature, 300 °C; capillary voltage, 4000 V; resolution, 0.7 FWHM; fragmentor voltage, 380 V; cell accelerator voltage, 7 V; carrier gas flow, 2.7 L/min; acquisition time, 2.5 min. The parameters were the same in negative ion mode except for the following: acquisition time, 1.4 min. Drying gas flow, pump suction, and distances (i.e., between the DART outlet and the ceramic tube inlet, and between the ceramic tube outlet and the capillary inlet) were optimized.