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Simultaneous determination of three pesticide adjuvant residues in plant-derived agro-products using liquid chromatography-tandem mass spectrometry

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ABSTRACT

Herein, an accurate and reliable isotope-labelled internal standard method was developed and validated for simultaneous determination of three polar pesticide adjuvants, namely 2-pyrrolidone, *N*-methyl-2-pyrrolidone, and *N*-ethyl-2-pyrrolidone in plant-derived agro-products. Matrices, including apple, cabbage, tomato, cucumber, rice, and wheat were extracted with a modified quick, easy, cheap, effective, rugged, and safe "QuEChERS" method and purified with a new clean-up sorbent (Z-Sep). A hydrophilic interaction liquid chromatography column (HILIC), exhibiting a lipophilic-hydrophilic character, was used to separate the three analytes over 10 min using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Matrix effects in various matrices were evaluated and an isotope-labelled internal standard method was employed to compensate for ion enhancement/suppression effects. At three fortification levels (2.0, 5.0, and 20.0 μg/kg), the mean recoveries ranged from 78.5 to 112.1% with relative standard deviations (RSDs) < 11.0% for all tested analytes. The limits of detection (LODs) and quantification (LOQs) were 0.04–0.45 and 0.12–1.58 μg/kg in various matrices, respectively. The developed experimental protocol was successfully applied to monitor different samples purchased from local markets in Beijing, China. In conclusion, the developed method exhibited both high sensitivity and satisfactory accuracy and is suitable for the simultaneous determination of the three tested pesticide adjuvant residues in agro-products of plant origin.

1. Introduction

Pesticide adjuvants are inert chemical ingredients that are intentionally added to pesticide formulations to enhance the activity, improve stability, and simplify the application process [1]. However, they exhibit various levels of toxicity (from non-toxic to highly toxic) and some are even more harmful than the active ingredients [2]. It has to be noted that the presence of pesticide adjuvant residues has received little attention. 2-Pyrrolidone (PYR), *N*-methyl-2-pyrrolidone (NMP), and *N*-ethyl-2-pyrrolidone (NEP) are versatile organic solvents with a wide variety of applications. NMP constitutes 100% of the solvent used in pesticide formulations [3] with a content up to 87% in some products [2]. Because of their developmental toxicity and teratogenic effects, NMP and NEP are categorised as reproductive toxicants by the European Commission [4,5]. Moreover, the three solvents cause skin and eye irritation. NMP and NEP were metabolized to 5-hydroxy-*N*-methyl-2-pyrrolidone and 2-hydroxy-*N*-methylsuccinimide in humans [6]. However, to the best of our knowledge, reports addressing the environmental metabolism of these three adjuvants are currently unavailable. It is therefore assumed that they may accumulate in agro-products, owing to the extensive use of pesticides containing such adjuvants. In turn, the Institute for the Control of Agrochemicals in China has included NMP, PYR, and NEP in its "prohibited and restricted lists of pesticide adjuvants" and has restricted their contents to 5% (w/w) in pesticide formulations [7], to enhance the management of pesticide adjuvants and protect human health. It is therefore necessitated to develop a reliable and sensitive quantita-

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Table 1
Tandem mass spectrometry parameters of NMP, PYR, NEP, and NMP-d_9.

Analyte	Quantification ion transition	CE1 (eV)	Confirmatory ion transition	CE2 (eV)	DP (V)
NMP	100.1 > 58.0	32	100.1 > 69.1	27	90
PYR	86.1 > 43.9	31	86.1 > 68.9	22	70
NEP	114.2 > 86.0	25	114.2 > 69.0	26	60
NMP-d_9	109.3 > 62.1	32			100

CE: collision energy; DP: declustering potential.

tive method for the determination of NMP, PYR, and NEP trace levels in various food matrices to assess their risks to the consumers. The generated data would assist the regulatory authority in China to manage the pesticide adjuvants.

To date, several methods have been reported for the determination of NMP [8–11], and PYR [12], however, no analytical methods are available for the simultaneous determination of the 3 solvents in single chromatographic run. LC–MS/MS is a powerful and efficient tool for the simultaneous determination of various organic pollutants, because of its high selectivity and sensitivity [13]. However, the effect of co-eluting compounds arising from the matrix results in signal enhancement or suppression. Such matrix effects can adversely affect the reproducibility, accuracy, sensitivity, and reliability of a method, leading to erroneous quantitation. The isotope-labelled internal standard (ILIS) method is the most effective approach to overcome matrix effect, because it has a comparable performance during sample preparation and possesses the same ionization efficiency, retention time, and ion suppression or enhancement same as the tested analytes [14].

The QuEChERS method is widely accepted for multiresidue analysis of pesticides in fruits and vegetables. It has been modified and applied for the determination of endocrine disrupting chemicals [15,16], chlorinated hydrocarbons [17,18], pharmaceutical compounds [19,20], and polychlorinated biphenyls [21,22]. So far, the QuEChERS approach has not been applied for the analysis of the tested analytes; NMP and PYR previously determined using solid-phase extraction (SPE) method [9,10]. Compared with SPE, the QuEChERS method is simple, rapid, consume low solvents, and produce good reproducibility. Therefore, the main objective of the present study was to develop and validate a simple, reliable, and effective analytical method based on ILIS-LC–MS/MS combined with a modified QuEChERS sample preparation method for simultaneous quantification of NMP, PYR, and NEP in plant-derived agro-products (apple, cabbage, tomato, cucumber, rice, and wheat). The novel clean-up sorbent (Z-sep) was evaluated for effective purification of the tested analytes during sample extraction. The assessment and elimination of the matrix effect using ILIS method were also considered.

2. Materials and methods

2.1. Reagents and materials

NMP (99% purity) was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), whereas PYR (98% purity) and NEP (98% purity) were purchased from ANPEL (Shanghai, China). The isotopically-labelled NMP-d_9 (99.6% purity) internal standard was obtained from Toronto Research Chemicals (Toronto, Canada). LC grade acetonitrile (ACN) was supplied by Fisher Scientific (Pittsburgh, USA) and LC–MS grade formic acid (FA) was provided by Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Primary secondary amine (PSA), octadecylsilyl (C18), and graphitized carbon black (GCB) were purchased from Agela (Tianjin, China), and the Supel™ QuE Z-Sep was supplied by Supelco (Bellafonte, PA, USA). Anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China).

Individual stock solutions (1.0 mg/mL) of NMP, PYR, NEP, and NMP-d_9 were prepared in ACN and stored in the dark at 4 °C. Standard solutions containing NMP, PYR, and NEP were mixed and diluted with ACN to prepare a series of working solutions, used for fortification and constructing the calibration curves. A solution employed for working internal standard (0.5 mg/L) of NMP-d_9 was freshly prepared from the stock solution by serial dilution on a daily basis.

2.2. Sample preparation

Apple, cabbage, cucumber, tomato, rice, and wheat samples were purchased from local markets. All edible portions were chopped and homogenized using a Multiquick-3 food processor (Braun, Neu-Isenburg, Germany), and stored at −20 °C pending analysis. Representative portions (10 g for apple, cabbage, cucumber, and tomato, and 5 g for rice and wheat) of homogenized samples were weighed into a 50-mL polypropylene centrifuge tube (Eppendorf AG 22331, Hamburg, Germany) and then spiked with 5 μg/kg of the ILIS. The samples were vortex-mixed (Vortex-Genie 2, Scientific industries, USA) for 30 s and equilibrated for 30 min before extraction, to allow distribution of the analytes and interaction with matrices. Subsequently, 10 mL ultrapure water (only for rice and wheat samples) and 10 mL ACN were added. The mixtures were vortexed for 3 min followed by the addition of 4 g $MgSO_4$ and 1 g NaCl. The mixtures were shaken vigorously for 2 min and then centrifuged (Biofuge Stratos, Heraeus, Germany) for 5 min at 2798 rcf. A portion of each extract (1.0 mL) was transferred into a 5-mL centrifuge tube containing the sorbents (50 mg Z-Sep and 10 mg GCB for apple, tomato, and cucumber; 50 mg PSA for cabbage and rice; 50 mg C18 for wheat) in addition to 150 mg $MgSO_4$. Each tube was then vortexed for 1 min and centrifuged for 5 min at 2798 rcf. Finally, the supernatant was filtered through a 0.22-μm membrane filter (Nylon 66, Jin Teng, Tianjin, China) into a glass autosampler vial (Agilent, USA) for LC–MS/MS analysis.

2.3. LC–MS/MS analysis

An Agilent 1200SL Series HPLC system (Agilent, Waldbronn, Germany) equipped with an autosampler (G1367C), and an API 5000 tandem quadrupole mass spectrometer (AB SCIEX, Chromos, Singapore) equipped with an electrospray ionization source (ESI^+) were used for analysis. Chromatographic separation was carried out using a hydrophilic interaction chromatography column (XBridge HILIC column, 150 mm × 2.1 mm × 5 μm, Waters, Ireland). The mobile phase consisted of 0.1% (v/v) FA aqueous (solvent A) and 0.2% (*v*/*v*) solution of FA in ACN (solvent B); pumped at a flow rate of 0.2 mL/min. Elution was completed in gradient mode as follows: 0–1.0 min, 10% solvent A; 1.0–3.0 min, linear gradient of 10% solvent A to 90% solvent A; 3.0–5.0 min, 90% solvent A; 5.0–5.1 min, linear gradient to return to 10% solvent A; 5.1–10.0 min, 10% solvent A. A sample injection volume of 5 μL was employed for each run.

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