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Determination of Paraquat Residues in Adzuki Beans (*Vigna angularis*)

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Abstract

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We developed a rapid and novel method to prepare and determine paraquat samples in adzuki beans using liquid chromatography -- tandem mass spectrometry (LC-MS/MS). The method is specifically used for adzuki beans since QuPPe-method (quick polar pesticides method) gave poor recovery of paraquat residues in adzuki beans. Sample preparation involves extraction with 1% formic acid/methanol, shaking of sample mixture, ultrasonication in a hot water bath with a temperature of 80°C, and centrifugation. The extracts were then passed through a 0.22-μm syringe filter into a plastic storage vial and subjected to LC-MS/MS analysis. The validity of the method was assessed by evaluating intraday and interday recoveries at low (0.05 mg/kg), medium (0.20 mg/kg), and high (0.50 mg/kg) levels of paraquat in adzuki beans. Recoveries ranged from 63.3% to 98.3%. The limit of quantification (LOQ) was 0.05 mg/kg, which may satisfy current regulatory needs in Taiwan.

Key words: adzuki beans, LC-MS/MS, paraquat, residue analysis.

Introduction

In this study, we developed a rapid and sensitive method for the preparation and determination of paraquat samples in adzuki beans using liquid chromatography -- tandem mass spectrometry (LC-MS/MS). Paraquat is a non-selective contact herbicide used for broad-spectrum control of broad-leaved weeds and grasses in various crops[(4)]. It is also used as a harvest-aid for dry beans, including adzuki beans[(3)]. Adzuki beans (*Vigna angularis (Willd.) Ohwi et Ohashi*), also known as small red beans, are an important agricultural product in

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southern Taiwan. These beans are often boiled with sugar and consumed as a sweet bean paste or a sweet soup. However, paraquat is toxic to humans and has the ability to cause irreversible pulmonary cellular damage[2]. Therefore, paraquat residues in adzuki beans have become an issue of concern. Paraquat is a highly polar pesticide and, due to its distinctive physico-chemical properties, it is difficult to analyze paraquat using multi-residue methods. Furthermore, its permanent ionic character and strong tendency to interact with glass surfaces hampers the analysis of paraquat residue in foods.

The QuPPe-method (quick polar pesticides method) was developed to analyze numerous highly polar pesticides, including paraquat, in plant-based foods[1]. However, we found that the QuPPe-method provided poor recovery rates when analyzing paraquat residues in adzuki beans. In addition, to our knowledge, no published literature has reported on the determination of paraquat in adzuki beans. Therefore, in this study, we developed a novel method for the determination of paraquat in adzuki beans. Our proposed method, which involves shaking the sample mixture, ultrasonication in a hot water bath, centrifugation, and analysis by LC-MS/MS, is simple, sensitive, and rapid.

Material and Methods

Locally grown adzuki beans were used in this study. Specifically, two grams of pulverized adzuki beans and 2 mL of water were added into a 15-mL plastic centrifuge tube. The mixture was then left to stand for 10 min. Subsequently, 10 mL of 1% formic acid/methanol (1/1, v/v) was added, and the mixture was shaken at 1,000 strokes/min for 5 min using a Geno-Grinder 2010 (SPEX SamplePrep, Metuchen, NJ, USA). Following this, the mixture was ultrasonicated for 30 min in a hot water bath with a temperature of 80°C before being shaken again for 1 min. The mixture was then allowed to cool to room temperature. After centrifugation (4,500 ×*g*, 15°C) for 30 min, a 0.9-mL aliquot of supernatant was mixed with 0.1 mL of 1% formic acid/methanol (1/1, v/v). Finally, this supernatant solution was passed through a 0.22 μm PTFE syringe filter into a plastic storage vial, and one aliquot was analyzed by LC-MS/MS.

Chromatographic separation was performed at 40°C using an Agilent 1200 series high-performance liquid chromatography (HPLC) system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Shiseido Capcell Pak ST column (150 × 2.0 mm ID, Shiseido, Tokyo, Japan). For this, the solvents were 10 mM ammonium acetate in 0.1% (v/v) formic acid (eluent A) and acetonitrile (eluent B). The gradient program was: 70% B at 400 μL/min (0 min), 10% B at 400 μL/min (1 min), 10% B at 500 μL/min (3 min), 10% B at 500 μL/min (6 min), 70% B at 400 μL/min (6.1 min), and 70% B at 400 μL/min (10 min). The injection volume was 10 μL, and all reagents were HPLC grade.

Data were acquired using a triple quadrupole mass spectrometer (Applied Biosystems 4000 QTRAP, Applied Biosystems, Warrington, UK) with the following parameter settings: electrospray ion-source in positive mode; multiple reaction monitoring scan type; an ion spray voltage of 5,500 V; an ion source temperature of 500°C; curtain gas of 15 psi; collision gas set to high; nebulizer gas of 50 psi; and auxiliary gas of 60 psi. Two transitions of paraquat, including 186/171 (with a decluster-

ing potential [DP] of 40 V, an entrance potential [EP] of 10 V, collision energy [CE] of 29 eV, and a collision cell exit potential [CXP] of 10 V) and 171/71 (DP of 86 V, EP of 10 V, CE of 55 eV, and CXP of 14 V), were selected and used as the quantitative transition and qualitative transition, respectively.

Results and Discussion

To evaluate the validity of our proposed method, five replicate recovery studies were performed on adzuki beans at three fortification levels: low (0.05 mg/kg), medium (0.20 mg/kg), and high (0.50 mg/kg). Intraday repeatability was verified over three days. On each of these days, five fortified samples were analyzed. Interday repeatability was verified by analyzing five fortified samples on three different days. Recovery levels were calculated by comparing the peak areas of fortified samples with those of matrix matched calibration standards. Intraday and interday recovery levels are shown in Table 1. Under low fortification (0.05 mg/kg), the intraday recoveries of paraquat were 74.5%, 72.5%, and 90.7% with relative standard deviations (RSDs) of 10.9%, 4.4%, and 12.6%, respectively. Considering intraday recoveries under medium (0.20 mg/kg) and high (0.50 mg/kg) fortification, our method also yielded acceptable recovery values, ranging from 63.3% to 98.3%, with RSDs within 11.1%. In addition, acceptable interday recoveries were achieved, with RSDs within 17.1%. These results indicate that our proposed method is applicable for the determination of paraquat in adzuki beans. Compared to the method described by Zou et al.[6], our method is more streamlined as it does not require a column clean-up step (i.e., solid phase extraction purification). Meanwhile, the limit of quantification (LOQ) of our method (obtained by spiking various levels of paraquat in adzuki beans) was 0.05 mg/kg (S/N = 112.7). In Taiwan, the maximum paraquat residue limit in dry beans, including adzuki beans, is 0.2 mg/kg[5]. Therefore, the method proposed in this report may satisfy current regulatory needs in Taiwan.

Conclusion

In this study, we developed a novel method for the determination of paraquat residues in aduzki beans. Our proposed method can be performed using a rapid, simple, sensitive, and efficient procedure and should be suitable for the routine inspection of aduzki beans.

Table 1. Recovery levels of paraquat in adzuki beans

Fortification level	Intraday recovery (n = 5)						Interday recovery	
	1		2		3			
	Mean (%)	RSD[1] (%)	Mean (%)	RSD[1] (%)	Mean (%)	RSD[1] (%)	Mean (%)	RSD[1] (%)
Low (0.05 mg/kg)	74.5	10.9	72.5	4.4	90.7	9.0	79.2	12.6
Medium (0.20 mg/kg)	98.3	3.0	70.6	8.7	79.3	4.6	82.8	17.1
High (0.50 mg/kg)	72.1	11.1	63.3	5.0	68.4	8.1	68.0	6.5

[1] RSD: relative standard deviation.

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Literature Cited

1. Anastassiades, M., Kolberg, D. I., Eichhorn, E., Benkenstein, A., Lukačević, S., Mack, D., Wildgrube, C., Sigalov, I., Dörk, D., and Barth, A. 2015. Quick method for the analysis of residues of numerous highly polar pesticides in foods of plant origin involving simultaneous extraction with methanol and LC-MS/MS determination (QuPPe-Method), Version 8.1. EU Reference Laboratory for Pesticides Requiring Single Residue Methods (EURL-SRM), Fellbach, Germany. 60 pp.
2. Dinis-Oliveira, R. J., Duarte, J. A., Sanchez-Navarro, A., Remiao, F., Bastos, M. L., and Carvalho, F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit. Rev. Toxicol. 2008. 38: 13-71.
3. Taiwan Food and Drug Administration. 2015. Standards for pesticide residue limits in foods. Food No. 1041300338 amended. Taiwan Food and Drug Administration, Ministry of Health and Welfare, Taipei, Taiwan.
4. Tomlin, C. D. S. 2003. The e-pesticide manual: a world compendium, 13th ed. British Crop Protection Council, Farnham, UK.
5. Syngenta Crop Protection, Inc. 2005. Supplemental label for Gramoxone Max herbicide. Available at http://www.syngentacropprotection.com/pdf/special/scp1074as31202.pdf.pdf
6. Zou, T., He, P., Cao, J., and Li, Z. 2015. Determination of paraquat in vegetables using HPLC-MS-MS. J. Chromatogr. Sci. 53: 204-209.

紅豆 (*Vigna angularis*) 中巴拉刈殘留之樣品前處理與檢測

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摘要

莊瑋臻、林嘉威、黃鎮華、徐慈鴻、林韶凱。2016。紅豆 (*Vigna angularis*) 中巴拉刈殘留之樣品前處理與檢測。臺灣農藥科學 1: 178-182。

本研究開發一套樣品前處理技術，搭配液相層析串聯式質譜儀 (LC-MS/MS)，提供快速且全新方法，作為分析紅豆中巴拉刈農藥殘留量使用。過去以國際間通用之 QuPPe (quick polar pesticides method) 方法，雖可進行農作物中巴拉刈殘留分析，但應用 QuPPe 方法於紅豆中的巴拉刈殘留回收率極差。本方法以 1% 甲酸／甲醇溶劑進行樣品萃取，以震盪方式將樣品與溶劑混和，以 80° C 超音波水浴萃取後離心，經 0.22 μm 過濾後置入塑膠樣品瓶，再以液相層析串聯式質譜儀進行分析。本方法經同日內與不同日之重複試驗進行確效評估，紅豆樣品前處理分別添加低濃度 (0.05 mg/kg)、中濃度 (0.20 mg/kg) 與高濃度 (0.50 mg/kg)，結果顯示回收率介於 63.3% 至 98.3%，定量極限 (limit of quantification, LOQ) 為 0.05 mg/kg，本方法適用於臺灣地區紅豆中巴拉刈之殘留檢驗 (法規容許量為 0.2 mg/kg)。

關鍵詞：紅豆、液相層析串聯式質譜儀、巴拉刈、農藥殘留分析。

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