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Analytical Methods

Determination of validamycin A in agricultural food samples by solid-phase extraction combined with liquid chromatography–atmospheric pressure chemical ionisation–tandem mass spectrometry

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ABSTRACT

For the first time, a rapid, sensitive and accurate liquid chromatography–atmospheric pressure chemical ionisation–tandem mass spectrometry (LC–APCI–MS/MS) method was developed for determination of validamycin A in agricultural food samples (rice, agaric, almond, cabbage, green onion, carrot, tomato, cucumber and spinach). The validamycin A residue was extracted with methanol–water (9/1, v/v) or methanol by vortex, and a HLB solid-phase extraction cartridge was used for cleaning up the extracts. LC–APCI–MS/MS data acquisition was carried out in multiple reaction monitoring (MRM) mode. A series of matrix-matched calibration solutions ranging from 2.5 to 50 ng mL^{-1} were used to record calibration curve. The limit of quantification (LOQ) was 10 μg kg^{-1}. The average recoveries, measured at three concentrations levels (10.0, 50.0, 100.0 μg kg^{-1}) were in the range 83.5–109.6%. The proposed method offers the best sensitivity and specificity for the routine analysis of validamycin A in agricultural food samples.

1. Introduction

Validamycin, or called as jinggangmycin in China, is an important agricultural antibiotic produced by fermentation of *Streptomyces hygroscopicus var. limoneus* (Iwasa, Yamamoto, & Shibata, 1970) or *S. hygroscopicus var. Jinggangensis* (Agriculture Antibiotic Group, Shanghai Institute of Agriculture Pesticides of China, 1975), and is widely used in Asia as rice protectant since 1970s. Both gene and chemical structure analysis have further comformed validamycin and jinggangmycin were completely identical (Jian, Pang, Yu, Zhou, & Deng, 2006). For example, rice sheath blight caused by *Rhizoctonia solani* is a major disease of rice that greatly reduces yield and grain quality, and validamycin is a non-systemic antibiotic with fungistatic action and exhibits remarkable therapeutic effects on this disease by inhibiting the hyphal extension of *R. solani* without growth inhibition (Iwasa, Higashide, Yamamoto, & Shibata, 1971; Shibata, Mori, & Hamashima, 1982). Validamycin can also be used for the control of *R. solani* in potatoes, vegetables, strawberries, tobacco, ginger and other crops, and damping-off diseases of cotton, rice and sugar beet, etc (He et al., 2003). Besides its excellent control effect, low price, low drug-resistance and low toxicity are the other outstanding merits of validamycin, and it is now one of the most important agricultural antibiotic with the biggest production in China.

However, because of widespread use on large agricultural areas and kinds of crops, consumer health and safety risks may be of concern. In fact, validamycin is a mixture of aminoglycoside compounds. There have been eight structural forms termed A to H been isolated from the metabolites of the fungus *S. hygroscopicus var. limoneus*, with validamycin A [1L-(1,3,4/2,6)-2,3-dihydroxy-6-hydroxymethyl-4-[(1S,4R,5S,6S)-4,5,6-trihydroxy-3-hydroxymethylcyclohex-2-enylamino] cyclohexyl β-D-glucopyranoside (Fig. 1)] the main form and the most active (Asano, Kameda, Matsui, Horii, & Fukase, 1990). So the content of validamycin A is the most important quality parameter of the commercial products of validamycin (GB/T 9553, 1993). And the Japanese positive list system tentatively lists validamycin A residue limit at 60 μg kg^{-1} in rice, 50 μg kg^{-1} in dry soybean, and 50 μg kg^{-1} in vegetables and fruit (Japanese Positive List System for Agricultural Chemical

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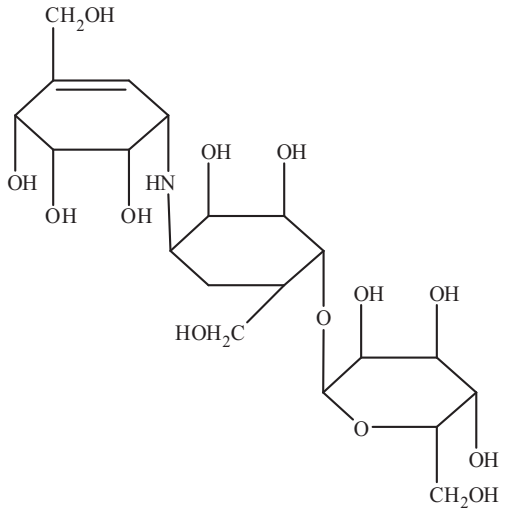

Fig. 1. Chemical structure of validamycin A.

Residues in Foods). Thus, monitoring validamycin A is crucial for proper assessment of human exposure from foods.

There have been several methods been developed for validamycin A analysis. The first and earliest official method used for the analysis of validamycin A in commercial formulations is the bioassay method called the "reversed layer method" (Iwasa, Kameda, Asai, Horii, & Mizuno, 1971). However, this method is complex and time-consuming, and cannot distinguish the actual antibiotic from false products (Hsiao & Lo, 1999; Lo & Hsiao, 1996). Later, gas chromatography (GC) was used for quantitative analysis of validamycin A in commercial formulations (Horii, Kameda, & Kawahara, 1972) and soil (Xu, Zhan, Tao, Zhang, & Jiang, 2008). By comparison with the bioassay method, GC is sensitive and precise. However, since validamycin A is difficult to volatilise, a laborious derivatisation step was necessary in all cases. So the GC method is also complex and time-consuming. In China, high-performance liquid chromatography–ultraviolet detector (HPLC–UV) is used as the national standard method for the routine analysis of validamycin A in commercial formulations (GB/T 9553, 1993). It is more sensitive than the GC method, but the pretreatment procedure is tedious, too. In this case, a series of relative simple capillary electrophoresis (CE) method have been established and used for validamycin A quantitative analysis in both commercial formulations (He, Liu, Liu, & Yu, 2005; He et al., 2003; Hsiao & Lo, 1999) and the validamycin A transformation broth for preparation of valienamine (Wei, Zhao, Gu, Zhao & Yao, 2010). However, for validamycin A has no characteristic ultraviolet absorption and its maximum absorption wavelength is 210 nm, whether HPLC or CE, the detection wavelength were all set at near 210 nm. At such wavelength, the spectra interference, which may come from other coexistence compounds and even the mobile phase or the running buffer, would be a serious problem, for the accuracy and sensitivity, as well.

In fact, considering that validamycin A is coexisting with other analogue validamycins, it is very difficult to obtain satisfactory resolution using only GC, HPLC or CE, while poor sensitivity might be another issue since the residue concentration is so low and the matrix of real food samples is complex just as Japanese positive list system lists requested. Therefore, it is necessary to develop more powerful and sensitive analytical methods to overcome these possible drawbacks for both qualitative and quantitative analysis of residues in food. Hyphenated techniques chromatography, especially tandem mass spectrometry (MS/MS) might be an ideal choice and has become the most widely used analytical methods for the identification and quantification of residues in food because of their excellent selectivity, sensitivity and the necessity for confirmation. Therein, for more extensive applicability and derivatisation free, LC–MS/MS has attracted more attention and been used more widely (Campillo, Viñas, Férez-Melgarejo, & Hernández-Córdoba, 2013; Han et al., 2011; Martínez-Villalba, Moyano, & Galceran, 2013; Murty, Sridhara Chary, Prabhakar, Prasada Raju, & Vairamani, 2009). However, as far as we know, there has no literature focused on this method for validamycin A analysis in any agricultural food samples.

Herein we report our effort on developing a rapid, sensitive and accurate liquid chromatography–atmospheric pressure chemical ionisation–tandem mass spectrometry (LC–APCI–MS/MS) method for both qualitative identification and quantitative determination of validamycin A in kinds of representative agricultural food samples.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were of analytical-reagent grade unless mentioned otherwise. Standard of validamycin A, (CAS No. 37248-47-8, purity > 91%), was purchased from Shanghai Pesticide Research Institute and the structure is shown in Fig. 1.

HPLC grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Ammonium acetate (purity 98%, *w/w*) was from Sigma–Aldrich (Steinheim, Germany). The water used was purified with a Milli-Q water purification system from Millipore (Bedford, MA, USA). High speed homogenizer was performed using a Retsch GM 200 Grindomix (Haan, Germany).

A stock standard solution of validamycin A was prepared at concentration of 100 μg mL^{-1} in water, stored in a refrigerator at 4 °C in the dark. Matrix-matched calibration standards ranged from 2.5 to 50 ng mL^{-1} were used to record calibration curve, and they were prepared by adding appropriate volumes of standard working solution to the extract-clean-up solution of the agricultural blank samples.

2.2. Sample extraction

2.2.1. Rice, agaric, almond

Accurately weigh 2.5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 20 mL methanol–water (9/1, v/v). Extract for 2 min in a high speed homogenizer. After centrifugation at 8875*g* for 5 min, the supernatant was collected in a graduated tube. The residues were re-extracted once more time with about 10 mL methanol–water (9/1, v/v) with the same procedure. Then combine the supernatants and the extract solution was evaporated to about 2.5 mL under nitrogen flow at 45 °C.

2.2.2. Cabbage, green onion, carrot, tomato, cucumber, spinach

Accurately weigh 2.5 g of the test sample (accurate to 0.01 g) into a 50 mL centrifuge tube, add 20 mL methanol. Extract for 2 min in a high speed homogenizer. After centrifugation at 8875*g* for 5 min, the supernatant was collected in a graduated tube. The residues were re-extracted once more time with about 10 mL methanol with the same procedure. Then combine the supernatants and the extract solution was evaporated to about 2.5 mL under nitrogen flow at 45 °C.

2.3. Solid-phase extraction

Solid-phase extraction was performed with Oasis HLB SPE cartridges. Before using, the cartridge was conditioned with 5 mL of methanol and 5 mL of ultra-pure water. The sample extract was