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Short Communication

Determination of metaflumizone residues in cabbage and soil using ultra-performance liquid chromatography/ESI-MS/MS

A simple confirmatory method for the determination of metaflumizone in cabbage tissues and soil samples using ultra-performance liquid chromatography-ESI/MS/MS is presented. Metaflumizone residues in cabbage and soils were extracted with acetonitrile and an aliquot was cleaned up with primary and secondary amine. Two precursor product ion transitions for metaflumizone were measured and evaluated to provide the maximum degree of confidence in results. Under ESI negative conditions, quantitation was achieved by monitoring the fragment $m/z = 302$ and qualitative fragment $m/z = 116.5$ while also collecting their parent ion $m/z = 505.5$. Average recoveries for cabbage and soil at four different levels (0.01, 0.05, 1 and 5 mg/kg) ranged from 77.6 to 87.9% with RSD of 3.5–7.9% and RSD_R of 4.5–7.1%. The coefficients of determination of $R^2 \geqslant 0.9991$ were achieved for metaflumizone calibration curves from 0.01 to 5.0 μg/mL. The metaflumizone LODs in cabbage and soil were determined to be both 0.001 mg/kg with a LOQ of 0.004 mg/kg. This method was able to demonstrate quantitative recoveries and provide confirmatory data for the identification of metaflumizone residues in cabbage tissues and the surrounding soils following commercial application.

Keywords: Dispersive SPE / Metaflumizone / Ultra-performance liquid chromatography-MS/MS
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1 Introduction

Metaflumizone, (EZ)-2′-[2-(4-cyanophenyl)-1-(α,α,α-trifluoro-m-tolyl)ethylidene]-4-(trifluoromethoxy) carbanilohydrazide, is a novel sodium channel blocker insecticide. This semicarbazone compound provides good to excellent control of most of the economically important lepidopterous pests and certain pests in the orders Coleoptera, Hemiptera, Hymenoptera, Diptera, Isoptera and Siphonaptera [1]. Metaflumizone provides long-lasting control of fleas on animals with a single spot-on application [2]. At the same time, it offers a low risk to non-target organisms including beneficial insects and pollinators, as well as humans and the environment [3]. Metaflumizone in its native state consists of two isomers (90% *E*-isomer and 10% *Z*-isomer) (Fig. 1). Compared with the *Z*-isomer, the *E*-isomer showed tenfold higher activity against lepidopterous larvae, and one isomer was easily converted to the other (*E* to *Z*, or *Z* to *E*) when exposed to light and in the presence of acid catalyst [4]. Although metaflumizone has been marketed globally for several years, to our knowledge, no residue data in food and environmental samples have ever been reported in the literature. Furthermore, metaflumizone was in the registration period for use on cabbage in China at present, and hence it is timely and important to establish reliable analytical methods of metaflumizone residue detection in cabbages and soils.

The outstanding Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method for monitoring the pesticides in vegetables (a high water content >75%) cleaned up by dispersive SPE was introduced by Anastassiades *et al.* [5]. The merits of this method include decreased costs of experimental apparatus and solvents as well as high sample throughout. The main disadvantage of the QuEChERS method compared with other common methods is that the 1 g/mL final extract concentration is lower than the 2–5 g/mL concentrated extracts of most traditional methods [6]. Thus, it often requires a highly sensitive and selective analytical instrument to determine the small extraction samples.

LC-MS has been a widely used tool for the analysis of pesticide residues in vegetables. Especially in MS/MS, the use of the multiple reaction monitoring mode permits an improvement in the LODs, due to increased S/N [7, 8].

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Abbreviations: QuEChERS, Quick, Easy, Cheap, Effective, Rugged, and Safe; **UPLC,** ultra performance liquid chromatography

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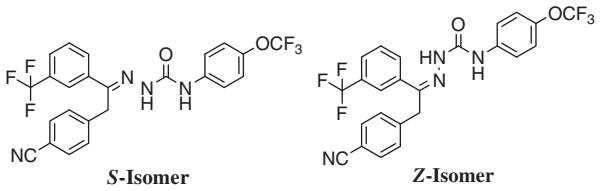

Figure 1. Chemical structures of metaflumizone.

This article describes an ultra-performance liquid chromatography (UPLC)-MS/MS method for determining the residues of metaflumizone in cabbage and soil. The residue determination method developed here was validated by excellent recovery values for authentic samples. The LOQ of the method was 0.004 mg/kg for the metaflumizone. The reliability of the developed analytical method was confirmed with real commercially treated samples.

2 Materials and methods

2.1 Chemical and reagents

Metaflumizone standard (purity $\geqslant$ 97.3%) and its commercial formulation (24% suspension concentrate) were obtained from BASF (Beijing, China). LC grade acetonitrile was purchased from Sigma-Aldrich (Steinheim, Germany); NaCl and $MgSO_4$ were analytical grade purchased from Beihua Fine Chemicals (Beijing, China). Ultra-pure water was obtained from a Milli-Q system (Bedford, MA, USA).

2.2 Instrumentation

The UPLC system used, Waters Acquity UPLC (USA), consisted of a binary solvent manager and Acuity column heater equipped with a Waters Acquity UPLC BEH C18 column (2.1 mm $\times$ 50 mm, 1.7 μm particle size) (Milford, MA, USA). Gradient UPLC elution was performed with acetonitrile (LC grade) as mobile phase A and ultra-pure water as mobile phase B. Separation of the analytes was performed at 45°C at a flow rate of 0.3 mL/min with an elution gradient of 10–95% A in 3.2 min, then 10% A in 0.1 min, holding at 10% A for 2 min. The system was re-equilibrated at 10% A for 2 min. The separation and the stabilization were achieved in 5.3 min. The temperature in the autosampler was set at 10°C and the sample volume injected was 10 μL.

A triple-quadrupole mass spectrometer (Waters Corp., Milford, Massachusetts, USA) equipped with an ESI source was used for metaflumizone detection. MS/MS detection was performed in negative mode, and the monitoring conditions were optimized for metaflumizone. Acquisition parameters were as follows: capillary voltage 3 kV, source temperature 120°C and desolvation temperature 350°C. The cone and desolvation gas flows were 40 V and 800 L/h, respectively. In total m/z 505.5 was selected as precursor ion and its product quantitative ion was m/z 302 and qualitative ion was m/z 116.5, when the collision energy was set at 20 and 42 V, respectively. The scan mode was selected for multiple reaction monitoring. Under above conditions, the retention times of metaflumizone isomers (*Z*-isomer and *E*-isomer) were about 2.73 and 2.83 min, respectively.

2.3 Preparation of standards and calibration curve

The stock solution of metaflumizone (5000 mg/L) was prepared in acetonitrile and serially diluted to produce working solutions of 0.01, 0.05, 0.1, 0.5, 1.0 and 5.0 μg/mL in acetonitrile/water (50:50 v/v). All solutions were protected against light with aluminum foil and stored in a refrigerator at 4°C. A calibration curve was generated by plotting peak area *versus* the concentration of the metaflumizone. Linear regression analysis was performed using Microsoft® Excel.

2.4 QuEChERS extraction and purification

Approximately 500 g of cabbage leaf was chopped and homogenized in an Ultra-Turrax homogenizer (IKA-Werke, Staufen, Germany). Soil samples were passed through a 2 mm sieve. In total 10 g aliquots of homogenized samples (cabbages) or soils were weighed into 50 mL Teflon tubes, and water (5 mL) and acetonitrile (10 mL) were added. The mixtures were shaken vigorously for 30 min at 25°C in a water bath shaker (Dongming Medical Instrument, Haerbin, China), then 4 g anhydrous magnesium sulfate ($MgSO_4$) and 1 g sodium chloride (NaCl) were added. The tubes were capped and immediately vortexed vigorously for 2 min and then centrifuged for 5 min at 2077 $\times$ *g*. Then, 1 mL of the upper layer (acetonitrile) was transferred into a single-use centrifuge tube and 200 mg anhydrous $MgSO_4$ and 30 mg primary and secondary amine were added. The samples were vortexed for 1 min and centrifuged for 5 min at relative centrifugal force (RCF) 2077 $\times$ *g*. The resulting supernatants were filtered through 0.22 μm Nylon syringe filters for UPLC/MS/MS injection.

2.5 Recovery assay

Recovery experiments were carried out with five replicates at four spiked levels (0.01, 0.05, 1 and 5 mg/kg) by adding known volumes of metaflumizone standards in acetonitrile to different matrixes (cabbage tissues and soils). Blank analyses (tissues and soils only) were performed in order to check interference from the matrix.

2.6 Practical sample test

The utility and performance of the method were tested by determining the residues of metaflumizone in practical sample (cabbages and soils). The cabbages and soil samples

were collected from the vegetable trial base at 1 and 3 days after spraying the metaflumizone. The samples were put into polyethylene bags and transported to the laboratory, where they were deep frozen (−20°C) until analysis.

3 Results and discussion

3.1 Linearity of the method

A standard calibration curve of metaflumizone was constructed by plotting analyte concentrations against peak areas with regression analyses performed using Microsoft® Excel. When the quantitative ion (m/z) was 302, the calibration range was linear from 10 to 5000 ng/mL; the equation of the standard curve was $y = 362.04x + 25\,254$ ($R^2 = 0.9991$), where y = peak area and x = concentration (ng/mL). The linearity correlation is shown in Fig. 2.

3.2 Recovery, repeatability and reproducibility

Soil and cabbage samples were all spiked at four different levels of metaflumizone (0.01, 0.05, 1 and 5 mg/kg based on five replicates) before extraction by adding the appropriate volume of the working standard solution. The recoveries

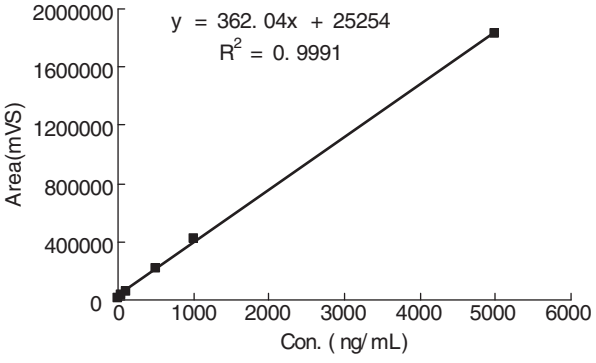

Figure 2. Linearity correlation for metaflumizone calibration curves.

obtained were in the acceptable range of 77.6–84.7% and 79.0–87.9% in soil and cabbage tissues, respectively, and the coefficient of variation (RSD_r) value of repeatability of the method ranged from 3.5 to 7.9%, as listed in Table 1. Figure 3 shows the chromatograms of the metaflumizone standard, blank and spiked samples.

The repeatability of the instrument was determined by analyzing the same cabbage sample spiked at 0.1 mg/kg. When the sample was injected ten times at 1 h intervals, the RSD values obtained for peak areas and retention times by UPLC/MS/MS were 2.5 and 0.4%, respectively. The precision of the method was determined by the repeatability and reproducibility studies of method and expressed by the RSD. The repeatability RSD_r was measured by comparing SD of the recovery percentage spiked samples run on the same day. The reproducibility RSD_R was determined by analyzing spiked samples for three different days by three operators. In general, the reproducibility RSD_R ranged from 4.5 to 7.1% for the spiking levels, as summarized in Table 1.

3.3 LOD and LOQ

The LOD for metaflumizone was the concentration that produced an S/N of 3. The LOQ was defined as an S/N ratio of 10 using the lowest point on the calibration curve. In this article, the LOD was estimated to be 0.001 mg/kg for metaflumizone from both the five replicate extractions and the analyses of spiked samples (soil and cabbages) containing metaflumizone at low concentration levels. The LOQ was 0.004 mg/kg for both soil and cabbage matrixes based on the five replicates.

3.4 Matrix effect

The matrix effect was investigated by comparing standards in solvent with matrix-matched standards for ten replicates at 0.1 mg/kg. The relative responses (response matrix/response solvent) were 0.94 and 1.02 for soil and cabbage,

Table 1. Recovery, RSD_r and RSD_R values obtained for metaflumizone at four spiked levels

Sample	Spiked level (mg/kg)	Day 1[a)]		Day 2[a)]		Day 3[a)]		RSD_R
		Average recoveries (%)	RSD_r (%)	Average recoveries (%)	RSD_r (%)	Average recoveries (%)	RSD_r (%)	
Soils	0.01	78.1	5.8	78.6	4.3	77.6	4.8	4.7
	0.05	81.5	4.7	82.0	5.4	81.1	4.3	4.5
	1	82.0	7.8	83.1	7.7	81.6	7.3	7.1
	5	84.7	5.4	82.3	7.5	83.7	4.6	5.6
Cabbages	0.01	79.3	5.6	79.0	4.1	79.7	7.9	5.6
	0.05	81.3	6.7	80.4	5.5	80.6	6.8	5.9
	1	87.9	4.9	86.3	4.6	87.3	6.0	4.9
	5	87.3	3.5	87.2	5.7	85.9	5.5	4.7

a) $n = 5$.

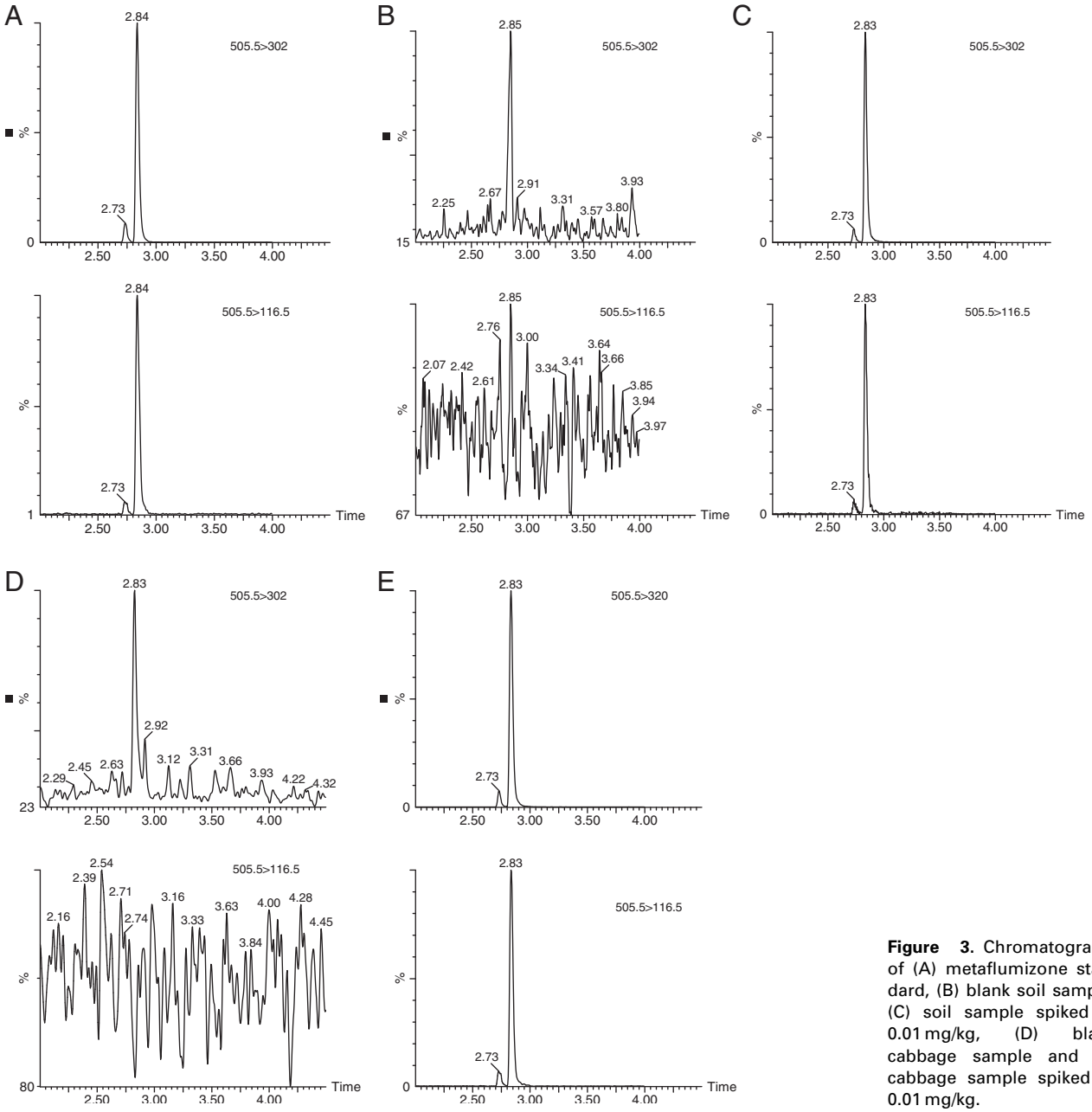

Figure 3. Chromatograms of (A) metaflumizone standard, (B) blank soil sample, (C) soil sample spiked at 0.01 mg/kg, (D) blank cabbage sample and (E) cabbage sample spiked at 0.01 mg/kg.

respectively. The matrix did not significantly suppress or enhance the response of the instrument.

3.5 Application to practical samples

Metaflumizone in cabbage was degraded from 0.61 to 0.12 mg/kg by 3 days after a commercial formulation treatment. The corresponding soil residues dropped from 0.48 to 0.30 mg/kg. As shown in Fig. 4, some conversions between the *E*-isomer and the *Z*-isomer of metaflumizone were noted, but further research should be carried out to clarify the conversion of metaflumizone isomers in different environments.

4 Concluding remarks

A rapid and simple UPLC/MS/MS method was developed and validated for the determination of residues of metaflumizone in cabbage tissues and soil samples following commercial applications. The method shows satisfactory validation parameters in terms of linearity, lower limits, accuracy and precision. The mean recoveries in cabbage and soil for the metaflumizone ranged between 77.6 and 87.9%, and the coefficient of variation (RSD_r) value of repeatability of the method ranged from 3.5 to 7.9%. The LOQs of metaflumizone in both cabbage and soil were 0.004 mg/kg.

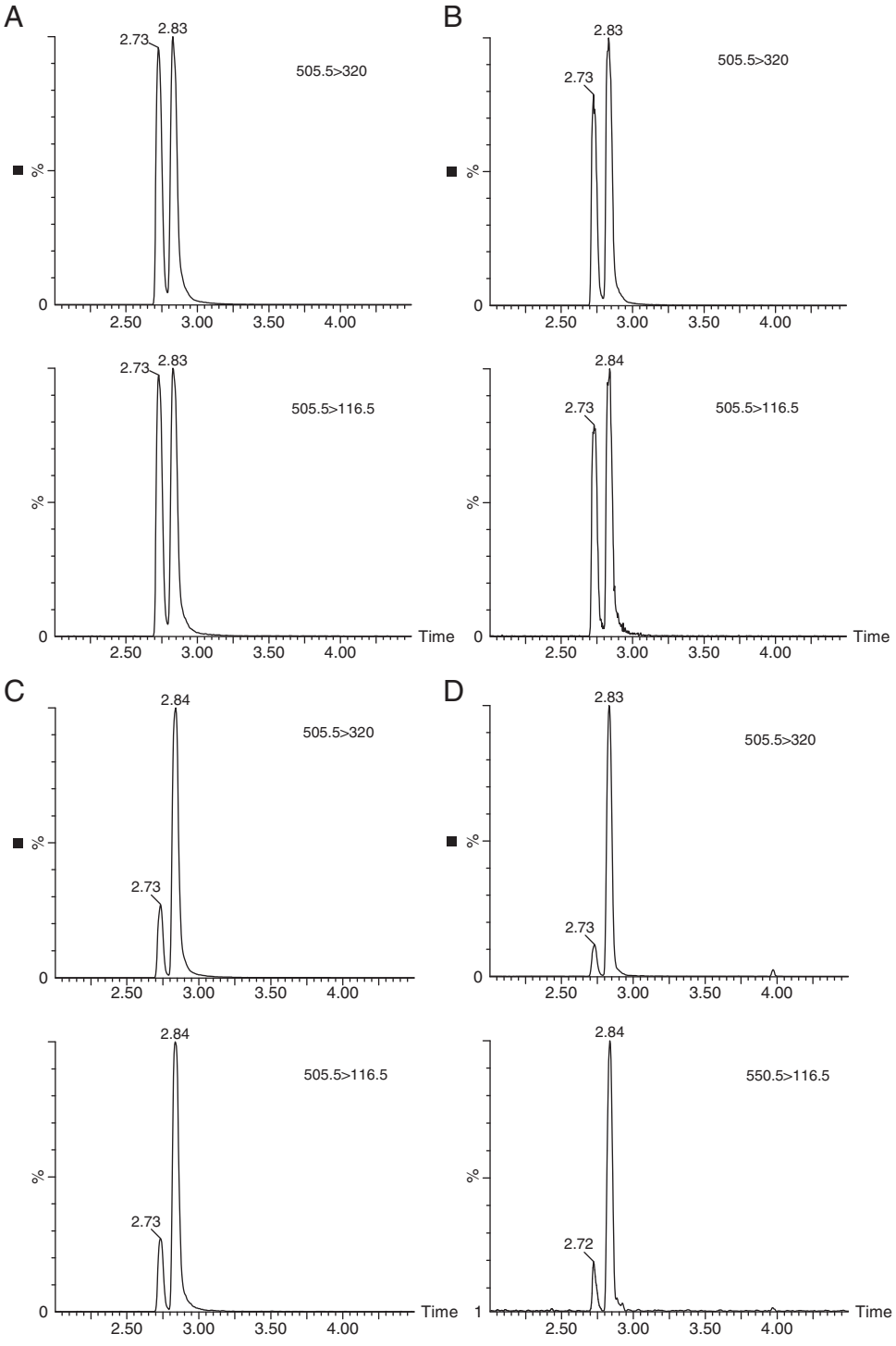

Figure 4. Chromatograms of (A) cabbage samples collected 1 day after application; (B) cabbage samples collected 3 days after application; (C) soil samples collected 1 day after application and (D) soil samples collected 3 days after application.

The maximum residue limit of metaflumizone in head cabbages has been established at 1 mg/kg by the European Union [9]. Therefore, this proposed analytical method is sensitive enough for the detection of residues at this level. The method is also accurate, fast and sufficiently easy to perform that it could be used for regular monitoring of metaflumizone residues in soil samples and in cabbages destined for market.

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The authors have declared no conflict of interest.

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