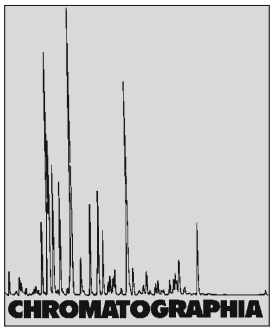
A Method for Quantifying Azoxystrobin Residues in Grapes and Soil Using GC with Electron Capture Detection



2008, *67*, 761–766

Wei Li[1], Yi-Jun Wu[1,✉], Dong-Mei Qin[3], Yan Ma[1,2], Ying-Jian Sun[2], Shao-Ping Qiu[1]

[1] State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, 100101 Beijing, People's Republic of China; E-Mail: wuyj@ioz.ac.cn
[2] Beijing Agricultural College, 102206 Beijing, People's Republic of China
[3] Institute for the Control of Agrochemicals, Ministry of Agriculture, 100026 Beijing, People's Republic of China

Received: 23 September 2007 / Revised: 18 January 2008 / Accepted: 22 January 2008
Online publication: 17 April 2008

Abstract

A relatively simple method for the determination of azoxystrobin residues in grapes and soil using gas chromatography equipped with electron capture detector (GC-ECD) is described. Samples were extracted with acetone, and further partitioned with dichloromethane and petroleum ether. The extracts were then cleaned up in a glass clean-up column filled with active charcoal and silica gel, and eluted with dichloromethane/ethyl acetate (70:30, *v/v*). The eluate was collected and concentrated for GC-ECD analysis. The results showed good linearity (r^2 = 0.9998) over the concentration range of 6.25–400 ng mL^{-1}. The limits of detection (LOD) and quantification (LOQ) of azoxystrobin were 3 and 10 ng mL^{-1}. Recovery from soil and grape samples was in the range of 83.52–107.36 and 82.21–107.31%, with corresponding relative standard deviations (RSD) of 5.21–9.11 and 4.53–5.90% for the three fortified levels. Inter- and intra-day RSDs were in the range of 0.87–6.76 and 2.01–5.46%. The accuracy and sensitivity of the GC-ECD method was independently confirmed by LC and GC-MS. It was demonstrated that the proposed method was simple and efficient, and particularly suitable for detecting azoxystrobin residues in grapes and soil.

Keywords

Gas chromatography
Electron capture detection
Fruit crops
Pesticides

Introduction

Azoxystrobin (methyl (E)-2-{2-[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate) is a broad spectrum fungicide from the strobilurin group of compounds (Fig. 1). At room temperature, it is a white crystal solid with a melting point between 118 and 119 °C and vapour pressure < 10^{-5} Pa (20 °C). Its solubility in water at 25 °C is 10 mg L^{-1} [1].

The acute toxicity of azoxystrobin is quite low; the LD_{50} for rats is > 5,000 mg kg^{-1} when ingested and 2,000 mg kg^{-1} for skin contact [2]. Azoxystrobin can inhibit fungal spore growth and microspore formation, as well as spore bourgeoning [3]. It has also been proposed as a means of controlling downy and powdery mildew in grapes, and brown patch in apples [4, 5]. Azoxystrobin has been applied as a fungicide on numerous crops in China since 2001 [6]. Although the FAO/WHO has not set a maximum residue limit (MRL) for azoxystrobin in grapes, the European Union has recommended an acceptable daily intake (ADI) of not more than 0.18 mg g^{-1} [7].

Several methods for quantifying azoxystrobin residues have been reported [8–13]. Gel filtration chromatography has been used to quantify azoxystrobin in grapes, wheat, and apples. In this case, the sample was prepared and cleaned up by solid phase diffusion and solid phase extract methods [14]. In other methods, the residues of azoxystrobin in apples and muskmelons were determined by mass spectrometry and liquid chromatography

DOI: 10.1365/s10337-008-0565-y
0009-5893/08/05

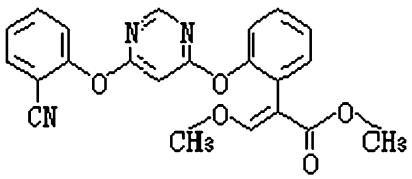


Fig. 1. Chemical structure of azoxystrobin

with a UV detector [15–18]. In this paper, gas chromatography coupled with an electron capture detector (GC-ECD) was used to quantify azoxystrobin residues in grapes and soil.

Experimental

Reagents and Materials

Petroleum ether (99%), acetone (99.5%), dichloromethane (99.5%), ethyl acetate (99.5%), glacial acetic acid (99.5%), sodium chloride (99.5%), and sodium sulfate anhydrous (anhydrous Na2SO4) (99%) were purchased from Beijing Chemical Reagent Company (Beijing, China). Acetonitrile (99.9% purity), Celite 545 and active charcoal were obtained from Merck Chemicals (Darmstadt, Germany). Methanol (99.9%) was purchased from Fluka Chemical Co. (Buchs, Switzerland). Silica gel (60–100 mesh) was obtained from Haiyang Chemical Plant (Qingdao, China). Standard azoxystrobin (99.7% purity) was obtained from Syngenta Co. (Cambridge, UK).

Equipment

Samples were weighed on a Mettler AE200 electronic analytical balance (Greifensee, Switzerland). Sample extracts were evaporated in an EYELA Model NE-IS vacuum rotary evaporator (Tokyo, Japan). Analyses were performed using an HP 5890 II gas chromatograph (GC) equipped with an electron capture detector (ECD) and electronic pressure controller (Agilent Technologies, Palo Alto, CA, USA), an HP-1100 liquid chromatograph equipped with UV detector (Agilent Technologies), and an Agilent-6890N-5973N mass spectrometer (MS) equipped with EI detector (Agilent Technologies).

Chromatographic Conditions

The GC parameters were: oven temperature 295 °C, injector temperature 300 °C, detector temperature 350 °C. The GC was operated under a constant pressure of 7.9 psi. The injection volume was 1 μL at a carrier gas flow of 1 mL min^{-1} nitrogen (>99.999%) with a split ratio of approximately 1:7. Compounds were separated on an HP-1 capillary gas chromatographic column (100% polydimethylsiloxane; 25 m × 0.32 mm × 0.25 μm) (Agilent Technologies). For comparison experiments, the GC was carried out under the same conditions except for the chromatographic column, which was a BPX50 column (50% phenyl equivalent polysilphenylene-siloxane; 30 m × 0.53 mm × 0.5 μm) (SGE International, Ringwood, Australia).

In addition to using a different polarity column for the GC method, LC and GC-MS analyses were also conducted for comparison with the GC-ECD method. For LC analysis, an HP-1100 liquid chromatograph equipped with a ZORBAX 300SB-C_{18} column (4.6 × 250 mm × 5 μm) was used for analysis. The mobile phase was acetonitrile/actic acid (0.17%, pH 3.0) (55:45, *v*/*v*). The column temperature was held at 30 °C and the flow rate was 1 mL min^{-1}. The UV wavelength was set at 255 nm. Injection volumes were 5 μL.

For GC-MS analysis, an Agilent-6890N-5973N mass spectrometer equipped with EI detector (70 ev) was used. Analytes were separated using a DB-1 chromatographic column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies), which was inserted directly into the ion source of the MS. The ion source temperature was 230 °C; quadrupole temperature was 150 °C. Splitless mode was used with an injector temperature of 280 °C. The GC oven temperature program was as follows: initial temperature was 50 °C for 1 min, then to 295 °C at 20 °C min^{-1} with the final temperature maintained for 5 min. Helium (99.99%) was used as the carrier gas. The injection volume was 1 μL. Full-scan chromatograms were obtained by scanning from 50 to 410 *m*/*z*.

Solutions

A stock solution of azoxystrobin was prepared by dissolving 50 mg of the pesticide in 50 mL of acetone-petroleum ether mixture (35:65, *v*/*v*) (APEM) (final concentration, 1 mg mL^{-1}). Appropriate dilutions of azoxystrobin were made in APEM to produce a working standard solution of 400 ng mL^{-1}. The prepared stock solution was stored at 4 °C until required.

Sample Preparation

Two types of samples, grape pulp and soil, were prepared in the experiments. The grapes were collected from the Kyoho variety grown in a well-drained red loam soil in the vineyards of Hangzhou, China. The soil samples were collected in the 0–15 cm soil layer from the vineyards and mixed well. The soil sample was air-dried, and then was sifted through a 40-mesh sieve. The water content of the soil samples was determined. The grapes were stemmed manually and crushed using a vegetation disintegrator. A 20 g homogenized grape pulp or soil sample was accurately weighed (precision 0.01 g). The samples were then spiked with known quantities of standard azoxystrobin at three concentration levels (0.01, 0.20 and 2.00 mg kg^{-1}). All prepared samples were stored at 4 °C. The sample was gradually warmed to room temperature before analysis.

Extraction

Twenty grams of the treated grape pulp or soil sample were transferred into a conical flask with stopper and mixed with 30 mL acetone overnight. The conical flask with stopper containing the mixture was shaken for 1 h in a shaker next day. The sample was filtered through a Buchner funnel into a filtrate flask by air pump with 80 mL acetone, and then the process was repeated with 70 mL acetone. The combined filtrate

was transferred to a separatory funnel. 200 mL 5% sodium chloride and 50 mL dichloromethane were added, then agitated for 2 min. After standing for 1 h, the dichloromethane phase was collected. The water phase was extracted with 2×30 mL dichloromethane. All dichloromethane phases were transferred into a round-bottomed flask, and then evaporated at 25 °C to near dryness on a rotary vacuum evaporator.

Table 1. Recovery of azoxystrobin from fortified grape and soil samples ($n = 5$)

Sample	Added amount (ng g^{-1})	Average recovery (%)	RSD[a] (%)
Soil	2000	91.41 ± 3.23	7.89
	200	94.38 ± 2.20	5.21
	10	98.58 ± 4.01	9.11
Grape	2000	86.12 ± 2.27	5.90
	200	90.75 ± 2.14	5.27
	10	103.38 ± 2.09	4.53

[a] Relative standard deviation

Clean-Up

The above extracts from 20 g grape pulp or soil samples were processed through the column for clean-up. A glass column (30×1 cm i.d.) was filled with 4 g anhydrous sodium sulfate (approximately 4 cm), 3.5 g of a mixture of silica gel, filtration aid and active charcoal (2:1.5:1), and finally, 4 g anhydrous sodium sulfate (approximately 4 cm). Four additional glass clean-up columns were used to compare the clean-up ability; the glass column was filled with an anhydrous sodium sulfate plus 1 g silicon gel, or 5 g florisil, or 1 g mixture of florisil and active charcoal (4:1), or 5 g florisil and 1 g mixture of florisil and active charcoal (4:1). The active charcoal and silica gel were baked for 4 h at 135 °C before use, and the active charcoal was acidified. The column was prewashed with 30 mL dichloromethane [19]. Then the extracts were transferred to the column with 30 or 15 mL dichloromethane (the first elution was discarded). Then the grape or soil sample extracts in the column were eluted by 100 or 45 mL dichloromethane-ethyl acetate mixture (70:30, *v*/*v*) (DEAM), respectively. The eluate was collected and vacuum evaporated at 35 °C to near dryness. The concentrated sample was transferred to a volumetric flask by APEM. The final volume of the sample was 10 mL and used for chromatographic analysis.

Results and Discussion

Optimal Clean-Up Conditions

Samples eluted through a glass clean-up column filled with silica gel showed the presence of the interferents, indicating that this procedure is not appropriate to detect azoxystrobin. Filling the column with florisil gave a similar result. Filling the column with florisil and active charcoal allowed azoxystrobin to be separated from the sample matrices by GC analysis; however, the recovery rate of azoxystrobin was low and elution was not completed with a 100 mL APEM elution solution. Filling the column with florisil plus a mixture of florisil and active charcoal both separated azoxystrobin in the sample matrices and achieved good recovery (above 80%). Filling the column with silica gel plus filtration aid and active charcoal was also effective in separating azoxystrobin from the sample matrices (Table 1). These data showed that active charcoal combined with either florisil or silica gel was effective in the clean-up of the samples for the GC detection of azoxystrobin. Silica gel was applied in the sequent experiments because it was easier to use than florisil.

Elution

After the column had equilibrated to room temperature the sample extract was loaded onto it. The column was then eluted with a 100 mL mixture of DEAM. The maximum azoxystrobin reading was obtained with a 30 mL elution solution, whereas azoxystrobin levels were below the detection limit when a 90 mL eluting solution was used. However, since azoxystrobin could not be eluted from samples with either dichloromethane or APEM alone, these chemicals can be used to wash impurities from samples. Therefore, dichloromethane was used for pre-filtration of the column and DEAM, rather than APEM, to elute samples loaded onto the column.

Method Validation

Calibration Curves and Limit of Detection

The calibration curve was obtained by analyzing seven standards in APEM. Linear regression analysis was performed using the external standard method with six replicate injections of standards at each concentration level. There was a linear relationship between the peak area (y) for azoxystrobin and the concentration (x) of the pesticide in the range of 6.25–400.00 ng mL^{-1}. The regression equation was $y = 182.64x - 446.39$. It displayed good linearity ($r^2 = 0.9998$) over a relatively wide range of concentrations. Relative retention time was about 6.606 min (Fig. 2a).

The limits of detection (LOD) and quantification (LOQ) were determined by comparing measured response levels of serials of diluted standard solutions with those of blank samples, in accordance with the criteria given elsewhere [20]. The concentrations for LOD and LOQ were 3 and 10 ng mL^{-1} respectively.

Repeatability

The repeatability of the method was assessed by measuring intra- and inter-day variability. The standards solutions were stored at 4 °C and warmed gradually to room temperature before analysis. Intra-day repeatability was examined in seven individual samples within one day; inter-day repeatability was determined for three independent days. The relative standard deviation (RSD) was

Table 2. Intra- and inter-day repeatability and accuracy of azoxystrobin assays

Nominal concentration (ng mL^{-1})	Inter-day (n = 7)			Intra-day (n = 7)		
	Mean ± SD	RSD[a] (%)	RME[b](%)	Mean ± SD	RSD[a] (%)	RME[b](%)
6.25	7.34 ± 0.40	5.40	17.44	6.16 ± 0.42	6.76	1.44
25	26.047 ± 0.52	2.01	4.20	27.08 ± 0.55	2.01	8.32
250	263.50 ± 5.29	2.01	5.40	248.10 ± 2.15	0.87	0.76

[a] Relative standard deviation
[b] Relative mean error

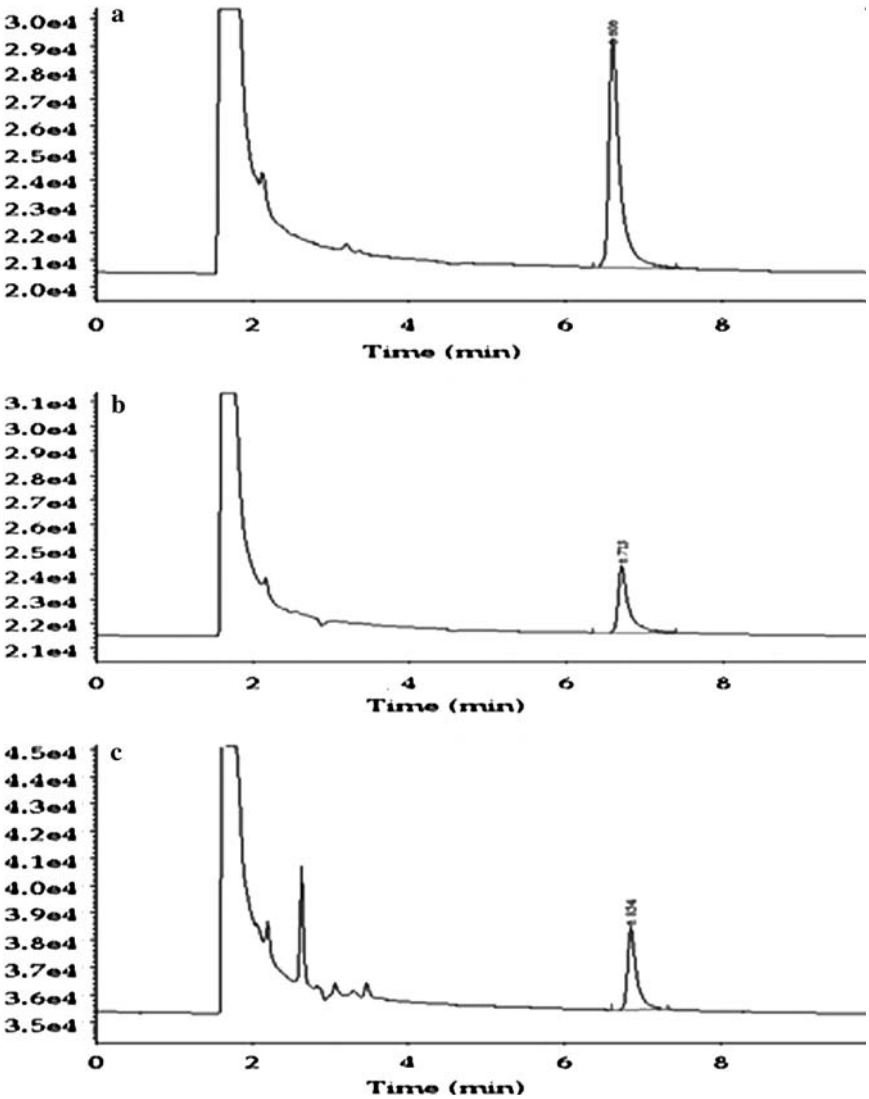


Fig. 2. Typical GC chromatograms of azoxystrobin obtained from: (**a**) standard solution (the concentration was 200 ng mL^{-1}); (**b**) grape sample (160 ng mL^{-1}); and (**c**) soil sample (160 ng mL^{-1})

calculated as a measure of repeatability. Intra- and inter-day RSD values were ca. 2% for all concentrations tested, except for the concentration of 6.25 ng mL^{-1} (5.40 and 6.76% for intra- and inter-day assay respectively) (Table 2).

Recovery

The recovery assay was carried out by adding known quantities of the standard solution to grape and soil samples (see Sample Preparation section). Quantification of azoxystrobin residues was carried out by the external standard method and percent recovery calculated. Recovery for soil and grape samples was 83.52–107.36 and 82.21–107.31% respectively, with corresponding RSD values of 5.21–9.11 and 4.53–5.90% (Table 1; Fig. 2b, c). All recoveries and RSD values were within the permissible range.

Stability

To test stability, a standard solution (25 ng mL^{-1}) and soil sample solutions (blank soil sample spiked with azoxystrobin at 25 ng mL^{-1}) that had been stored at 4 °C were analyzed every 24 h for 7 and 14 days respectively. No significant differences were observed between successive analyses (data not shown).

Comparison with Other Analytical Methods

Gas Chromatography with a BPX50 Column

The different polarity columns were used to validate the advantage of the pretreatment method coupling the ECD detector. The results of using GC-ECD with the BPX50 column method showed a linear relationship between peak area (y) for azoxystrobin at concentrations (x) in the range of 5–160 ng mL^{-1}; $y = 917.7x - 846.83$ ($r^2 = 0.9997$). The recoveries for soil and grape samples at concentrations of 200 and 10 ng g^{-1} azoxystrobin were 91.31 and 95.52%, respectively, which were very similar to the results obtained using GC-ECD with the HP-1 capillary column; no significant difference was found between the two columns. These data suggested that there

was no strict requirement for the column polarity when using this GC-ECD method to determine azoxystrobin.

LC

Supporting qualitative data may be provided by LC detection techniques. The result was obtained by analyzing the standard at 2,000 ng μL^{-1}. The retention time of azoxystrobin in this condition was 5.358 min (Fig. 3). A short retention time and a stable baseline were the merits of the LC analysis; however, the LOQ was 30 ng mL^{-1}, which was much higher than that of GC-ECD. This result confirmed the sensitivity of the GC-ECD method for detecting azoxystrobin.

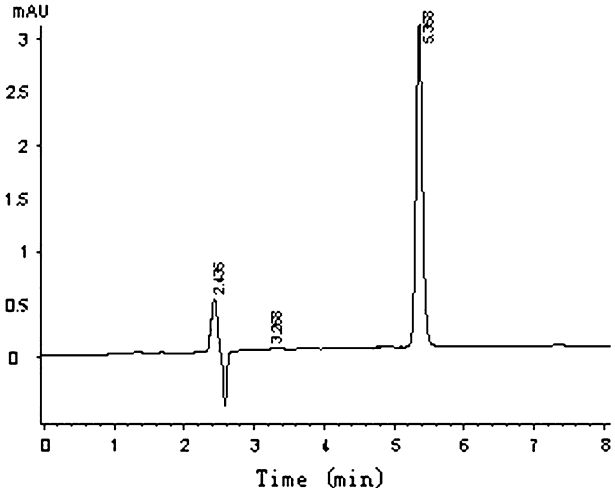


Fig. 3. Typical LC chromatograph of a standard solution of azoxystrobin (2,000 ng mL^{-1})

GC-MS

GC-MS, which is a powerful analytical tool for confirmatory analysis, was carried out for comparison with other techniques. In the GC-MS conditions described above, the compound was detected with the retention time of 16.33 min. The molecular ion peak of azoxystrobin was 403 (Fig. 4). The characteristic ion ratios were 344(999), 207(482), 345(320), 388(319), and 403(140), which is a 99% match for the data in the MS database (NIST base Ref#157185). This result was further confirmation that the chemical detected was azoxystrobin, and that the GC-ECD method is effective in detecting this chemical.

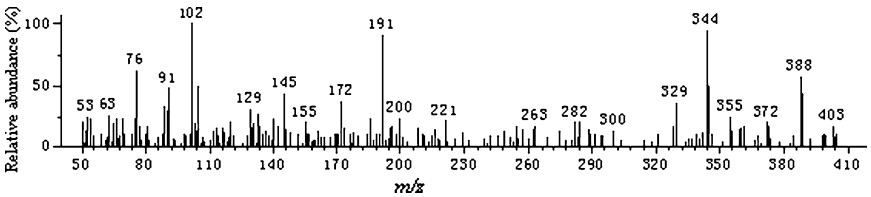


Fig. 4. The MS spectrum of a standard solution of azoxystrobin (5,000 ng mL^{-1})

Conclusion

After sample pretreatment, reliable results were obtained with the GC-ECD method. The method had a fast 6.6 min retention time analyzing a large number of samples. All statistical values (percentage recoveries, RSD, confidence limits of the slope and intercept, LOD and LOQ) were within acceptable limits and the results obtained were confirmed by other analysis methods. GC-ECD was simple, sensitive and reliable for quantifying azoxystrobin residues in soil and crops for routine assays of this pesticide.

Acknowledgments

This research was partly supported by the grants from the National Key Technologies R&D Program of China (2006BAK02A02), and the National 863 Program (No. 2006AA06Z423). We express our sincere gratitude to Prof. C.-F. Qian for her critical review of the manuscript.

References

1. Environmental Protection Agency (EPA) of USA. (2003) Rules and regulations Azoxystrobin. Federal Register, 68(117): 36480–36487[EB/OL]. http://www.epa.gob/fedrgstr
2. Pest Management Regulatory Agency, Health Canada. Regulatory Note: Section 17 Temporary registrations: REG 2000-15 Azoxystrobin [EB/OL]. http://www.hc-sc.gc.ca/pmra-arla/english/pdf/reg/reg200-15-e.pdf
3. Karaoglanidis GS, Karadimos DA (2006) Crop Prot 25:977–983
4. Bella G di, Saitta M, Salvo F, Nicotina M, Dugo G. (2003) Ital J Food Sci 15(3):427–432
5. Schirra M, Cabras P, Angioni A, Brandolini V (2002) J Agric Food Chem 50:1461–1464
6. Bo HB Bi Y, Ma LY, Chen LR (2005) China J Pestic 44:28–30
7. EU Directorate (1998) General for Agricutural, DG VI-B-II-1. Review report for the active substance azoxystrobin, Document 7581/V1/97-Rev. 5 [EB/OL]. http://eruopa.eu.int/comm/food/fs/ph_ps/pro/eva/newactive/list2_en.htm
8. Lentza-Rizos C, Avramides EJ, Kokkinaki K (2006) J Agric Food Chem 54:138–141
9. Giza I, Sztwiertnia U (2003) Acta Chromatogr 13:226–229
10. Christensen HB, Granby K (2001) Food Addit Contam 18:866–874
11. Cabras P, Angioni A, Garau VL, Pirisi FM, Brandolini V (1998) J AOAC Int 181(6):1185–1189
12. Henriet F, Pigeon O, Moreau JM (2006) Commun Agric Appl Biol Sci 71(2 Pt A):193–195
13. Furzer GS, Veldhuis L, Hall JC (2006) J Agric Food Chem 54(3):688–693
14. Flores JL, Díaz AM, Fernández de Córdova ML (2007) Anal Chim Acta 585(1):185–191

15. Polati S, Bottaro M, Frascarolo P, Gosetti F, Gianotti V, Gennaro MC (2006) Anal Chim Acta 579: 146–151
16. Sannino A, Bolzoni L, Bandini M (2004) J Chromatogr A 1036(2):161–169
17. De Melo Abreu S, Correia M, Herbert P, Santos L, Alves A (2005) Food Addit Contam 22(6):549–556
18. Boudina A, Emmelin C, Baaliouamer A, Paisse O, Chovelon JM (2007) Chemosphere 68(7):1280–1288
19. Yin F, Lu Z, Lin M (2004) Pestic Sci Admin 26(2):17–20
20. US Food and Drug Administration (2003) Guidance for approval of a method of analysis for residues [EB/OL]. http://www.fda.gov/cvm/guidance/1731.htm