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Analytical Methods

Validation of quantitative method for azoxystrobin residues in green beans and peas

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ABSTRACT

This study presents a method validation for extraction and quantitative analysis of azoxystrobin residues in green beans and peas using HPLC-UV and the results confirmed by GC–MS. The employed method involved initial extraction with acetonitrile after the addition of salts (magnesium sulfate and sodium chloride), followed by a cleanup step by activated neutral carbon. Validation parameters; linearity, matrix effect, LOQ, specificity, trueness and repeatability precision were attained. The spiking levels for the trueness and the precision experiments were (0.1, 0.5, 3 mg/kg). For HPLC-UV analysis, mean recoveries ranged between 83.69% to 91.58% and 81.99% to 107.85% for green beans and peas, respectively. For GC–MS analysis, mean recoveries ranged from 76.29% to 94.56% and 80.77% to 100.91% for green beans and peas, respectively. According to these results, the method has been proven to be efficient for extraction and determination of azoxystrobin residues in green beans and peas.

1. Introduction

According to UN's Food and Agriculture Organization (FAO), Egypt occupies the sixth position in the production world ranking of green beans and peas. The production quantities (tons) of Egypt in 2012 are 251.279 and 180.631 (FAO, 2014).

Azoxystrobin (Fig. 1) is a systemic, broad-spectrum fungicide belonging to the class of methoxyacrylates, which are derived from the naturally-occurring strobilurins. It exerts its fungicidal activity by inhibiting mitochondrial respiration in fungi. It is absorbed through the roots and translocated in the xylem to the stems and leaves, or through leaf surfaces to the leaf tips and growing edges. Azoxystrobin Controls foliar and soil-borne diseases including downy and powdery mildew, early and late blight, and pathogens *Sclerotinia*, *Alternaria*, *Ascochyta*, *Pythium*, and *Rhizoctonia* on many crops (PMRA, 2009). Several studies focused on the analytical methods of azoxystrobin in different matrices. In these studies, gas chromatography (GC) was the most commonly used method to determine azoxystrobin residues in different fruits and vegetables (Aguilera, Valverde, Camacho, Boulaid, & García-Fuentes, 2012; Bo, Bi, & Chen, 2005; Bo & Sun, 2008; Bo, Wang, Guo, Qin, & Lu, 2008; Ding et al., 2006; Gajbhiye et al., 2011; Han, Yao, Wu, Wang, & Qin, 2009; Huan, Xu, Lv, Xie, & Luo, 2013; Li et al., 2008; Liu, Sun, Zeng, & Liu, 2010; Sun, Bo, & Han, 2007; Wang, Hou, Zou, & Lu, 2010; Wang, Sun, & Liu, 2013; Wei, Lu, He, & Zuo, 2011; Wu, Wang, Wu, Zhao, & Yang, 2010; Yin et al., 2011; Zhang, Zhang, Lu, & Li, 2008). High-performance liquid chromatography (Abreu, Caboni, Cabras, Garau, & Alves, 2006; Polati et al., 2006; Shi, Zhao, Che, & Huang, 2010) and gas chromatography–mass spectrometry (GC–MS) (Bo, 2007; Bo et al., 2008; Melo et al., 2012) have also been introduced in the analysis of azoxystrobin residues. Moreover, liquid chromatography-tandem mass spectrometry was used to determine azoxystrobin residue (Itoiz, Fantke, Juraske, Kounina, & Vallejo, 2012; Polati et al., 2006; Wu et al., 2009). Photochemically induced fluorescence was developed to determine the presence of azoxystrobin (Javier, Antonio, & Maria, 2007). In this work, we aimed to develop and validate an effective method for extraction and quantitative determination of azoxystrobin in green beans and peas using high performance liquid chromatography with UV-detection; the results were confirmed by GC–MS.

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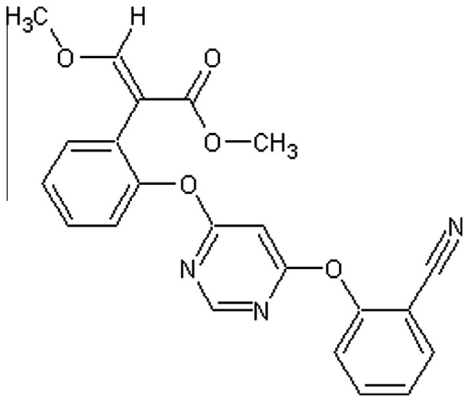

Fig. 1. The chemical structure of azoxystrobin.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile, isopropanol and methanol of HPLC quality were from J.T.Baker, USA. Double de-ionized water obtained by using an EasyPure LF (Compact Ultrapure Water System), USA. Anhydrous magnesium sulfate and sodium chloride were purchased from J.T.Baker, USA. Neutral Decolorizing Carbon from Fisher Scientific, USA. Azoxystrobin standard 1000 μg/mL ampoules (obtained from Absolute Standards, Inc., USA) were serially diluted in methanol to prepare stock standard of 100 μg/mL and other spiking concentrations. Pyrene D10 (obtained from Sigma–Aldrich, USA) was used as internal standard (ISTD) at 50 μg/mL in methanol.

2.2. Instruments and apparatus

A centrifuge (IEC/Centa GP8) was used for phase separation. Weighing was performed using a PG503 DeltaRange scale for masses above 0.1 g and an AG104 scale for masses below 0.1 g. Sample homogenization was conducting using a Cole Parmer, Analytical Mill. An ultrasonic bath (FS110H-Fisher Scientific) used for sonication during the extraction step. Chromatography consumables, certified glassware and personal protective equipment were used during all the analysis steps.

HPLC analysis of azoxystrobin was performed with Shimadzu HPLC system consisting of LC-10AS pump, SIL-9A auto injector and SPD-6AV UV–VIS spectrophotometric detector. A Biphenyl 100A Kinetex column (250 mm × 4.6 mm × 5 μm) from Phenomenex was kept at room temperature. Separation of azoxystrobin was done with isocratic elution using the mobile phase (50% acetonitrile: 50% water: 0.4% acetic acid). The flow rate was 0.8 mL/min and the injection volume was 40 μL. The detection wavelength set at 255 nm. The azoxystrobin residues identified by comparing the retention time of the sample peak with the retention time of the standards. The retention time of azoxystrobin was14.55 min. The GC–MS analysis of azoxystrobin was performed with gas chromatograph (HP6890 Series GC system) coupled to 5973 mass selective detector (Agilent Technologies, Inc., CA, USA) with detection system in the selective ion-monitoring mode (SIM). Sample ionization was achieved by electron impact at 70 k eV. The column used was an HP-5, 5% phenyl methyl siloxane (30 mm × 0.25 mm × 0.25 μm, Agilent Technologies). The oven was programed to start at 80 °C for 2 min, ramp at 20 °C/min until 180 °C, and a second ramp at 5 °C/min until 300 °C. The volume injected was 2 μL at 0 psi, the optimum vent pressure for each condition, calculated automatically by GC–MS software. Other operating conditions were explored by means of a simplex experimental design. Helium was used as carrier gas (1 mL/min). The transfer line was held at 280 °C. The retention time of Azoxystrobin was 30.10 min.

2.3. Sample preparation

The green beans and peas samples was purchased from the market in Athens, GA, USA, and analyzed before the validation to ensure that it was free from any pesticide residues. All required materials for the experiment were organized and labeled. Samples were roughly cut with a knife into small portions and homogenized for at least 30 s, the homogeneous matrix was stored in sealable plastic bag at −18 °C until the preparation day.

2.4. Extraction and cleanup

10 g (±0.1 g) of frozen sample homogenate were weighed into 50-mL centrifuge tubes. The samples were spiked with azoxystrobin standard solution while frozen to reach 3 concentration levels (0.1, 0.5, 3 mg/kg), 5 replicates for each level. The extraction involved the addition of 10 mL of acetonitrile followed by 100 μL of the internal standard solution (ISTD) containing 10 μg/mL of Pyrene D10. The tubes were closed and vigorously shaken by hand for 1 min. To induce separation and partitioning, salt mixture of 4 g of anhydrous magnesium sulfate and 1 g of sodium chloride was added. The tubes were re-closed, vigorously shaken by hand for 1 min, placed for 15 min in ultrasonic bath then centrifuged for 5 min at 4500 rpm. The extracts were subjected to freeze-out/-cleanup (aliquot placed in freezer for >2 h before cleanup). The cleanup was carried out by transferring 1 mL of the acetonitrile phase into 15 mL centrifuge tubes containing 0.002 g activated decolorizing neutral carbon. The tubes were closed, vigorously shaken by hand for 1 min followed by 5 min of centrifugation at 4500 rpm. The supernates were filtered using 0.2 μm nylon syringe filter (Chrom Tech Inc., Apple Valley, MN, USA) into auto-sampler vials which was tightly closed using a cap and septum and stored at −18 °C until the day of analysis.

2.5. Preparation of matrix-matched calibration solutions

Matrix-matched calibration was used to compensate for the matrix effects. Unspiked samples (blank) of green beans and peas were treated like the spiked samples without adding internal standard. One mL aliquots of the blank extracts were spiked by 100 μL of azoxystrobin at a concentration level corresponding to 120% of the spiking level using appropriately diluted working standard solutions. 100 μL of ISTD solution (1 μg/mL) (corresponding to one-tenth of the amount of ISTD added to the samples during the procedure) were also added to each of the matrix-matched calibration solution. Bracketing calibration was attained in which the matrix-matched calibration solutions were injected at the beginning and at the end of each sequence to insure that the determination system was free from any significant drift. According to document SANCO/12571/2013 (SANCO, 2013), the acceptable drift between two bracketing injections of the same calibration standard should not exceed 30%.

2.6. Method validation

According to Document SANCO/12571/2013 (SANCO, 2013), a within-laboratory method validation was performed to provide evidence that the method is fit for the extraction and quantitative determination of azoxystrobin in green beans and peas. Method validation is a requirement of accreditation bodies, and must be

supported and extended by method performance verification during routine analysis where all steps that are undertaken in a method should be validated. The method was validated following a conventional validation procedure that included the following parameters: linearity, matrix effects, limits of quantification (LOQ), specificity, trueness (bias) and repeatability precision (RSD*r*).

2.6.1. Linearity

Multi-level calibration (at levels 0.05, 0.25, 0.5, 1, 2, 4, 5 μg/mL and 0.1, 0.5, 1, 3, 5, 10 μg/mL for HPLC-UV and GC–MS analysis, respectively) and calibration function were used. The fit of the calibrations were plotted and inspected by calculation of the residuals, avoiding over-reliance on correlation coefficient, to insure that the fit is satisfactory within the concentration range of the pesticides detected.

The residuals were calculated as follows

$$\%\ \text{Residuals} = 100 \times (\text{SE} - \text{SC})/\text{SE}$$

where: % Residuals: residual of the point. SE: signal of the calibration point obtained experimentally. SC: signal of the calibration provided by calibration function.

2.6.3. Matrix effect

Matrix effects were defined as the influence of one or more co-extracted components from the sample on the measurement of azoxystrobin concentration. The presence of these effects is demonstrated by comparing the response produced from the azoxystrobin in a simple solvent solution with that obtained from the same quantity of azoxystrobin in the presence of the sample or sample extract. Extracts of blank matrix (peas and green beans) used for preparation of matrix-matched calibration solutions at levels 0.05, 0.1, 0.5, 1, 2, 3 mg/kg were used to compensate the matrix effects for both HPLC-UV and GC–MS analysis.

Matrix effects (%ME) were calculated using the equation:

$$\text{ME} = \frac{m_{\text{matrix}} - m_{\text{solvent}}}{m_{\text{solvent}}} \times 100\%$$

where ME is the matrix effect, and m_{matrix} and m_{solvent} are the slops of calibration curves in the matrix and in the pure solvent, respectively.

2.6.4. Limit of quantification LOQ and specificity

The limit of quantitation (quantification) was defined as the lowest concentration of the azoxystrobin that has been validated with acceptable trueness (70–120%) and precision (RSD$r \leqslant 20\%$) by applying the complete analytical method. According to the document SANCO/12495/2013 (SANCO, 2013) the Limit of quantification should be ⩽MRL. The maximum residue limit (MRL) for azoxystrobin is 3 mg/kg for green beans and peas, (European Union, 2014). Specificity was defined as the ability of the detector (supported by the selectivity of the extraction and clean-up) to provide signals that effectively identify the analyte (azoxystrobin), according to the document SANCO/12571/2013, these signals should be at levels ⩽30% of RL (reporting limit). Absolute numbers and at this level the detector provide signals that effectively identify azoxystrobin. It is equal to or higher than the LOQ.

2.6.5. Trueness (bias)

The measure of trueness is normally expresses as "bais". It was defined as the closeness of agreements between the average values obtained from a series of test results (the mean recovery). Five replicates used to check the recovery at the levels (0.1, 0.5, 3 mg/kg). According to the document SANCO/12571/2013 (SANCO, 2013), acceptable mean recoveries are those within the range of 70–120%.

Trueness was calculated using the following equation:

$$\%R = (\boldsymbol{X}/\mu) \times 100$$

$\%R$: recovery percentage.

$\boldsymbol{X}$: experimental concentration of azoxystrobin(mg/kg).

μ : calculated concentration of azoxystrobin(mg/kg).

2.6.6. Precision (RSDr)

The precision (Repeatability (r)) was defined as standard deviation of measurement of azoxystrobin obtained using the same method on the same samples in a single laboratory over a short period of time, during which differences in the materials and equipment used and analysts involved will not occur. The value of ⩽20% was used as the limit for RSD*r*. Five replicates for each recovery levels (0.1, 0.5, 3 mg/kg) per day on three different days were used to check the precision.

$$\%\ \text{RSD} = (\text{standard deviation of the replicates} / \text{mean value of the replicates}) \times 100$$

3. Results and discussion

3.1. Sample preparation

The samples were homogenized at low temperature (frozen) to avoid any significant influence of ambient temperature would result in degradation of certain pesticide residues. Frozen condition also helps to compensate for the heat generated (exothermic reaction) when magnesium sulfate and sodium chloride were added. On other hand, freezing-out removes most of lipids, waxes and sugars as well as other components with low solubility in acetonitrile that may negatively affect the robustness of GC and LC analysis (Anastassiaded, Tasdelen, Scherbaum, & Stajnbaher, 2007).

3.2. Extraction and cleanup

These were carried out under precise and timed steps for all samples and blanks. The time between spiking and extraction was fixed at 20 min and shaking time was 1 min. Table 1 shows that acceptable recoveries were obtained by using 0.003 g in the cleanup step.

3.3. Method validation

3.3.1. Linearity

The evaluations of calibration curve linearity of azoxystrobin were done based on injections of standard solutions prepared in organic solvent (methanol) at concentrations 0.05, 0.25, 0.5, 1, 2, 4, 5 μg/mL for HPLC-UV analysis and at concentrations 0.1, 0.5, 1, 3, 5, 10 μg/mL for GC–MS analysis. Figs. 2 and 3 show that the fit

Table 1
Effect of the amount of carbon on the accuracy and precision of azoxystrobin in green beans and peas in the cleanup step at the MRL value (3 mg/kg).

Amount of carbon (g)	Green beans		Peas	
	Mean recovery (%) (n^* = 5)	RSD (%)	Mean recovery (%) (n^* = 5)	RSD (%)
0.005	28.96	±2.21	45.87	±5.64
0.003	115.93	±4.55	89.61	±0.23
0.002	83.69	±12.06	107.58	±1.48

* Number of replicates.

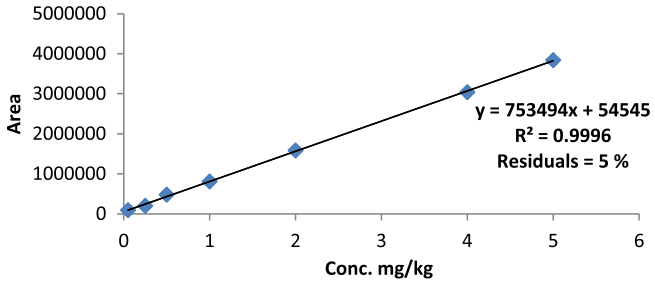

Fig. 2. Calibration curve of azoxystrobin with HPLC-UV analysis.

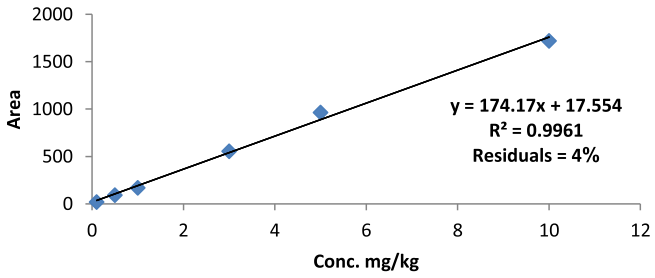

Fig. 3. Calibration curve of azoxystrobin with GC–MS analysis.

Table 2
Matrix effect and LOQ values of azoxystrobin in green beans and peas.

Matrix	Matrix effect (ME%)		LOQ (mg/kg)	
	HPLC-UV analysis	GC–MS analysis	HPLC-UV analysis	GC–MS analysis
Green beans	−31.58%	−32.78	0.1	0.1
Peas	+63.16%	+17.76	0.1	0.1

of the calibrations are satisfactory with Residuals ±5% and ±4% for HPLC-UV and GC–MS analysis, respectively. According to the document SANCO/12571/2013 (SANCO, 2013), the acceptable limit of residuals deviation is ⩽±20%, where the fit of calibration inspected by calculation of the Residuals avoiding over-reliance on correlation coefficient.

3.3.2. Matrix effect (ME%), Limit of quantification LOQ and specificity

The matrix-matched calibration solutions were used to circumvent errors associated with matrix-induced enhancement and suppressions effects in both GC and LC determinations. The matrix effect was evaluated by comparing the slops of calibration curves (at levels 0.05, 0.1, 0.5, 1, 2, 3 mg/kg) of azoxystrobin in matrix (green beans and peas) and in pure solvent. The matrix effect for HPLC-UV analysis for both green beans and peas were −31.58% and +63.16%, respectively. The matrix effect for GC–MS analysis for both green beans and peas were −32.78% and +17.76%, respectively. The negative values of ME% in case of green beans for both HPLC-UV and GC–MS analysis reflect matrix induced suppression and positive values in case of peas reflect matrix induced enhancement. The lowest validated level of azoxystrobin with acceptable precision and trueness (LOQ) was 0.1 mg/kg for HPLC-UV and GC–MS analysis in both green beans and peas. According to the document SANCO/12571/2013 (SANCO, 2013), the LOQ values are acceptable where LOQ ⩽ MRL (3 mg/kg for both green beans and peas, (European Union, 2014)). Table 2. Shows the Matrix effect and LOQ values of azoxystrobin in green beans and peas. For HPLC-V and GC–MS analysis, The specificity of the detectors were attained where the detectors provided signals that effectively identify azoxystrobin at level 0.05 mg/kg for both green beans and peas, so the reporting limits of azoxystrobin will be >0.16 mg/kg. Blank control samples for both green beans and peas provided response <30% RL.

3.3.3. Trueness and precision (RSDr)

The trueness, bais or mean recovery was carried out in five replicates at levels (0.1, 0.5, 3 mg/kg) by spiking 10 g of blank sample with standard solutions. For HPLC-UV analysis, the obtained mean recoveries ranged from 83.69% to 91.58% with RSD ranging from 2.95 to 15.11 for green beans. For peas, mean recoveries ranged from 81.99% to 107.85% with RSD ranging from 1.48 to 17.24. For GC–MS analysis, mean recoveries for green beans ranged from 76.29% to 94.56% with RSD ranging from 5.11 to 13.27. For peas, mean recoveries ranging from 80.77% to 100.91% with RSD ranging from 6.22 to 11.12. According to the document SANCO/12571/2013 (SANCO, 2013), the obtained mean recoveries were within the acceptable range (70–120%). The repeatability precision (RSD*r*) involved repeat of recovery levels (0.1, 0.5, 3 mg/kg), five replicates for each level per day on three different days. For HPLC-UV analysis, The (RSD*r*) values ranged from 10.99 to 12.88% and from 0.67% to 9.19% for green beans and for Peas, respectively. For GC–MS analysis, the (RSD*r*) values ranged from 6.94% to 14.63% and from 8.15% to 15.47% for green beans and for peas, respectively. According to the document SANCO/12571/2013 (SANCO, 2013), the obtained (RSD*r*) values were within the acceptable range ⩽20% (Table 3).

4. Conclusion

A simple quantitative method for azoxystrobin residues in green beans and peas using HPLC-UV and GC–MS was validated. The method involved initial extraction step after addition salts (magnesium sulfate and sodium chloride), followed by a cleanup step, utilizing dispersive solid-phase extraction by activated neutral carbon. Validation was performed according the document SANCO/12571/2013 (SANCO, 2013). Validation parameters linearity, matrix effect, LOQ, specificity, trueness and repeatability precision were attained.

Table 3
Mean recoveries and repeatability precision of azoxystrobin in green beans and peas for HPLC-UV and GC–MS analysis.

Spiking level (mg/kg) (n^* = 5)	Green beans				Peas			
	HPLC-UV analysis		GC–MS analysis		HPLC-UV analysis		GC–MS analysis	
	Mean recovery (% ± RSD)	RSD*r* (%)	Mean recovery (% ± RSD)	RSD*r* (%)	Mean recovery (% ± RSD)	RSD*r* (%)	Mean recovery % ± RSD))	RSD*r* (%)
0.1	91.58 ± 15.11	12.88	76.29 ± 6.54	6.94	81.99 ± 17.24	0.67	100.09 ± 9.58	12.31
0.5	86.98 ± 2.95	10.99	85.19 ± 5.11	14.63	100.31 ± 8.86	7.93	80.77 ± 11.12	8.15
3	83.69 ± 12.06	11.55	94.56 ± 13.27	13.33	107.58 ± 1.48	9.19	100.91 ± 6.22	15.47

* Number of replicate.

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References

Abreu, S. M., Caboni, P., Cabras, P., Garau, V. L., & Alves, A. (2006). Validation and global uncertainty of a liquid chromatographic with diode array detection method for the screening of azoxystrobin, kresoxim-methyl, trifloxystrobin, famoxadone, pyraclostrobin and fenamidone in grapes and wine. *Analytica Chimica Acta, 573–574*, 291–297.

Aguilera, A., Valverde, A., Camacho, F., Boulaid, M., & García-Fuentes, L. (2012). Effect of household processing and unit to unit variability of azoxystrobin, acrinathrin and kresoxim methyl residues in zucchini. *Food Control, 25*, 594–600.

Anastassiaded, M., Tasdelen, B., Scherbaum, E., & Stajnbaher, D. (2007). Recent developments in QuChERS methodology for pesticide multiresidue analysis. In H. Ohkawa, H. Miyagawa, & P. W. Lee (Eds.), *Pesticide chemistry: Crop protection, public health, environmental safety*. Weinheim: Wiley-VCH.

Bo, H. B. (2007). Determination of azoxystrobin residues in fruits and vegetables by GC chromatography/ms spectrometry with solid-phase extraction. *Chinese Journal of Chromatograph, 25*(6), 898–901.

Bo, H. B., & Sun, J. (2008). Determination of azoxystrobin residue in muskmelon and apple by gas chromatography. *Food Research and Development, 29*(3), 113–115.

Bo, H. B., Bi, Y., & Chen, L. R. (2005). Determination of azoxystrobin residue in muskmelon and apple by gas chromatography. *Chinese Journal of Analytical Chemistry, 33*(5), 695–698.

Bo, H. B., Wang, J. H., Guo, C. H., Qin, R., & Lu, X. Y. (2008). Determination of strobilurin fungicide residues in food by gas chromatography–mass spectrometry. *Chinese Journal of Analytical Chemistry, 36*(11), 1471–1475.

Ding, R. Y., Li, H. D., Du, H. X., Li, M. L., Ji, J., & Chen, Z. L. (2006). Determination methods of azoxystrobin residue in cucumber by gas chromatography. *Chemical Analysis and Meterage, 15*(6), 76–77.

Document SANCO/12571/2013. Guidance document on analytical quality control and validation procedures for pesticide residues analysis in food and feed. <http://www.eurl-pesticides.eu/library/docs/allcrl/AqcGuidance_Sanco_2013_12571.pdf>.

European Union (2014). Maximum residue limits for pesticides. <http://ec.europa.eu/sanco_pesticides/public/?event=substance.selection&ch=1>.

Gajbhiye, V. T., Gupta, S., Mukherjee, I., Singh, S. B., Singh, N., Dureja, P., et al. (2011). Persistence of azoxystrobin in/on grapes and soil in different grapes growing areas of India. *Bulletin of Environment Contamination and Toxicology, 86*, 90–94.

Han, B., Yao, A. Q., Wu, H. M., Wang, M. C., & Qin, L. (2009). Degradation of azoxystrobin residue in orange and soil. *Agrochemicals, 48*(12), 899–901.

Huan, Z., Xu, Z., Lv, D., Xie, D., & Luo, J. (2013). Dissipation and residues of difenoconazole and azoxystrobin in bananas and soil in two agro-climatic zones of China. *Bulletin of Environment Contamination and Toxicology, 91*, 734–738.

Itoiz, E. S., Fantke, P., Juraske, R., Kounina, A., & Vallejo, A. A. (2012). Deposition and residues of azoxystrobin and imidacloprid on greenhouse lettuce with implications for human consumption. *Chemosphere, 89*, 1034–1041.

Javier, L. F., Antonio, M. D., & Maria, L. F. C. (2007). Determination of azoxystrobin residues in grapes, musts and wines with a multicommuted flow-through optosensor implemented with photochemically induced fluorescence. *Analytica Chimica Acta, 585*, 185–191.

Li, W., Wu, Y., Qin, D., Ma, Y., Sun, Y., & Qin, S. (2008). A method for quantifying azoxystrobin residues in grapes and soil using GC with electron capture detection. *Chromatographia, 67*, 761–766.

Liu, Y., Sun, H. B., Zeng, F. J., & Liu, J. M. (2010). Study on residual dynamics of azoxystrobin in mango and soil. *Guangdong Agricultural Science, 10*, 106–107.

Melo, A., Cunha, S. C., Mansilha, C., Aguiar, A., Pinho, O., & Ferreira, I. M. P. L. V. O. (2012). Monitoring pesticide residues in greenhouse tomato by combining acetonitrile-based extraction with dispersive liquid–liquid microextraction followed by gas-chromatography–mass spectrometry. *Food Chemistry, 135*, 1071–1077.

PMRA (2009). Pest Management Regulatory Agency. <http://www.hc-sc.gc.ca/cps-spc/pest/registrant-titulaire/tools-outils/label-etiq-eng.php>.

Polati, S., Bottaro, M., Frascarolo, P., Gosetti, F., Gianotti, V., & Gennaro, M. C. (2006). HPLC-UV and HPLC-MSn multiresidue determination of amidosulfuron, azimsulfuron, nicosulfuron, rimsulfuron, thifensulfuron methyl, tribenuron methyl and azoxystrobin in surface water. *Analytica Chimica Acta, 579*, 146–151.

Shi, F., Zhao, K. H., Che, J., & Huang, A. T. (2010). Determination of residual azoxystrobinin in citrus shatangju by HPLC. *Journal of Hebei Agricultural Sciences, 14*(2), 161–163.

Sun, J., Bo, H. B., & Han, S. Y. (2007). Studies on determination methods of azoxystrobin residue in fruits and vegetables by gas chromatography. *Journal of Gansu agricultural university, 42*(2), 112–115.

Wang, S. W., Hou, Z. G., Zou, J., & Lu, Z. B. (2010). Residual decline of azoxystrobin 25%SC in ginseng environment. *Agrochemicals, 49*(6), 436–438.

Wang, S., Sun, H., & Liu, Y. (2013). Dissipation and residue of azoxystrobin in banana under field condition. *Environmental Monitoring and Assessment, 185*, 7757–7761.

Wei, Y. J., Lu, W. H., He, Y. F., & Zuo, F. H. (2011). Determination methods of azoxystrobin residue in agricultural products by gas chromatography. *Agrochemicals, 50*(6), 434–435.

Wu, J. L., Wang, H. C., Wu, X. T., Zhao, J., & Yang, N. N. (2010). Determination of azoxystrobin residue in *Panax ginseng* and *Panax quinquefolium* and dietary intake risk assessment. *Chinese Journal of Pesticide Science, 14*(1), 67–73.

Wu, Y. X., Lin, F., Lin, H. D., Shao, L. Z., Jiao, H., & Li, X. (2009). Determination of azoxystrobin residues in Legume using high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry. *Journal of Instrumental Analysis, 28*(5), 617–620.

Yin, L. D., Hou, Z. G., Chen, C., Ji, H. M., Wei, X. H., & Lu, Z. B. (2011). Residues and decline study of azoxystrobin in soybean. *Chinese Journal of Pesticide Science, 13*(3), 304–309.

Zhang, H., Zhang, T. C., Lu, Z. B., & Li, Y. (2008). The residue dynamic of azoxystrobin 25 % SC in *Pleurotus ostreatus*. *Agrochemicals, 47*(12), 905–906.