ANALYTICAL METHODOLOGY FOR THE ESTABLISHMENT OF MAXIMUM RESIDUE LIMITS (MRLs) FOR SPINETORAM IN AVOCADO (*PERSEA AMERICANA*)

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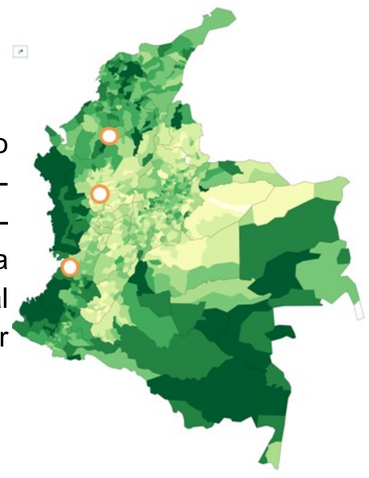
Avocado (Persea americana) is an exotic fruit of economic importance on the global market and has become a major part of the diet in many countries. In Colombia, Avocado fruit is an alternative for the small growers because of its export potential. Currently, Spinetoram, one of the insecticides registered in Colombia for *Thrips* spp. control in avocado, does not have maximum residue limit (MRL) in this commodity and is making it difficult to gain access to the international market because of safety regulations. Colombia developed technical studies to determine maximum residue limits (MRLs) for Spinetoram in avocado through a STDF supported project and with assistance from the USDA and IR-4 minor use project.

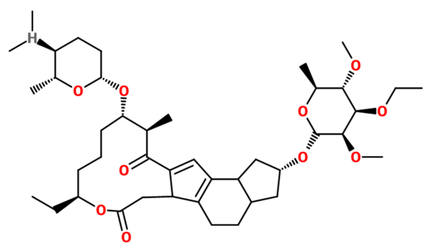
FIELD SAMPLES

Untreated samples of tree diferent varieties of avocado (Hass, Reed and Papelillo), with known production histories were provided from six farms in the three main avocado production regions of Colombia: Antioquia, Risaralda and Cauca. The farms were certified in Good Agricultural Practices (GAP) and field trials were developed under OECD-GLP standards.



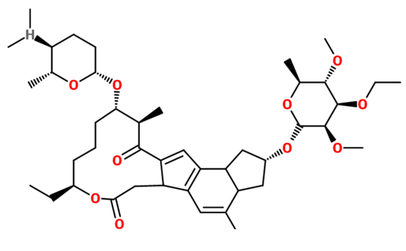
ANALYTICAL PROCEDURE

Spinetoram is a broad-spectrum insecticide used to control crop-damaging insects; is a mixture of chemically modified spinosyns J and L. The chemical structures are shown below.



3'-O-ethyl-5,6-dihydro spinosyn J

SPINETORAM J

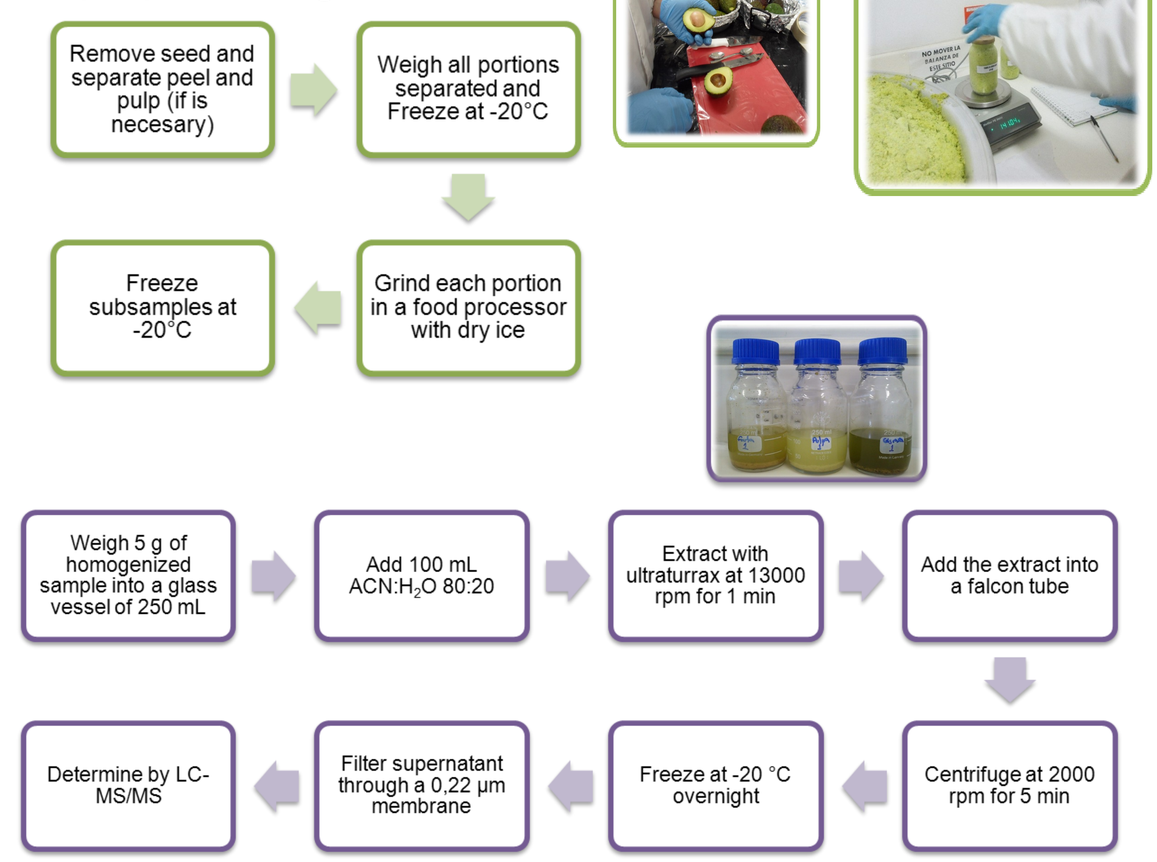


3'-O-ethyl spinosyn L

SPINETORAM L

Major considerations for the analytical method performance include accuracy, precision, sensitivity and selectivity. The residue definition includes Spinetoram (Spinetoram-J and Spinetoram-L) and also its metabolites (N-Demethyl-Spinetoram-J and N-Formyl-Spinetoram-J). The methodology was validated for the four compounds in peel, pulp and whole fruit of avocado

Sample processing



ACKNOWLEDGMENTS

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INSTRUMENTAL CONDITIONS

For LC-MS/MS analysis, the UPLC system was an Acquity UPLC® (Waters®, Milford, USA) with the MassLynx software. The column was an ACQUITY C_{18} (1,7 µm, 2.1 × 100 mm) maintained at 25 °C. The mobile phase consisted of a combination of phase A (ACN/MeOH (1:1) with amonium acetate 2 mM) and phase B (Amonium acetate 2 mM). The conditions of chromatography were as follows: phase A was initiated at 66% increased linearly to 100% at 2.2 min, hold until 3.5 min and then back to 66% at 4 min until the end of the run (5 min), flow rate of 0.35 ml/min. The MS/MS analysis conditions are shown below in the table 1.

Table 1. MRM. transitions and retention times of analytes

Analyte	Retention time (min)	Precursor ion(m/z)	Quantitation ion (m/z)	Confirmation ion (m/z)	CV (V)	CE (eV)
Spinetoram J	3.30	748.5	142.11	98.04	46	32
Spinetoram L	3.34	760.53	142.11	98.04	44	28
N-Demethyl Spinetoram J	2.66	734.53	128.01	84.06	34	24
N-Formyl Spinetoram J	2.91	762.4	156.15	203.08	26	20
Spinetoram J IS	3.25	757.55	146.08	102.00	42	32
Spinetoram L IS	3.40	769.55	146.14	102.07	44	32
N-Demethyl Spinetoram J IS	2.64	739.49	128.07	84.06	40	32

RESULTS

For the recoveries, untreated samples were fortified whit a fixed volume of a mixture of Spinetoram and its metabolites at 0.003, 0.01, 0.20 and 2.0 mg/kg. The quantitation was done using matrix matched calibration curves and deuterated standards due to the presence of matrix effects.

Figure 1 shows the calibration curves obtained for pulp, peel and whole fruit. The difference between the sensitivity in the three matrices can be noted, given the presence of more coextracted fat substances in the pulp and the greater amount of pigments coextracted

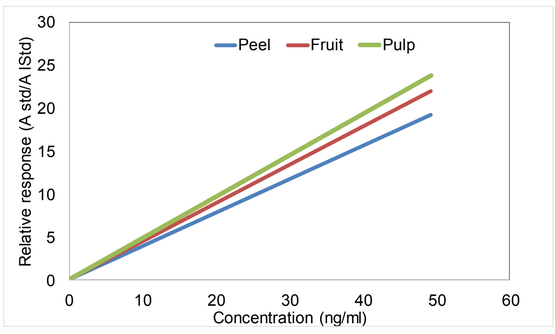

Figure 1. Calibration curves for Sipnetoram J in Pulp,Peel and Whole fruit.

Table 2. Average recoveries and CV

Compound	Matrix	% Rec*	CV
Spinetoram J	Fruit	107	6
	Peel	101	13
	Pulp	110	5
Spinetoram L	Fruit	108	9
	Peel	102	12
	Pulp	109	7
N-Demethyl Spinetoram J	Fruit	111	4
	Peel	103	10
	Pulp	103	11
N-Formyl Spinetoram J	Fruit	106	10
	Peel	101	19
	Pulp	109	7

Table 2 shows the average recoveries for Spinetoram and its metabolites in the three different matrices. The experiment was developed at 0.003, 0.01, 0.20 and 2.0 mg/kg. All the recoveries were in the range 70-120% and CV below 20%. Quantitation and detection limits (LOQ and LOD) were calculated for Spinetoram and its metabolites using the SD from the 0.01 mg/kg recovery experiments in pulp, whole fruit and peel.

The calculated LOQ were below to 0,01 mg/kg for all compounds and matrices. A representative chromatogram for the MRM of the quantitation transition for matrix matched calibration standard at 0,01mg/kg is shown in the figure 2.

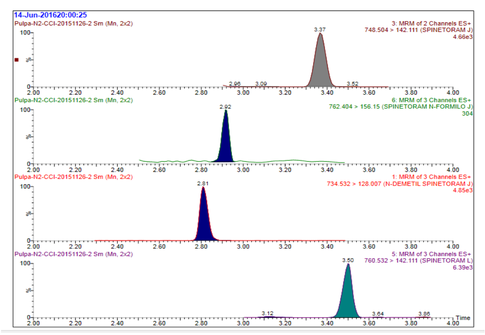

Table 2. Recovery percentage and

Figure 2. MRM quantitation transition for Spinetoram and its metabolites. Peel 0,01 mg/kg

CONCLUSION

The results from this study show this methodology can be adopted for the analysis of samples to establish maximum residue limit (MRL) for Spinetoram in avocado.

REFERENCES

Hastings, M. Dow AgroSciences Methods GRM 05.03 and GRM 015.04. 2005. Unpublished report of Dow AgroSciences LLC