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POSITION PAPER No. 16 - 01

"GUAZATINE"

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Abstract
This position paper is related to the residue analysis and the corresponding reporting of levels of the fungicide "Guazatine" on citrus fruits and other crops. Guazatine is a mixture of reaction products from polyamines, comprising mainly octamethylenediamine, iminodi(octamethylene)diamine and octamethylenebis(imino-octamethylene)diamine, and cyanamide. It consists of polymorphic compounds, containing guanidines and polyamines. Guazatine is always produced in the form of acetate salts. These aspects illustrate the challenges for the establishment of a common analytical approach to receive reliable and comparable analytical residue data.
The analytical procedure described by Scordino et al. is taken as reference to discuss the approach of this paper. The method is based on an extraction with mixture of formic acid (1%) in water and acetone (1:2) followed by the chromatographic separation and determination by LC/MSMS.
As the commercial available reference materials are technical mixtures as well, it is of high importance to describe and indicate the reference material and the approach for identification and calculation of the amounts of the particular compounds and at least the calculation of the final result of "Guazatine". This final result must of course correspond to the residue definition as published by the regulation (EC) no. 396/2005 in its current version. To meet this requirement, this position paper provides the necessary information in order to achieve a common approach on selecting the most appropriate reference material, specific compounds and the related factors to calculate the final result of "Guazatine".

Introduction
With regulation (EU) no. 2015/1910 of 21. October 2015 the European Commission has set new MRLs (Maximum Residue Levels) for Guazatine and has introduced a new residue definition. The regulation shall apply from 13 May 2016.
The new MRLs are all set at the "specific limit of determination (SLD)" related to every commodity listed in annex 1 of reg. (EC) 396/2005. For all commodities of plant origin the new MRL is 0.05 mg/kg, which is similar to the SLD (so called "asterix-MRL").
For citrus fruits the new MRLs (0.05 mg/kg) differ significantly from the old MRLs (5 mg/kg). All other commodities keep the "asterix-MRLs" but due to the change of the residue definition the new MRLs are lowered from 0.1 mg/kg to 0.05 mg/kg.
The new residue definition is:
Guazatine (guazatine acetate, sum of components).
The question to be answered is: What are the "components"?
For better understanding, once again the description of Guazatine: Guazatine is a mixture of reaction products from polyamines, comprising mainly octamethylenediamine, iminodi(octamethylene)diamine and octamethylenebis(imino-octamethylene)diamine, and cyanamide.
Therefore, it is essential to work out and agree on a harmonised analytical approach of the identification and quantification of the Guazatine-"components".

Analytical approaches
Two different analytical resp. quantification approaches are known to be used within the relana® laboratory circle.

Approach 1 (basic approach)
This approach was introduced by 4 members of relana® as a joint proposal. It is the basic approach of this Position Paper. It was introduced first by Karsten Ott during the annual relana® meeting in Stuttgart in June 2015. For more details contact afr@labor-friedle.de and/or Karsten.Ott@bilacon.de.

Analytical conditions are
- extraction: water (acidified with 1 % HCOOH) / acetone (1:2)
- separation/detection: LC-MS/MS (ISTD Dodine)
- column (f. ex.): Aquasil C18 (Thermo Fisher);
- literature: LC-MS/MS detection of fungicide guazatine residues for quality assessment of commercial citrus fruits; Monica Scordino et al.; Eur Food Res. Technol (2008) 227:1339-1347

Quantification:
The quantification is based on 4 main components of the technical mixtures:
Guazatine-GG
Guazatine-GGG
Guazatine-GGN
Guazatine-GNG.
All components are present as the acetate salts. Therefore, the related cations (f.ex. GG^+) are analysed and quantified. At the end, the calculation has to take into consideration the presence of the Guazatine components as the *acetate salts*.
"G" stands for Guanidine groups within the related molecule (component), while
"N" stands for Amino groups (N = nitrogen = main part of Amino-groups).

The relevant mass-transitions related to the four selected components are published by Scordino et al. (see literature above). The following table show mass-transitions used by Karsten Ott, bilacon GmbH, Berlin (Germany):

Coumpond	$[M+2H]^+$	$[M+2H]^{2+}$	$[M+2H]^{3+}$	Fragment ions m/z
GG		115		170; 128
GGG			133	170; 128
GGN		179		187; 170
GNG		179		187; 170

The reporting limits (RL) of the 4 Guazatine indicator cations are 0,01 mg/kg each according to Mr. Ott.

Reference Materials
The most critical aspect is the batch/lot of reference material used by the particular laboratory. As discussed above, Guazatine is a technical mixture which might show different levels of the several components from batch to batch (depending on the preparation conditions). The data of the following table was as well provided by Karsten Ott, bilacon GmbH, Berlin (Germany):

Compound	SIGMA BATCH: SZBB102XV Content [%]	SIGMA BATCH: SZBE090XV Content [%]
GG	9,81	9,80
GNN	0,084	-
GNG (IMO)	1,060	0,28
GGN	11,690	14,5
GGG	27,434	30,2
Main four coumponds	**50,1 (81,7 %)**	**54,8 (83,2%)**
total	**61,3**	**65,9**
water	**39,0**	**38,0**

A. Nitsopoulos, Labor Friedle GmbH, Tegernheim (Germany), proposed the following approach for quantification and calculation of the total amount of "Guazatine", using the reference material batch no. SZBE090XV by Sigma Aldrich. This proposal focuses on 4 cations only, as they cover > 50% of the total compounds of the technical Guazatine mixtures:

Approach for the calculation of guazatine acetate

Reference material in use (f.ex. of Sigma Aldrich)
Product-No.: 37915
Batch: SZBE090XV
Date of prparation: 31.03.2014
Expiry date: 31.03.2019

percentage of water in reference material	38	%
percentage of acetate in reference material	20,6	%

percentage of single cations in the mixture (related to cations)		
GG	9,802	%
GNG	0,281	%
GGN	14,522	%
GGG	30,19	%
sum (percentage) of the four cations in the mixture (related to cations)	54,795	%

factor to calculate total sum of cations by the sum of 4 cations	1,825	= 1/(sum YELLOW)*100
factor to calculate the sum of guazatine acetate from the sum of Cations	1,498	=(100-ORANGE)/(100-ORANGE-RED)
factor to calculate the total sum of guazatine acetate related to the used reference material	**2,733**	= 1/(sum YELLOW)*100*(100-ORANGE)/(100-ORANGE-RED)

Value taken from the Reference Material Certificate
Value taken from the Reference Material Certificate
Value taken from the Reference Material Certificate

A.Nitsopoulos, Nov. 2015

The factor above (green space) must be calculated for the reference material in use. It has to be re-calculated, if the laboratory makes use of a new batch/lot or any different reference material.

Conclusively, The **final result** is calculated by **summarising** the results of the **4 selected cations** ONLY (Guazatine-Indicator-Cations; GIC) in mg/kg, and then by **multiplying** them with the a.m. **factor**:

Guazatine (Guazatine-acetate, sum of components) = (x,xxx mg/kg **GG-Cation** + x,xxx mg/kg **GGG-Cation** + x,xxx mg/kg **GGN-Cation** + x,xxx mg/kg **GNG-Cation**) **x factor** (green).

Reporting
The reporting of results should be done accordingly in this way:
Guazatine (Guazatine-acetate, sum of components): x,xx mg/kg;
calculated by the sum of the below mentioned four components, multiplied with a factor deriving from the used standard reference material (here f.ex.: f = 2,733):
Guazatine-GG-cation: x,xxx mg/kg
Guazatine-GGG-cation: x,xxx mg/kg
Guazatine-GGN-cation: x,xxx mg/kg
Guazatine-GNG-cation: x,xxx mg/kg.

Approach 2 (alternative approach)
This approach was introduced by Jens Luetjohann, GALAB Laboratories GmbH, Hamburg (Germany). Details are available at Jens.Luetjohann@galab.de. This approach is also provided in order to offer a second analytical method to analyse for Guazatine, f.ex. for confirmation analyses.

Analytical conditions are
- single residue method (SRM) using the indicator component "1,8-Diguanidin-1-yl-octane" (GG) as reference
- extraction with 1 M HCl (100°C/10 min)
- alkalinisation of supernatant with NH_3 solution
- clean up step by cation exchange SPE (f.ex. Chromabond HR-XCW; Macherey &Nagel); elution with methanol, acidified with 5% HCOOH (formic acid)

Two different chromatographic/mass-spec approaches are possible:

a. chromatographic separation and detection by UPLC/MS-MS of the SPE eluate, or

b. derivatisation of the eluate with hexafluoroacetylacetone solved in 10% sodium bicarbonate (100°C/ 2 h), followed by chromatographic separation and detection with GC/MS-NCI (negative chemical ionisation).

The reporting limit (RL) of the indicator component **GG** in matrix lemon is 0,01 mg/kg, independent of the selected analytical technique.

Remarks:
Avoid to make use of glassware, as the Guazatine cations tend to stick on glass surfaces. All labware (flasks, columns etc.) should consist of plastic material.

Literature

- regulation (EU) no. 2015/1910 of 21. October 2015
- The Pesticide Manual© BCPC (British Crop Production Council), 2014
- LC-MS/MS detection of fungicide guazatine residues for quality assessment of commercial citrus fruits; Monica Scordino et al.; Eur Food Res. Technol (2008) 227:1339-1347

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This Position Paper is a literary property of relana®, based on the contribution and the knowledge of the members of the relana® laboratory quality circle. The aim of this publication is to increase knowledge and to provide expertise to all relevant and interested stakeholders in order to achieve best practices on analytical services related to food and feed testing. Everybody is invited to make use of this Position Paper and to circulate it wherever meaningful. While using this Position Paper please make the reference as:
relana® Position Paper No. 16-01 "Guazatine" version 2016/01/19.