

## **POSITION PAPER No. 16 - 01**

### **“GUAZATINE”**

**Version 2016/07/21**  
**(update of vers. 2016/01/19)**

#### **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. Both approaches are based on reaction products of octamethylenediamine.

In the meantime approach 2 is withdrawn. The analytical protocol of the remaining approach as described below was evaluated by a "method"-ring test in April to June 2016, performed on behalf of the "Working Group Pesticides" of the German Association of Chemists (GDCh) and designed, organised and reported by PROOF-ACS GmbH ([www.proof-acs.de](http://www.proof-acs.de)).

For informations related to this ring test please contact Mr. Albrecht Friedle (Labor Friedle GmbH): [afr@labor-friedle.de](mailto:afr@labor-friedle.de).

### Analytical protocol

This protocol was introduced by 4 members of relana® as a joint proposal. It was introduced first by Karsten Ott during the annual relana® meeting in Stuttgart in June 2015. For more details contact Karsten Ott ([Karsten.Ott@bilacon.de](mailto:Karsten.Ott@bilacon.de)) or Albrecht Friedle ([afr@labor-friedle.de](mailto:afr@labor-friedle.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.

(The chemical structures of the components are published by E. Dreassi et. al.)

All components are present as the acetate salts. Therefore, the related cations (f.ex. GG<sup>+</sup>) 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] <sup>+</sup>	[M+2H] <sup>2+</sup>	[M+2H] <sup>3+</sup>	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
<b>Main four compounds</b>	<b>50,1 (81,7 %)</b>	<b>54,8 (83,2%)</b>
<b>total</b>	<b>61,3</b>	<b>65,9</b>
<b>water</b>	<b>39,0</b>	<b>38,0</b>

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 preparation: 31.03.2014 Expiry date: 31.03.2019
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percentage of water in reference material	38 %
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percentage of acetate in reference material	20,6 %
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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
factor to calculate the sum of guazatine acetate from the sum of Cations	1,498

= 1/(sum YELLOW)\*100  
 =(100-ORANGE)/(100-ORANGE-RED)

factor to calculate the total sum of guazatine acetate related to the used reference material	<b>2,733</b>
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= 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

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): deleted~~

#### 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
- E. Dreassi et. al, JPBA 2007 (43) 1499-1506.

**Editorial remark: Changes related to the previous version are in RED letters.**

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