



FOOD

Development and validation of a single residue method (SRM) for the determination of guazatine in citrus fruit

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Introduction and Objectives

Guazatine, a non-systemic contact fungicide, is a mixture of reaction products from polyamines, comprising mainly octa-methylenediamine, iminodi(octamethylene)diamine, octamethylenebis(imino-octamethylene) diamine and carbamonitrile. In the guazatine mixture diamine derivatives account for 40% of the constituents of guazatine, triamines for 46%, tetramines for 11% and other amine derivatives for 3%. The most abundant individual components are the fully guanidated triamine (GGG 30,6%) and the fully guanidated diamine (GG 29,5%) followed by the monoguanidated diamine (GN 9,8%) and a diguanidated triamine (GGN, 8,1%). [1]

Guazatine is no longer authorized for an application in plant protection products within the EU. EFSA reviewed the existing MRLs in the framework of the MRL review under Article 12 of Regulation (EC) No 396/2005 and proposed to lower all MRLs to the appropriate LOQ as no import tolerances were notified [2].

The actual EU MRLs according to Reg. (EC) No 149/2008 (1.3.2008) for citrus fruits are 5 mg/kg, for cereals 0,2 mg/kg and for all remaining plant- and animal-derived commodities 0,1 mg/kg.

The aim of our study was to develop a single-residue method for the determination of guazatin in citrus fruit. We focused at the marker molecule 1,8-diguanidin-1-yl-octane (GG) comprising about 30 % of the technical guazatine mixture.

Materials and methods

Due to the lack of extractability by MRMs/SRMs like QuEChERS or QuPPE an individual extraction/cleanup procedure for the marker molecule 1,8-diguanidin-1-yl-octane (GG) from the technical guazatine mixture was designed.

1 g of homogenized citrus fruit sample was extracted with 1 M HCl (100°C/10 min). The extract was cleaned via cation exchange SPE. The eluate was either subjected to UPLC-MS-MS analysis and/or derivatized with hexafluoroacetylacetone (HFAA; 100°C/2 h) and the GG derivative determined by GC-NCI-MSD.

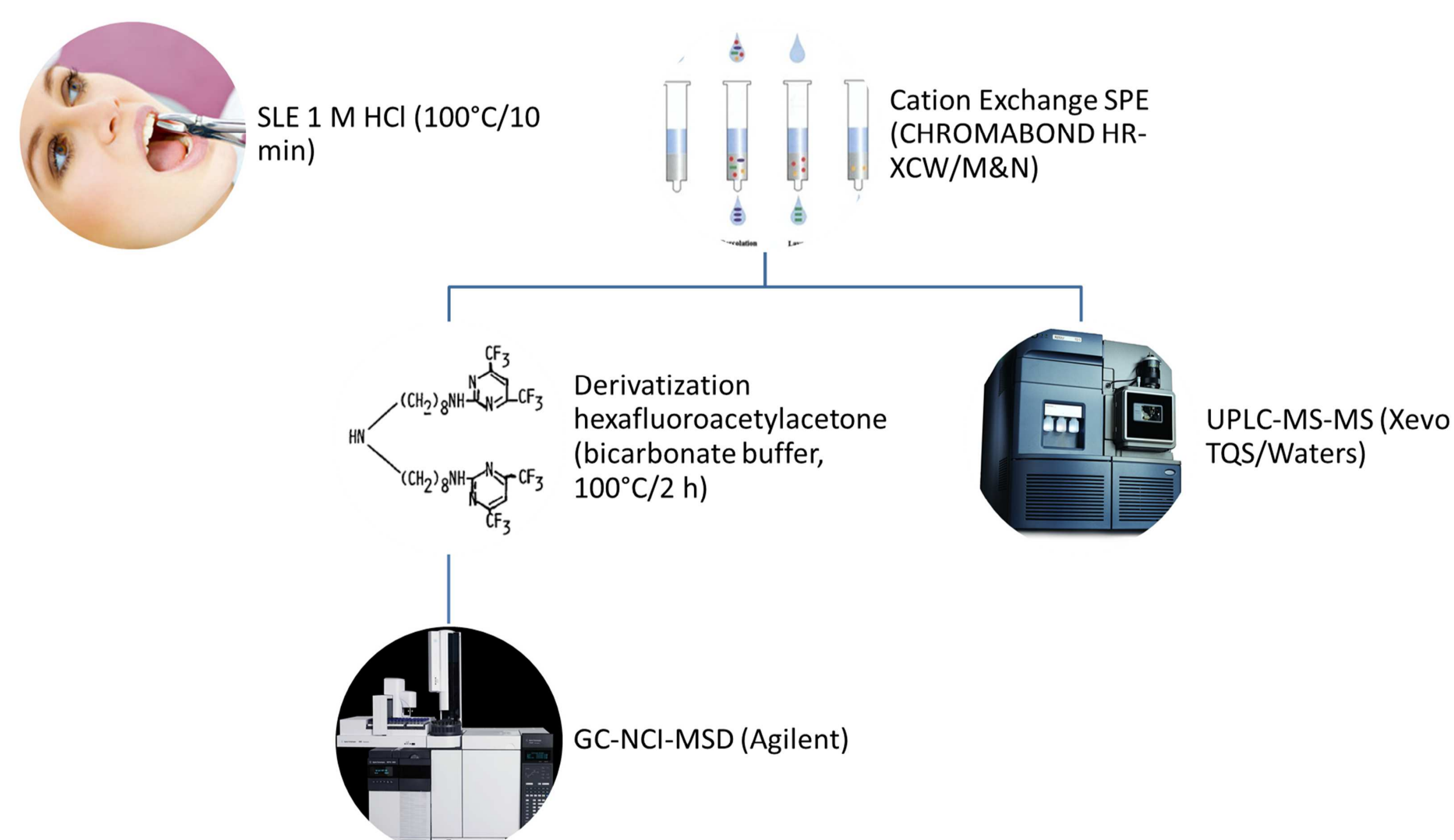


Fig. 1: Workflow of guazatine analysis in citrus fruit.

Results and Discussion

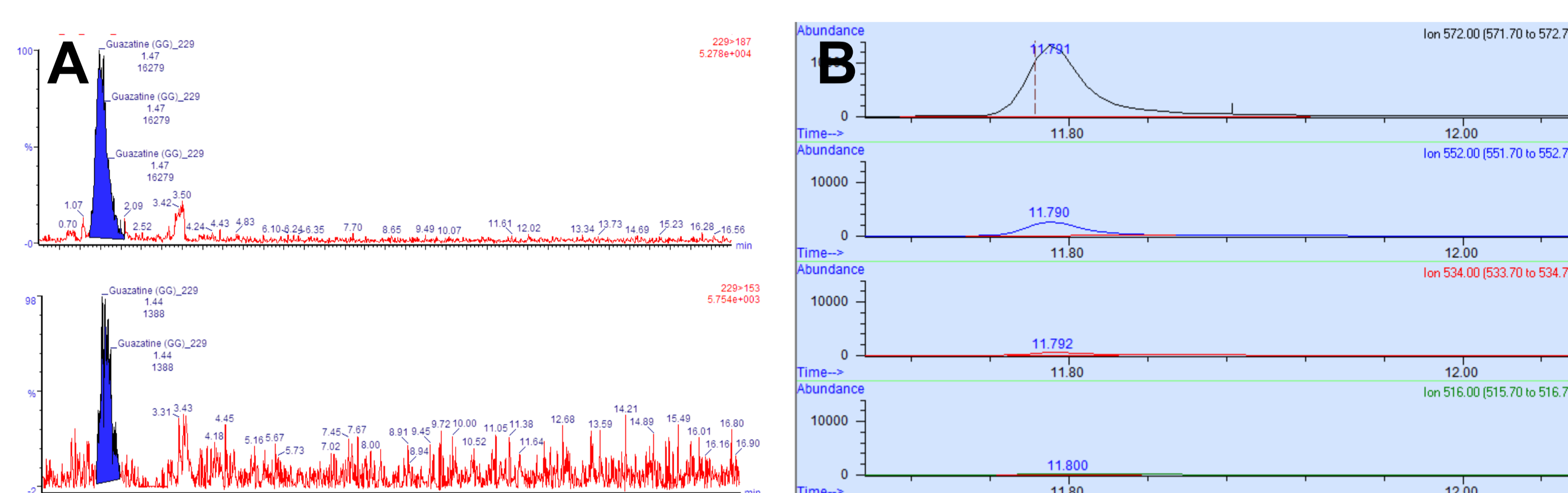


Fig. 2: A lemon sample with 0,01 mg/kg guazatine. A) UPLC-MS-MS of GG and B) GC-NCI-MSD of GG HFAA derivative.

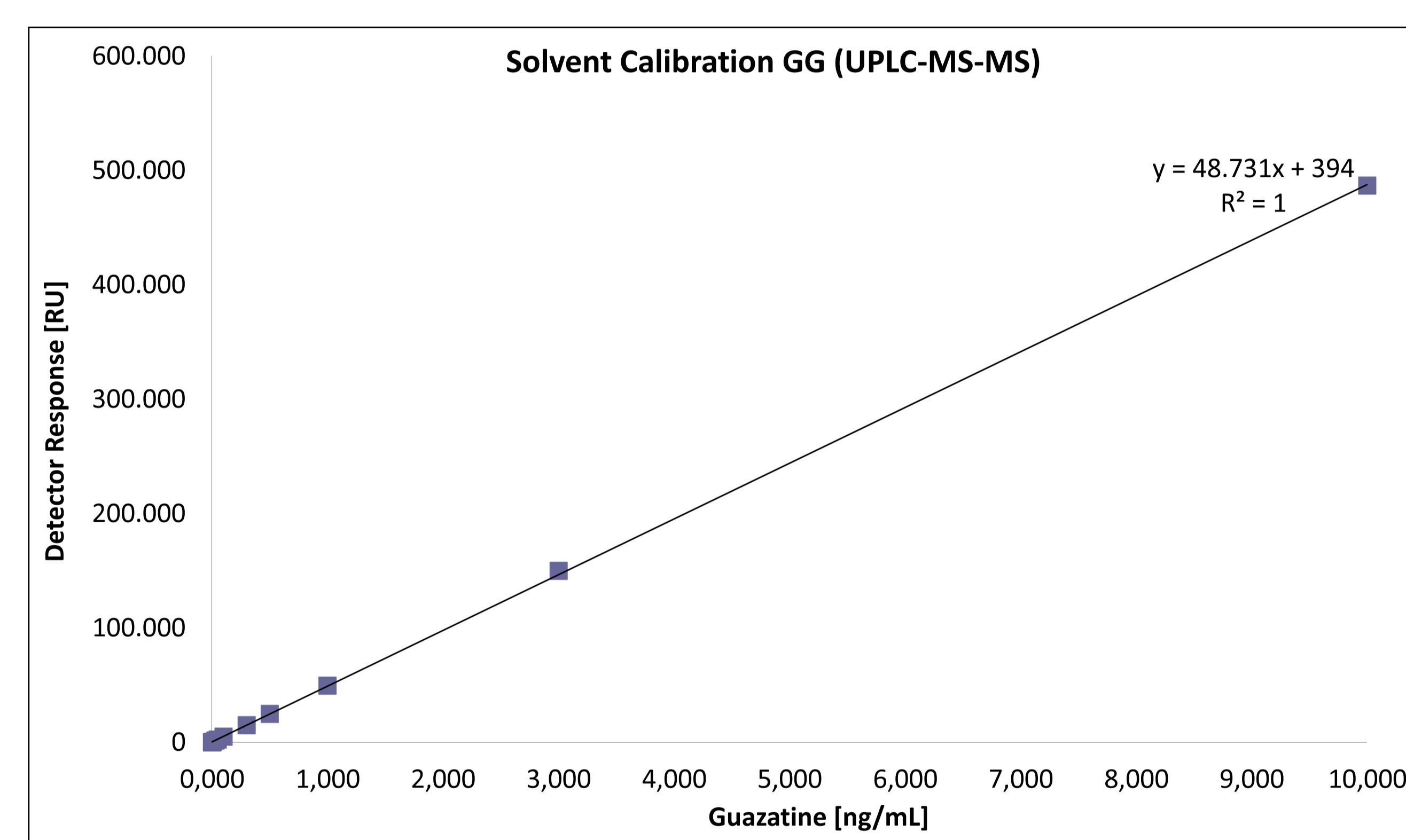


Fig. 3: Solvent calibration of guazatine (GG) in the range of 0.001 – 10 mg/kg in citrus fruit (UPLC-MS-MS data).

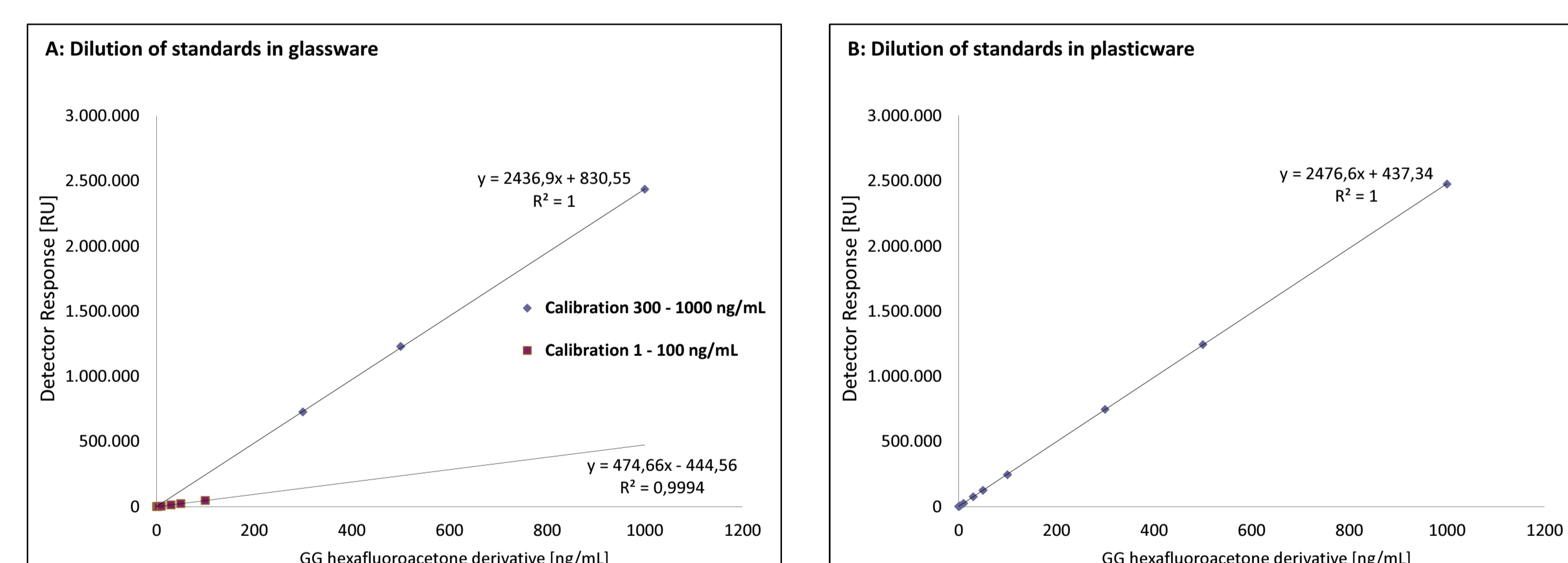


Fig. 4: Solvent Calibration of GG HFAA derivative GC-NCI-MSD data. A) Standards diluted in glassware and B) standards diluted in plasticware.

Tab. 1: Validation data guazatine analysis in citrus fruit according to SANCO 12571/2013

Validation Level [mg/kg]	GC-NCI-MSD		UPLC-MS-MS	
	VC [%]	Recovery [%]	VC [%]	Recovery [%]
0,01	9,5	97,4	9,7	89,9
0,05	4,8	104,4	4,7	96,9
0,50	3,0	99,7	8,0	102,8

Take Home Messages

- SRM for the determination of technical guazatine mixture via the marker molecule 1,8-diguanidin-1-yl-octane (GG)
- Sample prep requires acid SLE, cation exchange SPE (UPLC-MS-MS) + additional HFAA derivatization module (GC-NCI-MSD)
- LOD/RL: 0,003/0,01 mg/kg (UPLC-MS-MS) and 0,001/0,01 (GC-NCI-MSD), respectively.
- Plasticware a must for handling of guazatine solutions...

LITERATURE:

- [1] Dreassi E: Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1499–1506.
- [2] Reasoned opinion on the modification of the existing MRL for guazatine in citrus fruits EFSA Journal 2014;12(8):3818.
- [3] Kasumov T: Analytical Biochemistry 395 (2009) 91–99.