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POSITION PAPER No. 16 - 04

"Reliable analysis of Ethephon in processed food products"

Version 2016/08/01

Abstract

This position paper is related to the reliable analysis of the ripening and plant growth regulator "Ethephon" in processed (mainly dried) food products.

Organic control bodies are faced with Ethephon results of dried bio-mangos. Depending on the applied method Ethephon is detected, but the counter samples / samples of the same batch analysed using an independent different analytical method do not confirm these results to a certain extent.

Two analytical approaches are known and published in the literature for the analysis of Ethephon: an "older" method based on the indirect determination of Ethephon by its' degradation product Ethylene (using a Headspace-Gaschromatographic method "HS-GC") and a "newer" method, based on the QuPPe (Quick Polar Pesticides Method) protocol using a methanol extraction and subsequent determination of Ethephon itself by the LC/MSMS technique.

Several laboratories of the relana® quality circle have been involved to investigate these contradictory results, which mainly appears at concentration levels in the low µg/kg (ppb) range. The results of these investigations have been discussed during the annual relana® meeting 2016 in Bologna, Italy. This relana® Position paper presents the outcome and corresponding conclusions of the discussions and scientific exchange during this meeting.

Introduction

In the 1990ies, an official enforcement method to analyse residues of Ethephon in food products was established and published (ASU §64 LFBG L 00.00-47). As during that times the today well known and routinely applied LC/MSMS technique had not been established yet in analytical laboratories, an analytical protocol was developed including the use of the already well established gaschromatographic separation and detection of the Ethephon metabolite Ethylene. It is important to mention, that all the validation and quality assurance data, which was determined during the development of the method were related to fresh fruits and vegetables.
One main disadvantage of this method is the "indirect" determination of Ethephon as Ethylene. Such indirect methods (like also the analytical protocols related to dithiocarbamates as „CS_2 - Carbonsulfide" or the Methyl-Bromide as „total-bromide - Br^-") have to be used and especially interpreted very carefully.

Several years later, the LC/MSMS technique was introduced and applied in routine by analytical laboratories. As a consequence, new analytical protocols to determine several specific pesticides have been established in order to replace former methods with certain deficiencies. An analytical approach to analyse Ethephon by making use of this LC/MSMS technique was established, too. Later, the QuPPe method included Ethephon into the scope of this modular analytical method.

After the introduction of this new analytical approach, several method performance tests have been performed to check the comparability of the different analytical protocols.

The publication of the results of a method performance assessment using a spiked tomato purée (Bruns et.al. 2009) concludes, that the results of all three applied techniques (Headspace-GC; GC/MS after derivatisation; LC/MSMS) meet the performance criteria. The results of the labs applying the Headspace-GC method were a little bit higher compared to the other techniques (but not outside the performance criteria):

"Taking into consideration the different protocols and applied techniques, one tendency can be identified: The results of the headspace-GC methods are typically higher than those obtained using the GC or LC-MS/MS methods. Out of the 8 highest results reported, 5 are related to the headspace methods. All results of the headspace methods are higher than the spiked level but still very close to this level. Therefore, this is just a slight indication that there might exist influences increasing the values during the indirect determination of ethephon via ethylene (e.g. caused by natural ethylene concentrations).
The analysis of ethephon seems to be valid and robust - independent of the applied method. Differences in results of different laboratories analysing routine samples might be caused by other sources (e.g. non homogenous distribution of ethephon in the different pieces of the commodities sent to the different laboratories)."

As a conclusion, the analysis of Ethephon in fresh fruits and vegetables seems to be robust and reliable, independent of the applied analytical technique.

As more and more processed food products are produced and put on the shelves, an increased request of the analysis of such food products is coming up. The analytical laboratories are required to transfer the scope of the established analytical methods to other food products than originally validated during the set-up of the analytical protocols. This is often demanding and challenging as processed food products often behave differently compared to the unprocessed food during sample preparation and clean-up. Therefore, it has to be checked by the analytical laboratories whether their applied analytical protocols fit for the processed food products as well.

Several organic control bodies are faced with Ethephon results of dried bio-mangos. Depending on the applied method Ethephon is detected, but the counter samples / samples of the same batch analysed using an independent different analytical method do not confirm these results to a certain extent. These contradictory results have been investigated by several laboratories of the relana® quality circle.

Some results are summarised in the following table (results of Lab A using the GC method are indicated by 1A, etc. , results of Lab B using the LC/MSMS method by 1B etc.):

Operator -sample taker	GC results (etephon in mg/kg)	LC results (etephon in mg/kg)
Operator 1 -Control body	1A: 0,048 and 1B : 0,046	Remaining amount of sample 1B : < LOQ
Operator 2 -Control body	2A : 0,044 (2B not requested) => investigation (other drying units) 3A : 0,07 4A : 0,039 5A : 0,049 6A : 0,048	3B : < LOQ 4B : < LOQ 5B : < LOQ 6B : < LOQ
Operator 2 -operator simultaneously with control body (sample x and x' are identical)		3'A : < LOQ 4'A : < LOQ 5'A : < LOQ 6'A : < LOQ
	7 samples : 100% positive	9 samples : 100 % negative

Investigations of the analytical approaches related to the presence of Ethephon in dried mango

The two different analytical approaches are briefly identified by (for details please refer to ASU §64 LFBG L 00.00-47 and QuPPe method):

1. GC: The sample preparation for GC-Headspace analysis makes use of alkaline treatment of the sample material (➔ transferring the Ethephon to Ethylene). Determination of Ethylene by GC/FID or GC/MSD.
2. LC/MSMS: Ethephon is extracted from the sample with acidified methanol. After extraction the determination of unchanged Ethephon is performed using LC-MS/MS.

Several aspects are important to achieve reliable data using the LC/MSMS approach:

- For homogenisation two different techniques are possible:
  using dry ice or liquid nitrogen for milling to get a powdered sample;
  preparing a "slurry" by adding sufficient amounts of water followed by an appropriate comminution and milling.
- Ensure exhaustive extraction of Ethephon out of the matrix by adding sufficient amounts of water and using appropriate extraction techniques like f.ex. Ultraturrax® or Collomix® Viba (high frequent shaker).
- Avoid a too "acidic" environment as Ethephon quickly degrades to Ethylene and thus disappears during extraction (f.ex. 0.1m HCl is too strong). It is recommended to use a methanol/water mixture (1:1) acidified with 1% of formic acid or acidic acid.

The investigations related to extraction efficiencies indicated no significant differences related to variations of extraction technique (by hand or by Collomix), extraction times (3 / 5 / 10 min), swelling time before extraction (0 / 10 / 30 /

60 min). Recovery rates were between 82% and 101 % at a 20 µg/kg (ppb) level.
The analysis of several different products of **dried mango** which have been purchased at commercial food stores by one relana® lab and using both analytical approaches are summarised in the table below:

Results of Ethephon in µg/kg (ppb)

Sample code	Headspace-GC	QuPPe (LC(MSMS) with Collomix	QuPPe (LC/MSM) with Ultraturrax
Bio 1	38	< 10	< 10
Bio 2	148	123	124
Bio 3	32	< 10	n.a.
Conventional 1	42	< 10	n.a.
Conventional 2	41	< 10	n.a.
Conventional 3	44	< 10	n.a.
Conventional 4	89	10	< 10
Conventional 5	49	< 10	n.a.

n.a. not analysed; < 10: below reporting limit

Applying the Headspace-GC approach in **every** sample certain levels of "Ethylene" are detected. All results with levels between 32 µg/kg and 49 µg/kg have not been confirmed by the QuPPe method with direct detection of Ethephon. If much higher levels of "Ethylene" are present in the samples (ca. 100 µg/kg or higher), the confirmation of the presence of Ethephon seems to be evident. But even at these "higher" levels, the results of the Headspace-GC methods are higher compared to the Ethephon levels analysed with the direct LC/MSMS method. This also confirms the findings of the method performance assessment of Ethephon in tomato purée of 2009.

From these experiments it can be concluded, that food products and especially dried mango (but perhaps also other dried food products) do show levels of Ethylene at concentration ranges up to ca. 50 or more µg/kg, which can not be linked to Ethephon as the probable source.

Conclusions and Recommendations

Based on the information provided in this **relana® position paper** and the discussed aspects it seems to be evident, that the Headspace-GC method, which determines Ethephon in an indirect way by its' metabolite Ethylene, typically provides higher levels of Ethylene as expected by the related Ethephon levels. This effect seems to be even higher, if processed food products like dried mango are analysed.
At concentrations levels between 10 µg/kg and ca. 50 µg/kg this effect generates "false positive" results related to the presence of Ethephon. At this concentration range there seems to be present some kind of a "background" noise of Ethylene.

Taking all these facts and aspects into consideration, it is highly recommended to apply the QuPPe method (or a similar extraction/clean-up approach) and subsequent LC/MSMS determination of Ethephon in food products! This is even more recommended in case of processed (f.ex. dried) food products and if the expected levels of Ethephon are below ca. 50 µg/kg (ppb).

Literature

- ASU §64 LFBG L 00.00-47:1999:11 „Bestimmung von Ethephon durch Headspace-Gaschromatographie in pflanzlichen Lebensmitteln“ (official compilation of analytical methods in Germany)

- QuPPe method: http://www.crl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPe-PO_EurlSRM.pdf

- S Bruns, G Lach, and H Parlar: *Analysis of Ethephon Residues in Tomato Purée Method Performance Assessment*; Fresen. Environm. Bull. Vol. 18, no. 11a (2009); 2219-2223

Acknowledgment

The investigations and the related analytical data to this **relana® position paper** were mainly provided by

- Eckard Jantzen, GALAB Laboratories (Hamburg, Germany,),
- Marco Miebach, Eurofins Dr. Specht Laboratorien (Hamburg, Germany),
- Marvin Overbeeke, Eurofins Lab Zeeuws Vlaanderen (Graauw, The Netherlands),
- Roy Sperling, bilacon GmbH (Berlin, Germany).

All other laboratories of the relana® quality circle contributed with their knowledge and analytical experiences during the annual relana® meeting 2016 in Bologna, Italy.

relana® Position Papers

relana® Position Paper No. 16-04 “Reliable analysis of Ethephon” version 2016/08/01.