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relana®

c/o Lach & Bruns Partnerschaft
Tempowerkring 1
21079 Hamburg
Germany

Phone +49(0)40-79012-232/-233
relana@relana-online.de

www.relana-online.com

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"Analysis of Acidic Herbicides with respect to the related residue definitions"

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Abstract

This position paper is related to the correct analysis of the so-called "**acidic herbicides**", which mainly consists of Phenoxycarboxylic acids (like 2,4-D, Dichlorprop, Fluazifop, Haloxyfop etc.) in respect to their complex residue definitions as established and published by the pesticide residue regulation (EC) No 396/2005.

The issue has been discussed during the annual relana® meeting 2016 in Bologna, Italy. This relana® position paper presents the outcome and conclusions of these discussions and the scientific exchange during this meeting.

Introduction

During the approval of several pesticides within the framework of the pesticide legislation of the European Union (EU) some complex residue definitions were set up. To confirm compliance with the related maximum residue levels (MRL) of regulation (EC) No 396/2005 it is necessary to determine all listed components or groups of components by the applied analytical approach. If these components are exactly defined (like f. ex. for Captan: sum of Captan and Tetrahydrophthalimid, expressed as Captan, reg. (EU) 2016/452), the laboratories have to check, whether these components are covered when applying a multi-residue-method (MRM) like the QuEChERS-method - or by a single residue method (SRM). In case the definition is a more general one (not exactly defining the individual components), it might become a challenge for the laboratories.

Many residue definitions of herbicides are quite "general". Some examples are:

- 2,4-DB = "sum of 2,4-DB, its salts, its esters and its conjugates, expressed as 2,4-DB";
- 2,4-D = "sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D";
- Dichlorprop: sum of dichlorprop (including dichlorprop-P) and its conjugates, expressed as dichlorprop;
- Fluazifop-P-butyl (fluazifop acid (free and conjugate)).

This position paper is about the necessary analytical approaches in order to confirm compliance with the related MRLs.

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Analytical approaches to meet the requirements of the residue definitions of phenoxycarboxylic acids

The residue definitions of many of the Acidic Herbicides include the expressions:
"sum of ???, its salts, its esters and its conjugates, expressed as ???".

While applying a common MRM approach like the QuEChERS-method, the free acids resp. the salts and some esters of the particular phenoxycarboxylic acids are covered. Not covered by this "common" analytical approach are several esters and especially all the possible "**conjugates**" of the phenoxycarboxylic acids, which might be formed in the plants after application of the pesticide formulation. The formation of such conjugates might differ significantly from crop to crop and strongly depends on specific conditions like temperature, sunlight, humidity etc. Possible conjugates are f. ex. amino-acid conjugates (see lit. "Metabolism of 2,4-Dichlorphenoxyacetic acid ...") or glucoside conjugates (see lit. "Principles of weed science ..."). These conjugates cannot be analysed directly as

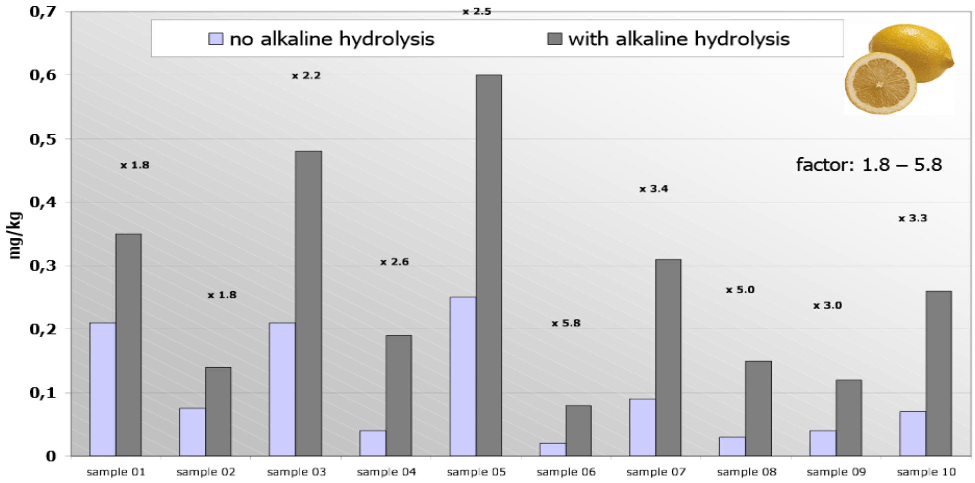
a) it is not clear which conjugates exactly are formed, and
b) usually, no standard reference material is available.

Actually, one comprehensive analytical approach for including all these possible conjugates is based on an additional **alkaline hydrolysis step**. Applying this hydrolysis step, all conjugates and all esters of a phenoxycarboxylic acid, which might be present in a sample are converted into the related "free" phenoxycarboxylic acid. Subsequently, this "free" acid (which is formed out of all esters, salts and conjugates) can be determined with the common steps of the MRM. Such an analytical approach is f. ex. the "Modified QuEChERS-method" as described on the QuEChERS website (see literature). It is a modified method because an alkaline hydrolysis step is included during sample preparation.

Analytical data of phenoxycarboxylic acids with and without alkaline hydrolysis step

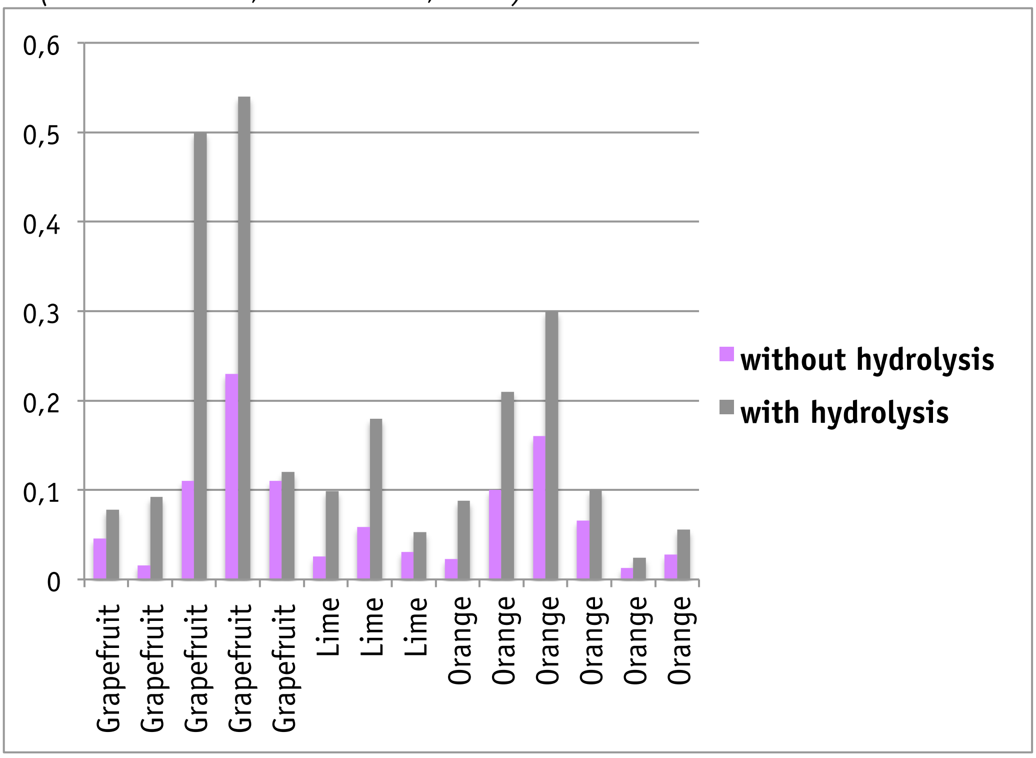
The experiences of laboratories confirm the assumption that in many cases the total quantity of acidic herbicides is significantly higher with an additional alkaline hydrolysis step as compared to the total quantity determined without a hydrolsysis step. Some examples:

1. *Levels of 2,4-D in lemons without and with hydrolysis*
   (Data: Acidic pesticides using QuEChERS method, CVUA Stuttgart)



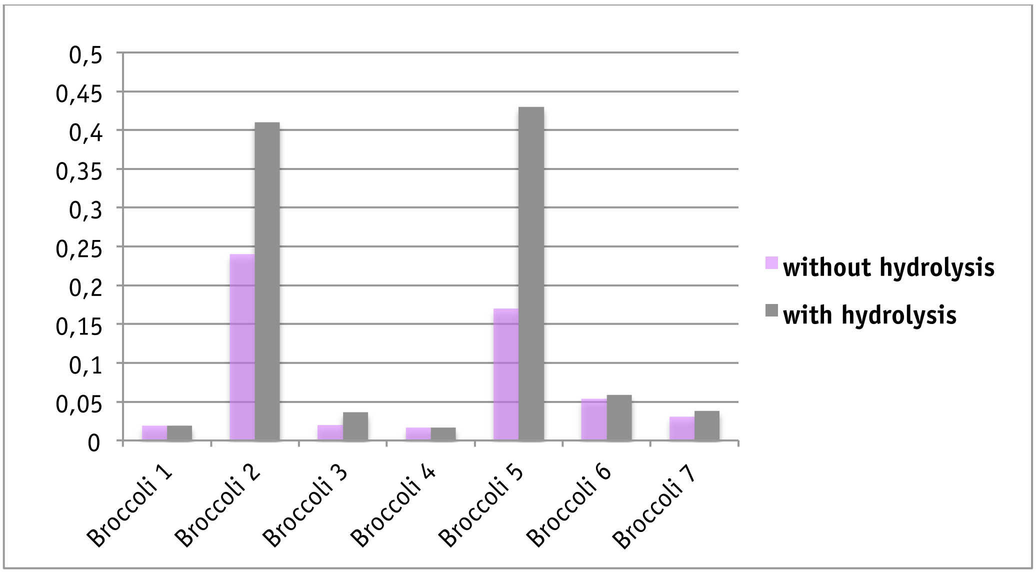
The results indicate a factor of 1,8 up to 5,8 between the results without and with application of the alkaline hydrolysis.

2. *Levels of 2,4-D in several citrus fruits without and with hydrolysis*
   (Data: Karsten Ott, bilacon GmbH, Berlin)



The results also indicate a factor of 1,1 up to 5,8 between the results without and with application of the alkaline hydrolysis (y-axes: level in mg/kg).

3. *Levels of Fluazifop in several broccoli without and with hydrolysis (Data: Karsten Ott, bilacon GmbH, Berlin)*



The results related to Fluazifop levels in broccoli indicate a factor of 1,0 up to 2,35 between the results without and with application of the alkaline hydrolysis (y-axes: level in mg/kg).

These examples are confirmed by the experiences of additional members of the relana® quality circle.

As a conclusion it can be summarised, that in many samples the total amount of residues of acidic herbicides can be only determined when applying the additional alkaline hydrolyses step during sample preparation.

Practical approach for routine samples

A practical approach to deal with these complex residue definitions in order to show compliance with the related MRLs of reg. (EC) no 396/2005 could be:
In case of a first finding of Phenoxycarboxylic acids (free acids and/or esters of the acids) during the analysis with the common multi-method approach, an extra analysis (hydrolysis) must be performed. Even if f.ex. the methyl-esters of a particular phenoxycarboxylic acid are detected only at low levels in the first finding, the total levels of Phenoxycarboxylic acids like Haloxyfop might be much higher with hydrolysis. This increase probably derives from the conjugates present in the sample. **Therefore, it is mandatory to perform an extra analysis with hydrolysis step, if the residue definition has to be met to confirm compliance with the related MRL.**

If only the common MRM approach is performed, the test report must include a comment, that the possible total amount of Phenoxycarboxylic acids is not covered by this analytical method (if a complex residue definiton exists for the particular Phenoxycarboxylic acid).

Conclusion

Based on the information provided in this **relana® position paper** and the discussed aspects it seems to evident, that in many samples the total amount of residues of acidic herbicides can be only determined correctly when applying an additional alkaline hydrolyses step.

In case of a first finding of Phenoxycarboxylic acids (free acids and/or esters of the acids) within the common multi-method approach, an extra analysis (hydrolysis) must be performed.

As a consequence, it is mandatory for members of the relana® laboratory circle to perform an extra analysis with a hydrolysis step in case the residue definition has to be met in order to confirm compliance with the related MRL.

Literature

- "Modified" Quechers-method: http://quechers.cvua-stuttgart.de/pdf/acidicpesticides.pdf
- Regulation (EC) No 396/2005
- Metabolism of 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Soybean Root Callus: Evidence for the conversion of 2,4-D amino acid conjugates to free 2,4-D, Plant Physiol. 1980 Oct; 66(4):537-40.
- Principles of weed science; Rao, V.S.; Science Publishers, Inc. Enfield (New Hampshire); 2000; pp. 213-216

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

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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. 16-05 "Acidic herbicides – residue definitions" version 2016/08/08.

Annex 1 – Residue definitions accord. to Reg. (EC) No 396/2005 of selected Phenoxycarboxylic acids

Phenoxycarboxylic acids	residue definition
2,4-D	2,4-D (sum of 2,4-D, its salts, its esters and its conjugates, expressed as 2,4-D)
2,4-DB	2,4-DB (sum of 2,4-DB, its salts, its esters and its conjugates, expressed as 2,4-DB)
2,4,5-T	2,4,5-T (sum of 2,4,5-T, its salts and esters, expressed as 2,4,5-T)
Bentazon	Bentazone (Sum of bentazone, its salts and 6-hydroxy (free and conjugated) and 8-hydroxy bentazone (free and conjugated), expressed as bentazone)
Clodinafop	Clodinafop and its S-isomers and their salts, expressed as clodinafop
Dalapon	Dalapon
Dicamba	Dicamba
Diclofop	Diclofop (sum diclofop-methyl and diclofop acid expressed as diclofop-methyl)
Dichlorprop-P	Dichlorprop (Sum of dichlorprop (including dichlorprop-P), its salts, esters and conjugates, expressed as dichlorprop
Fenoxaprop-P	Fenoxaprop-P
Fluazifop-P	Fluazifop-P-butyl (fluazifop acid (free and conjugate))
Haloxyfop-P (Haloxyfop-R)	Haloxyfop (Sum of haloxyfop, its esters, salts and conjugates expressed as haloxyfop (sum of the R- and S- isomers at any ratio))
MCPA	MCPA and MCPB (MCPA, MCPB including their salts, esters and conjugates expressed as MCPA)
MCPB	MCPA and MCPB (MCPA, MCPB including their salts, esters and conjugates expressed as MCPA)
Mecoprop (MCPP)	Mecoprop (sum of mecoprop-p and mecoprop expressed as mecoprop)
Quizalofop	Quizalofop, incl. quizalfop-P