Analysis of Carbofuran (Sum) via Modified QuEChERS Method

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Introduction

Carbofuran (CF) is an insecticide, nematicide and acaricide of the carbamate group. Carbosulfan (CS), furathiocarb (FT) and benfuracarb (BF) are pro-pesticides that degrade to the active substance CF. None of the four compounds is approved in the EU but there are still uses in other countries. A recent import-tolerance request for mushrooms was rejected. CF and its metabolite 3-OH-carbofuran (3-OH-CF) exhibit very high acute neurotoxicity and were thus allocated very low ARfDs and ADIs. Therefore, new, very low MRLs (down to 0.001 mg/kg) were established for all products with high, short-term consumption rates.

New Residue Definition

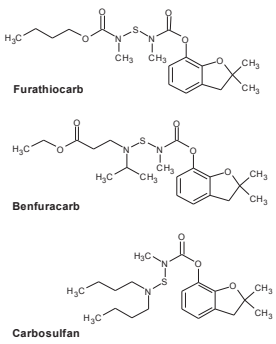
Given the tendency of CS, BF and FT to degrade during laboratory analysis and food processing (see EURL Analytical Observation Report) , a new residue definition (RD) was introduced which, for plant products, includes CF, its three pro-pesticides and 3-OH-CF expressed as CF. For foods of animal origin the RD includes only 3-OH-CF.

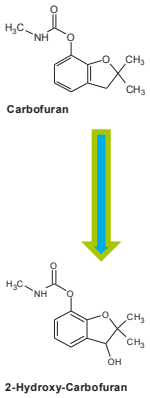
Challenge and Analytical Strategy

Given the very low MRLs, a conversion of CS, BF and FT to CF in the case of plant products was considered crucial, as it reduces the number of compounds to be analyzed from five to just two (CF and 3-OH-CF). It was considered further beneficial to conduct the conversion in the final QuEChERS extract (raw or cleaned up). Fortunately, both CF and 3-OH-CF can be very sensitively analyzed using standard LC-MS/MS equipment and conditions. The conversion to CF, within the same vial, would be conducted in case of positive screening under routine conditions.

Method Development

Initial experiments focused on achieving quantitative conversion of BF, CS and FT to CF in QuEChERS extracts. CS and BF hydrolyzed rapidly but the formation of CF was considerably delayed, apparently due to formation of intermediate products. Especially in acidic products this often leads to underestimated results. In contrast, FT showed remarkable stability even when further acidifying the raw QuEChERS extracts with formic acid (see figures below). Quantitative conversion of all components was finally achieved following the addition of 10 µL 5N H_2SO_4 to a 1 mL extract and heating the vial at 80°C for 2-3 hours.





Final Method

Extraction + Cleanup: Apply the citrate buffered QuEChERS (EN 15662) using an internal standard which is stable during acidic hydrolysis (e.g. Carbofuran-D3). Cleanup via dSPE is optional for fruits and vegetables.

Hydrolysis: Transfer 1 mL of the QuEChERS raw extract into a vial and add 10 µL 5N H_2SO_4. Nearly quantitative transformation of BF, CS and FT into CF is achieved by heating the vials for 3 h at 80 °C.

LC-MS/MS analysis: For screening purposes, CF and 3-OH-CF, as well as BF, FT and CS, may be analyzed by LC-MS/MS directly in QuEChERS raw extracts or cleaned-up extracts. In the case of positive findings the hydrolysis step can be conducted as described above and the LC-MS/MS analysis of CF repeated.

Results

The method including the hydrolysis step was validated at the 0.001 mg/kg level for BF, FT and CS in grapes and potatoes. It was also validated for CF and 3-OH at 0.0005 mg/kg each in milk (data not shown, for more details see [1]).

Matrix	Spiking level parent (mg/kg)	n	BF		FT		CS		MS/MS Instrument employed
			Spiked level corresponds to						
			0.00058 mg/kg CF		0.00058 mg/kg CF		0.00054 mg/kg CF		
			Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	
Grapes	0.001	5	112	2	111	2	113	2	ABSCIEX 5500Q
Potatoes	0.001	5	114	2	109	3	113	4	ABSCIEX 5500Q

Summary

Following the establishment of a new residue definition for CF (sum) and the setting of extremely low, toxicology based MRLs, we have developed a simple QuEChERS-based method entailing acidic hydrolysis of CS, BF and FT to CF, which is applied directly to the final QuEChERS extracts. Sensitive and selective measurement is achieved by LC-MS/MS. The method was validated down to 0.0005 mg/kg. For more details see [1].

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Direct analysis (n=2)

CF:	0.020 mg/kg
CS:	0.006 mg/kg
Sum (as CF):	0.023 mg/kg

After hydrolysis (n=2):

Sum (as CF) :	0.026 mg/kg

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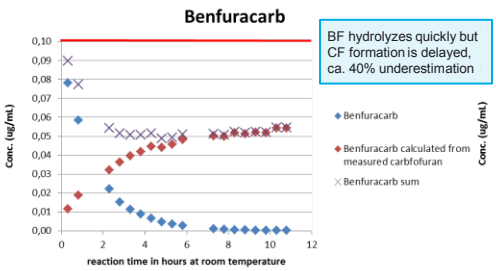
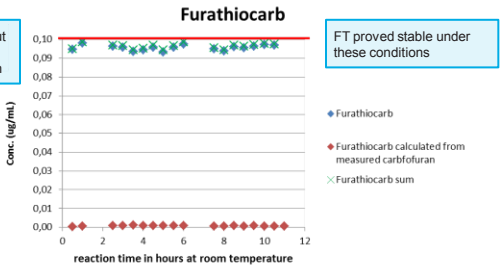


BF hydrolyzes quickly but CF formation is delayed, ca. 40% underestimation



FT proved stable under these conditions

Figure: Hydrolysis attempt of 0.1 µg/mL BF and FT to CF in 1 mL raw QuEChERS extracts from cucumber following acidification with 10 µL formic acid (98%)

CF and 3-OH-CF proved stable under the hydrolysis conditions. Direct injection without any neutralization proved feasible.

Reference

1. EURL Website, Observation and Method on CF

Baden-Württemberg