EURL-SRM - Analytical Observations Report

Concerning the following…

- **Compound(s)**: BAC (C_8-C_{18}), DDAC (C_8-C_{12})
- **Commodities**: Plant origin
- **Extraction Method(s)**: CEN-QuEChERS (EN-15662), QuOil (CEN/TS 17062:2019)
- **Instrumental analysis**: LC-MS/MS

Analysis of Quaternary Ammonium Compounds (QACs) in Fruits and Vegetables using QuEChERS and LC-MS/MS

Version 6 (last update: February 2023)

Background information

Quaternary ammonium compounds (QACs), also known as quaternary ammonium cations or "quats", are surface-active substances containing a quaternary cationic nitrogen atom, substituted by alkyl chains of varying length. They are diversely used, for example as biocides/disinfectants/sanitizers, as cationic surfactants, as additives in personal-care products (e.g. hair conditioners and shampoos for antistatic and biocidal purposes), as fabric softeners, and in many other areas including plant protection.

Most QACs are marketed as chloride salts (or solutions thereof) but bromides are also common. In many cases, the counter ion is included in the acronyms of the products (e.g. **BAC**[1] and **DDAC**), and these acronyms are used to report residue findings despite the fact that only the cation is being detected. In the following, these acronyms are used even if referring to the cations.

BAC is a mixture of alkyldimethylbenzylammonium chlorides having various even-numbered alkyl chain lengths (C_8-C_{18}). The greatest biocide activity is associated with the C_{12}-C_{14} derivatives, which are the main components of the mixture. **DDAC**[2] is a mixture of dialkyldimethylammonium salts with typical alkyl chain lengths of C_8, C_{10} and C_{12}. The congener with a chain length of C_{10} is the main DDAC component and typically makes more than 90 % of the mixture. In most cases, the term DDAC is thus used for the didecyldimethylammonium congener.

Further quarternary ammonium cations with biocidal properties include the following: benzethonium, methylbenzethonium, cetalkonium, cetylpyridinium, cetrimonium, quaternium-14 and tetraethylammonium. These are not yet covered in the present version of this document.

In a wider sense, the plant growth regulators chlormequat and mepiquat and the herbicides paraquat, diquat and difenzoquat are also regarded as QACs. These compounds are covered by the QuPPe-method.

[1] Alternative acronyms: ADBAC (alkyl dimethyl benzyl ammonium chloride); and BKC. Note that the term BAC is also used for the biocide "Bromide activated chloramine"

[2] In the ECHA database for biocides "Didecyldimethylammonium chloride (DDAC (C8-10)" is used for products with CAS No. 68424-95-3 and "Didecyldimethylammonium chloride (DDAC)" is used for products with CAS No. 7173-51-5

Regulatory Aspects:

DDAC used to be approved in the EU as a as bactericide, fungicide, herbicide and algaecide for indoor use on ornamental plants, whereas BAC did never receive EU-approval as active substance in plant protection products PPPs).

Both BAC and DDAC are classified as pesticides and regulation 396/2005/EC thus applies when it comes to residue levels in food and feed. Since Oct. 2014, both BAC and DDAC are regulated with the following residue definitions under Reg. (EC) No. 396/2005:

- **Benzalkonium chloride** (mixture of alkylbenzyldimethylammonium chlorides with alkyl chain lengths of C_8, C_{10}, C_{12}, C_{14}, C_{16} and C_{18})
- **Didecyldimethylammonium chloride** (mixture of alkyl-quaternary ammonium salts with alkyl chain lengths of C_8, C_{10} and C_{12})

Both DDAC and BAC were illegally used in agriculture but roughly a decade ago, following their introduction into the analytical scope of laboratories, and numerous legal cases, their use has ceased.

For a certain period in the past, some BAC- or DDAC-containing products were even marketed as suitable for organic agriculture, without the QACs being listed on the label or the specification. Some of the products containing high percentages of DDAC or BAC were for example marketed as biological "grapefruit-seed-extract-based" PPPs or as "plant strengtheners". Farmers, in fact mainly organic farmers, were using such products without knowing that they contained BAC or DDAC as the active ingredients.

The EU list of pesticide active substances also contains the generic entry **"Quaternary ammonium compounds"**, for which, in the strict sense, the default MRL of 0.01 mg/kg (according to Art 18(1)(b) Reg. (EC) No. 396 / 2005) applies. Strictly speaking, this entry refers to any quarternary ammonium compounds and there seems to be an overlapping and contradictive ruling. Eventually this aspect needs to be addressed. In any case, the specific MRL-provisions on BAC and DDAC should prevail over the generalized and unspecified provisions on "Quaternary ammonium compounds" (*lex specialis* derogate legi *generali)*.

BAC and DDAC are furthermore approved as **biocidal active substances** under the EU Biocidal Products Regulation (EU BPR, Reg. (EU) 528/2012) and may be used in various classes of biocidal products in industrial, household, medical and other areas (see **Table 1**):

Table 1: Overview of application areas of DDAC and BAC as biocides

Biocidal Product Type	"DDAC" / "DDAC (C8-10)"	ADBAC (C12-16) / ADBAC (C12-18)
PT01 (Human hygiene)	Approved	Application for approval in progress
PT02 (Disinfectants and algaecides not intended for direct application to humans or animals)	Approved	Application for approval in progress
PT03 (Veterinary hygiene)	Approved	Approved
PT04 (Food and feed area)	Approved	Approved
PT05 (drinking water)	not supported any more	not supported any more
PT08 (Wood preservatives)	Approved	Approved
PT06 (Preservatives for products during storage)	Application for approval in progress	Application for approval in progress
PT10 (Construction material preservatives)	Application for approval in progress	Application for approval in progress
PT11 (Preservatives for liquid-cooling & processing systems)	Application for approval in progress	Application for approval in progress
PT12 (Slimicides)	Application for approval in progress	Application for approval in progress
PT22 (Embalming and taxidermist fluids)	Application for approval in progress	Application for approval in progress

Sources of BAC and DDAC residues in food

Even though BAC- and DDAC-containing PPPs are not allowed within the EU, they are still in use in some other countries. But even if they are not used during cultivation the food products can be contaminated due to the use of BAC and DDAC as sanitizers in packing and processing facilities.

Nowadays, residues of BAC and DDAC in fresh produce are mainly attributed to their post-harvest use as biocides (santitizers). BAC and DDAC are widely used to wash the products themselves or the surfaces with which the products come into contact (e.g. conveyors) in packing stations. Processed products, such as dairy products, meat products, juices and others, are contaminated due to the QAC-residues remaining in sanitized pipes, tanks and other surfaces.

The dairy Industry is probably the most important food-related field of DDAC and BAC use. BAC and DDAC are popular among dairy farmers, where they are used to disinfect udder and milking equipment with the goal of preventing mastitis and producing raw milk with low bacterial count, which is preferential both from the hygienic and economic point of view. Unlike many chlorine-containing products, BAC and DDAC do not cause skin irritation. DDAC and BAC containing products are also used for the disinfection of milk storage tanks, ice-cream machines and other.

BAC is furthermore used in beekeeping for the treatment of rotten diseases of the brood, as well as a bactericide and algaecide in aquacultures (e.g. shrimp farming).

A rather unintentional pathway leading to the contamination of food products is the touching of food with contaminated hands, as QACs may be contained in various personal care products, such as hand sanitizers, wet wipes, eye-drops, soaps and hair-care products. Sampling officers should be aware of this aspect and avoid the use of such products or at least wear gloves during the sampling.

An overview of possible sources of BAC and DDAC residues in food products in given in **Table 2**.

Table 2: Overview of contamination pathways before the products enter the laboratory.

Stage of contaminantion	Pathway	notes
Cultivation	Use of QAC-containing PPs*	Used to be significant
Harvesting	Contact with contaminated hands	Spurious
Handling in packing stations	Contact with contaminated hands	Spurious
	Contact with washing solutions	Possibly the main contaminantion pathways nowadays
	Contact with contaminated conveyors	
Processing	Contact with surfaces of tanks and pipes that have been sanitized before	
Sampling	Contact with contaminated hands	Spurious

**PPPs=Plant Protection Products*

QAC-contaminations within the laboratory:

Lots of laboratories are complaining about periodic problems with BAC and/or DDAC background levels, that increase the risk of reporting false positives and that compromise their ability to analyze these compounds at low levels and to effectively control MRLs.

An overview of possible sources of BAC and DDAC contamination within the laboratory is given in **Table 3**.

Table 3: Overview of contamination pathways after the products enter the laboratory

Stage of contamination	Pathway	notes
Sample processing	Contact with contaminated hands	Spurious
	Use of contaminated equipment for sample comminution or storage	Spurious or systematic; If systematic, the source needs to be localized and eliminated (e.g. cleansing reagents).
Extraction	Use of contaminated equipment (containers, vessels etc.)	Spurious or systematic; If systematic, the source needs to be localized and eliminated (e.g. cleansing reagents). Need to run reagent blanks.
	Use of contaminated extraction solvents and other consumables*	
Measurement	Use of contaminated mobile phase*	Systematic; During re-equilibration time or flushing time between runs or sequences, QACs are enriched at the beginning of the column. This is typically the contamination-source of highest relevance and concern.
	Use of contaminated consumables*	Systematic; the source needs to be localized and eliminated.

* If the QAC-source can be localized as a primary contamination of a purchased chemical consider changing the provider. If a secondary contamination of a chemical or a vessel is suspected, try to localize the source (e.g. cleansing product of glassware, sanitation reagent of air-conditioning).

Managing contaminations in the lab

Finding the source(s) of contaminations in a lab can be laborious and tricky and may even lead to inconclusive results, especially if the contamination varies in abundance and composition, e.g. due to lot-to-lot variations of the reagents. Observations of the background levels over many years at the EURL-SRM have shown, that the contaminations occurring within the LC system are the most critical and the most crucial to address. Contaminations at the LC step do not only compromise the ability to measure the most relevant QACs at low concentrations and the ability to adequately control the compliance with MRLs but also disturb the ability to encounter other potential sources of contamination within the lab.

Implementation of the trap-column approach

As previously presented by P. Zomer *et al.* (2020)[3], QAC contaminations in the LC system and the LC-eluents can be easily separated from the actual analyte peaks by using a trap column between the pump and the autosampler of the LC system (see scheme in **Figure 1**).

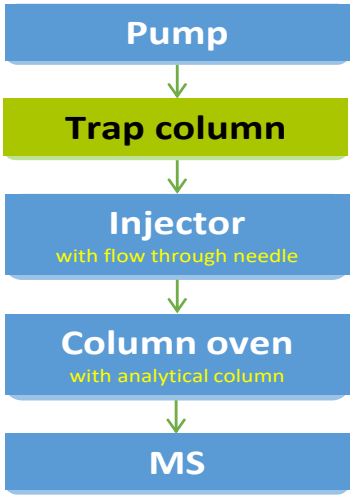


Figure 1: Schematical LC system set-up for using the trap column approach.

In initial experiments at the EURL-SRM, a sorted-out reversed phase LC-column, previously used for the analysis of QACs, was employed as trap-column. Other methods running on the same instrument, that do not actually require the trap column set-up, were only marginally affected by this setup with only minor retention time shifts being observed.

Prior to applying this procedure, our lab experienced highly fluctuating background levels, that for individual QACs (mainly BAC-C12 and DDAC-C10), sporadically reached levels as high as 0.05 mg/kg. This, seriously compromised the ability to monitor the affected QAC and to control MRL compliance. With the introduction of the trap-column approach the background levels could be almost fully eliminated, see **Figure 2** .

With the new set-up, we were able to successfully validate the whole group of BACs and DDACs at levels down to 0.005 mg/kg in several matrices of plant origin as the background contaminations could be chromatographically separated from the actual analyte peak (see also **Figure 3** and **Figure 4**). Just a small contamination signal of approx. 0.001 mg/kg remained, potentially deriving from another source (e.g. the extraction solvent, the matrix in the case of matrix blank, the usual cross-contamination at the injector).

[3] P.Zomer, R. Boerrigter-Eenling, H. Mol, Wageningen University & Research, Improvement of LC-MS/MS analysis of quaternary ammonium compounds by using a trap column, Poster presented at the 13th EPRW (online), 2020

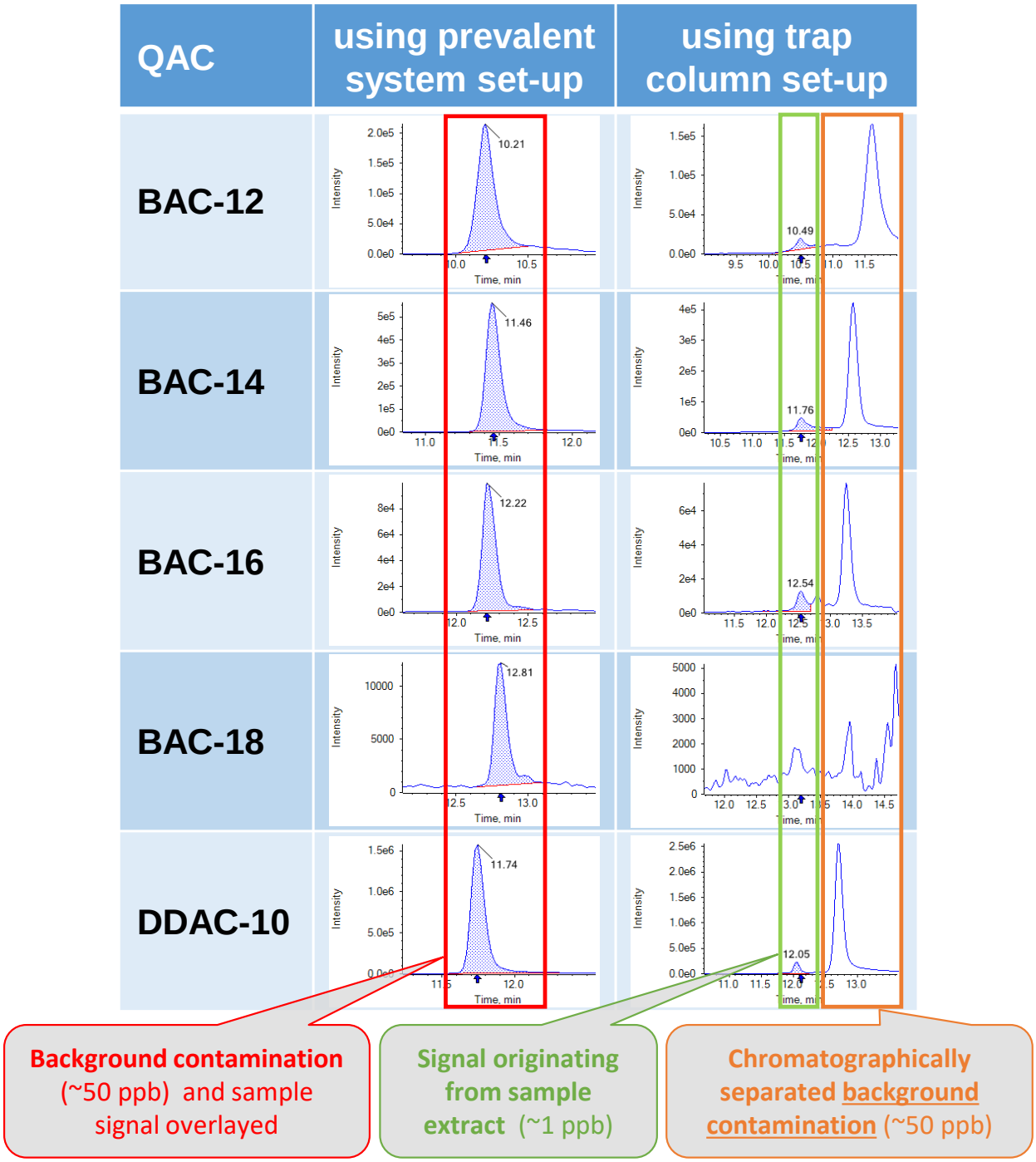
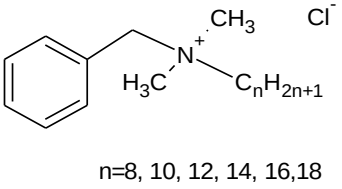
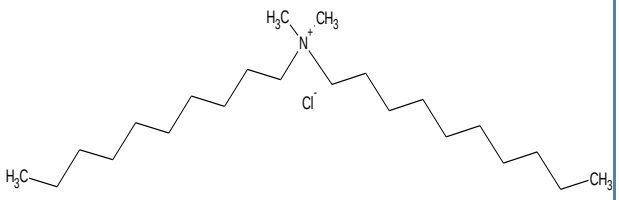


Figure 2: Extensive reduction of background contamination peak using the trap column set-up (on the right). The original system set-up without a trap column is shown on the left).

Analyte properties

Physicochemical properties and additional information on the BACs and DDACs are given in **Table 4**.

Table 4: Chemical Properties of alkylbenzyldimethylammonium chlorides and didecyldimethyammonium chlorides.

Alkylbenzyldimethylammonium chloride (CAS: 85-41-6, mixture of components with unspecified alkyl chain length) Other names: benzalkonium chloride, BAC, benzyldimethylalkylazanium chloride		
Parameter	Value/Notes	
Molecular Mass	Variable; 283.88 g/mol (BAC-C_8) up to 424.15 g/mol (BAC-C_{18})	 n=8, 10, 12, 14, 16,18
Formula	$C_9H_{13}ClNR$ (R=C_8H_{17} to $C_{18}H_{37}$)	
Boiling point	-	
pKa	No ionizable atoms present; permanent cations	
LogP	Chemicalize.com (computed): variable; 0.85 (BAC-C_8) up to 5.3 (BAC-C_{18})	
Water solubility	Up to 4000 g/L[4]	
Stability	Unknonwn[5]	
Residue definition (EU)	Benzalkonium chloride (mixture of alkylbenzyldimethylammonium chlorides with alkyl chain lengths of C8, C10, C12, C14, C16 and C18); Reg. (EU) No. 1119/2014	
Approved in …	Not approved	
Toxicity	BAC and its single components are not classified according to Reg. 1272/2008. No official ADI or ARfD are set for BAC and its single components. An ADI of 0.1 mg/kg bw per day and an ARfD of 0.1 mg/kg bw is recommended for BAC by the European Commission and EFSA[3]	
Other sources	• Usage as biocides for cleaning/sanitation of surfaces in the areas of food, feed and medical production • Personal care products	
Didecyldimethylammonium chloride (CAS 7173-51-5) Other names: DDAC-C_{10}, bis(decyl)dimethylazanium chloride		
Parameter	Value / Notes	
Molecular Mass	362,08 g/mol	
Formula	$C_{22}H_{48}ClN$	
Boiling point	-	
pKa	No ionizable atoms present; permanent cation	
LogP	Chemicalize.com (computed): 4.01	
Water solubility	0,39 g/L[3]	
Stability	unknown	
Residue definition EU	Didecyldimethylammonium chloride (mixture of alkyl-quaternary ammonium salts with alkyl chain lengths of C8, C10 and C12)	
Approved in …	Not approved	
Toxicity	Didecyldimethylammonium chloride is classified in cat. 4 as regards its acute toxicity and in cat. 1B as regards its skin corrosivity. No official ADI or ARfD are set for DDAC-C_{10}. An ADI of 0.1 mg/kg bw per day and an ARfD of 0.1 mg/kg bw is recommended by the European Commission and EFSA[3]	
Other sources	• Usage as biocide for cleaning/sanitation of surfaces in the areas of food, feed and medical production • Personal care products	

[4] GESTIS Substance Databse: https://gestis-database.dguv.de/data?name=491119 (last time checked: 03.06.2022 at 11:30 a.m.)

[5] EFSA, Reasoned opinion on the dietary risk assessment for proposed temporary maximum residue levels (MRLs) of didecyldimethylammonium chloride (DDAC) and benzalkonium chloride (BAC), EFSA Journal 2014;12(4):3675 (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3675)

Apparatus, Chemicals and Consumables

Chemicals and Materials

The used materials and apparatuses are listed in CEN-QuEChERS (EN-15662) and QuOil (CEN/TS 17062:2019) standard procedures. The suppliers of the used analytical standards are shown in **Table 5**.

Table 5: Sources of Analytical standards (exemplary; last time checked: 31.05.2022).

Acronym	Compound	CAS	Company	Order No.
Native standards				
BAC-C8	Benzyldimethyloctylammonium chloride	959-55-7	HPC	675698
BAC-C10	Benzyldimethyldecylammonium chloride	965-32-2	Sigma-Aldrich	13371
BAC-C12	Benzyldimethyldodecylammonium chloride	139-07-1	Sigma-Aldrich	13380
BAC-C14	Benzyldimethyltetradecylammonium chloride	139-08-2	Sigma-Aldrich	292796
BAC-C16	Benzyldimethylhexadecylammonium chloride	122-18-9	Sigma-Aldrich	B4136
BAC-C18	Benzyldimethyloctadecylammonium chloride	122-19-0	HPC	674644
DDAC-C8	Dimethyldioctylammonium bromide	3026-69-5	HPC	675696
DDAC-C10	Didecyldimethylammonium chloride	7173-51-5	HPC	674493
			LGC	DRE-C12588000
DDAC-C12	Didodecyldimethylammonium bromide	3282-73-3	HPC	674863
	Didodecyldimethylammonium chloride	139-07-1	Chemos	135628
DDAC-C14	Ditetradecyldimethylammonium bromide	68105-02-2	Sigma-Aldrich	40225
			HPC	674861
DDAC-C16	Dihexadecyldimethylammonium bromide	70755-47-4	Sigma-Aldrich	420220
DDAC-C18	Dioctadecyldimethylammonium bromide	3700-67-2	Sigma-Aldrich	40163
Internal Standards (Optional)				
BAC-C10 D7	D_7-Benzyldimethyldecylammonium chloride		HPC	674610
BAC-C12 D6	D_6-Benzyldimethyldodecylammonium iodide		HPC	674572
BAC-C14 D7	D_7-Benzyldimethyltetradecylammonium chloride		HPC	674611
DDAC-C10 D6	D_6-Didecyldimethylammonium iodide		HPC	674541

Disclaimer: Names of companies are given for the convenience of the reader and do not indicate any preference by the EURL-SRM towards these companies and their products

Stock solutions of the substances (e.g. 1 mg/mL) are prepared in acetonitrile, taking the purity of the standard substances into account. They were stored in a refrigerator for typically up to 48 months. Working solutions, e.g. mixtures, are prepared as necessary in acetonitrile and may be stored in the refrigerator for many months.

Sample Preparation and Measurement

The sample homogenates are extracted according to the CEN-QuEChERS (citrate-buffered) method (EN-15662) or, in case of high-oil content commodities, according to the QuOil method (CEN/TS 17062:2019) including a dispersive SPE clean-up (25 mg PSA, 25 mg ODS and 150 mg $MgSO_4$ per mL extract). As internal standards chlorpyrifos-D_{10} and propyzamide-D_3 (e.g. 100 µL of a mixture in acetonitrile at 10 µg/mL each) may be used. The internal standards are added to the sample portion before extraction. Isotope labelled internal standards (see **Table 5**) may also be used to correct for matrix effects even when using a calibration standard based on a different matrix or based on solvent.

The extract is directly subjected to LC-MS/MS separation and measurement. Exemplary LC-MS/MS conditions are given in **Table 6**.

Table 6: Instrumentation and method details (LC: Agilent 1290 Infinity II; MS: Sciex QTrap 6500+)

Instrument parameters	Conditions					
Column/temperature	Phenomenex Aqua C18, 20x50 mm, 5 µm, 125 A at 40 °C					
Pre-column	Aqua C18 125A 4mm x 2mm (Phenomenex AJO-7510)					
OPTIONAL: Trap column *(in case of background contamination deriving from contaminated eluent components or otherLC- consumables)*	Waters XBridge BEH C18, 2.1 mm X 50 mm, 5 µm, 130Å *Please be aware, that the column should also be compatible with the eluent conditions used for possible other methods run on this system!*					
Eluent A	5 mmol NH4-formiate in H2O + 0,01 % formic acid					
Eluent B	5 mmol NH4-formiate in methanol (MeOH) + 0,01 % formic acid					
Gradient	**%A**	**Flow [mL/min]**	**Time [min]**			
	95	0.4	0			
	50	0.4	2			
	40	0.4	8			
	10	0.4	12			
	10	0.4	14			
	95	0.4	14.5			
	95	0.4	21			
Injection volume	5 µL					
Acquired mass transitions (m/z)	**Compound**	**Mass transitions and their MS-parameters**				
		Q 1 (m/z)	**Q 3 (m/z)**	**DP[1] (V)**	**CE[2] (V)**	**CXP[3] (V)**
	BAC-C_8	248	156	36	25	25
		248	91	36	41	8
	BAC-C_{10}	276	184	55	27	10
		276	91	55	37	36
	BAC-C_{12}	304	212	91	29	10
		304	91	91	37	16
	BAC-C_{14}	332	240	83	31	10
		332	91	83	59	8
	BAC-C_{16}	360	268	78	33	12
		360	91	78	67	10
	BAC-C_{18}	388	296	31	33	8
		388	91	31	111	26
	DDAC-C_8	270	158	46	35	6
		270	43	46	61	2
	DDAC-C_{10}	326	186	61	39	12
		326	41	61	93	6
	DDAC-C_{12}	382	214	121	43	6
		382	58	121	67	4
	Chlorpyrifos-D_{10} (internal standard)	360	199	95	23	12
	Propyzamid-D_3 (internal standard)	259	193	61	21	10
	Optional:					
	BAC-C_{10} D_7	283	98	81	51	4
	BAC-C_{12} D_6	310	218	91	37	16
	BAC-C_{14} D_7	339	98	96	61	4
	DDAC-C_{10} D_6	332	192	96	41	10

Ion Source Parameters	**Ionisation mode**	ESI Positive
	Curtain Gas Flow	40 psi
	Ion Spray Voltage	5500 V
	Temperature	470 °C
	Nebulizer Gas Flow	60 psi
	Heater Gas Flow	70 psi

1) DP: Declustering Potential, 2) CE: Collission Energy, 3) CXP: Cell Exit Potential

Validation data:

Validation experiments for BAC-C_8, -C_{10}, -C_{12}, -C_{14}, -C_{16} and –C_{18} as well as for DDAC-C_8, -C_{10} and –C_{12} were conducted using matrices representing for the four main commodity groups according to Document Nº SANTE/11312/2021. The analytes were spiked in quintuplicate to the respective portions of the sample homogenates using a standard mixture in acetonitrile prepared as described above.

In case of cucumber, grapes and wheat flour, the CEN-QuEChERS extraction without any clean-up was conducted. In the case of peanuts, the QuOil extraction involving an ODS-containing dSPE clean-up was conducted to remove the fat content of the matrix (see also at chapter “Sample Preparation and Measurement”). The obtained recovery rates and the observed matrix effects are shown in **Table 7** to **Table 10**. The trap column approach was used during the validation experiments.

The conducted validations were successful for all levels and matrices, except of DDAC-C_{10} in grapes and peanuts at 0.005 mg/kg see **Table 8** and **Table 10**. In these cases, the blank extract contained DDAC-C_{10} levels > 30 % of the spiking level, and thus not fulfilling the criteria of the Document Nº SANTE/11312/2021. As a result, these validations had to be considered invalid.
In case of BAC-C_{18} in wheat flour, recoveries slightly below the usual recovery range of 70 %-120 % were obtained for both validated levels, see **Table 9**. As the relative standard variations remained below 5 % for each validated level the recovery rates could be regarded as consistent, and the validation for BAC-C_{18} in wheat flour could be still regarded successful. Nevertheless, measures to reduce the bias by compensating for recovery losses would need to be applied in routine applications (see also chapter G6 in Document Nº SANTE/11312/2021 as a reference). It remains to be checked at which analytical steps BAC-C_{18} is getting lost, and whether this is related to its surfactant and its tendency to attach onto lipid surfaces (e.g. during extraction and cleanup) or to other lipophilic surfaces (e.g. sorbents).

Exemplary chromatograms for the validation experiments in cucumber using the trap column approach are shown in **Figure 3** and **Figure 4**.

Table 7: Recoveries, relative standard variations (RSD) and the matrix effect for the validation of BAC-C_8, -C_{10}, -C_{12}, -C_{14}, -C_{16} and –C_{18} as well as DDAC-C_8, -C_{10} and –C_{12} in cucumber at 0.005 mg/kg and 0.010 mg/kg, each n = 5.

Matrix	Spiking level (mg/kg)	Analyte	Mass trace	Calculation using matrix-matched calibration		Matrix effect[1)]
				Mean Rec.	RSD	
CUCUMBER	0.005	BAC-C_8	248/156	103 %	1 %	-1
			248/91	102 %	1 %	
		BAC-C_{10}	279/184	100 %	2 %	+5
			279/91	101 %	2 %	
		BAC-C_{12}	304/212	103 %	1 %	+9
			304/91	101 %	2 %	
		BAC-C_{14}	332/240	97 %	1 %	+16
			332/91	97 %	1 %	
		BAC-C_{16}	360/268	96 %	2 %	+13
			360/91	95 %	2 %	
		BAC-C_{18}	388/296	92 %	2 %	+15
			388/91	91 %	3 %	
		DDAC-C_8	270/158	101 %	1 %	+7
			270/43	103 %	3 %	
		DDAC-C_{10}	326/186	107 %	5 %	+13
			326/41	102 %	7 %	
		DDAC-C_{12}	382/214	91 %	3 %	+15
			382/58	94 %	4 %	
	0.010	BAC-C_8	248/156	100 %	1 %	-1
			248/91	100 %	1 %	
		BAC-C_{10}	279/184	100 %	1 %	+5
			279/91	99 %	1 %	
		BAC-C_{12}	304/212	98 %	2 %	+9
			304/91	99 %	1 %	
		BAC-C_{14}	332/240	97 %	1 %	+16
			332/91	97 %	1 %	
		BAC-C_{16}	360/268	95 %	2 %	+13
			360/91	93 %	1 %	
		BAC-C_{18}	388/296	91 %	2 %	+15
			388/91	91 %	2 %	
		DDAC-C_8	270/158	98 %	2 %	+7
			270/43	103 %	2 %	
		DDAC-C_{10}	326/186	99 %	4 %	+13
			326/41	97 %	3 %	
		DDAC-C_{12}	382/214	90 %	2 %	+15
			382/58	95 %	4 %	

1) *Calculated from the mean peak areas of the matrix-matched calibration vs. the mean peak areas of the solvent calibration (120 % calibration level. each) of the quantifier mass trace using the higher concentration level.*

Table 8: Recoveries, relative standard variations (RSD) and the matrix effect for the validation of of BAC-C_8, -C_{10}, -C_{12}, -C_{14}, -C_{16} and –C_{18} as well as DDAC-C_8, -C_{10} and –C_{12} in table grapes at 0.005 mg/kg and 0.010 mg/kg, each n = 5.

Matrix	Spiking level (mg/kg)	Analyte	Mass trace	Calculation using matrix-matched calibration		Matrix effect[1)]
				Mean Rec.	RSD	
GRAPES	0.005	BAC-C_8	248/156	99 %	3 %	+15
			248/91	100 %	1 %	
		BAC-C_{10}	279/184	100 %	3 %	+13
			279/91	101 %	3 %	
		BAC-C_{12}	304/212	102 %	7 %	+9
			304/91	99 %	4 %	
		BAC-C_{14}	332/240	100 %	3 %	+10
			332/91	97 %	5 %	
		BAC-C_{16}	360/268	97 %	3 %	+12
			360/91	103 %	2 %	
		BAC-C_{18}	388/296	102 %	3 %	+10
			388/91	102 %	2 %	
		DDAC-C_8	270/158	104 %	5 %	+11
			270/43	97 %	5 %	
		DDAC-C_{10}	326/186	109 % [2)]	3 % [2)]	+14
			326/41	106 % [2)]	10 % [2)]	
		DDAC-C_{12}	382/214	98 %	5 %	+12
			382/58	106 %	4 %	
	0.010	BAC-C_8	248/156	99 %	1 %	+15
			248/91	97 %	2 %	
		BAC-C_{10}	279/184	98 %	2 %	+13
			279/91	98 %	1 %	
		BAC-C_{12}	304/212	99 %	2 %	+9
			304/91	98 %	3 %	
		BAC-C_{14}	332/240	98 %	2 %	+10
			332/91	97 %	2 %	
		BAC-C_{16}	360/268	98 %	2 %	+12
			360/91	99 %	2 %	
		BAC-C_{18}	388/296	98 %	2 %	+10
			388/91	96 %	2 %	
		DDAC-C_8	270/158	101 %	1 %	+11
			270/43	103 %	5 %	
		DDAC-C_{10}	326/186	91 %	4 %	+14
			326/41	94 %	3 %	
		DDAC-C_{12}	382/214	98 %	3 %	+12
			382/58	102 %	3 %	

1) Calculated from the mean peak areas of the matrix-matched calibration vs. the mean peak areas of the solvent calibration (120 % calibration level. each) of the quantifier mass trace using the higher concentration level.

2) The blank extract contained DDAC-C_{10} levels >30 % of the spiking level. The validation was therefore considered invalid as the method criteria of the Document Nº SANTE/11312/2021 were not met.

Table 9: *Recoveries, relative standard variations (RSD) and the matrix effect for the validation of of BAC-C_8, -C_{10}, -C_{12}, -C_{14}, -C_{16} and –C_{18} as well as DDAC-C_8, -C_{10} and –C_{12} in wheat flour at 0.005 mg/kg and at 0.010 mg/kg, each n = 5.*

Matrix	Spiking level (mg/kg)	Analyte	Mass trace	Calculation using matrix-matched calibration		Matrix effect[1)]
				Mean Rec.	RSD	
WHEAT FLOUR	0.005	BAC-C_8	248/156	102 %	2 %	-36
			248/91	101 %	2 %	
		BAC-C_{10}	279/184	99 %	3 %	-20
			279/91	101 %	1 %	
		BAC-C_{12}	304/212	98 %	4 %	-18
			304/91	98 %	3 %	
		BAC-C_{14}	332/240	87 %	3 %	-11
			332/91	87 %	2 %	
		BAC-C_{16}	360/268	80 %	3 %	-4
			360/91	77 %	3 %	
		BAC-C_{18}	388/296	71 %	4 %	+20
			388/91	67 %	4 %	
		DDAC-C_8	270/158	101 %	2 %	-29
			270/43	97 %	5 %	
		DDAC-C_{10}	326/186	105 %	11 %	-20
			326/41	108 %	12 %	
		DDAC-C_{12}	382/214	74 %	4 %	+11
			382/58	75 %	3 %	
	0.010	BAC-C_8	248/156	92 %	1 %	-36
			248/91	95 %	2 %	
		BAC-C_{10}	279/184	95 %	1 %	-20
			279/91	95 %	2 %	
		BAC-C_{12}	304/212	92 %	1 %	-18
			304/91	92 %	2 %	
		BAC-C_{14}	332/240	84 %	2 %	-11
			332/91	85 %	1 %	
		BAC-C_{16}	360/268	75 %	2 %	-4
			360/91	72 %	2 %	
		BAC-C_{18}	388/296	67 %	1 %	+20
			388/91	67 %	3 %	
		DDAC-C_8	270/158	95 %	2 %	-29
			270/43	97 %	2 %	
		DDAC-C_{10}	326/186	88 %	6 %	-20
			326/41	87 %	5 %	
		DDAC-C_{12}	382/214	71 %	4 %	+11
			382/58	71 %	4 %	

1) *Calculated from the mean peak areas of the matrix-matched calibration vs. the mean peak areas of the solvent calibration (120 % calibration level. each) of the quantifier mass trace using the higher concentration level.*

Table 10: *Recoveries, relative standard variations (RSD) and the matrix effect for the validation of of BAC-C_8, -C_{10}, -C_{12}, -C_{14}, -C_{16} and –C_{18} as well as DDAC-C_8, -C_{10} and –C_{12} in wheat flour at 0.005 mg/kg and at 0.010 mg/kg, each n = 5.*

Matrix	Spiking level (mg/kg)	Analyte	Mass trace	Calculation using matrix-matched calibration		Matrix effect[1)]
				Mean Rec.	RSD	
PEANUTS	0.005	BAC-C_8	248/156	97 %	4 %	+6
			248/91	98 %	3 %	
		BAC-C_{10}	279/184	98 %	1 %	+9
			279/91	98 %	1 %	
		BAC-C_{12}	304/212	101 %	7 %	+29
			304/91	103 %	10 %	
		BAC-C_{14}	332/240	96 %	6 %	+35
			332/91	99 %	5 %	
		BAC-C_{16}	360/268	95 %	1 %	+7
			360/91	97 %	5 %	
		BAC-C_{18}	388/296	91 %	5 %	+20
			388/91	94 %	3 %	
		DDAC-C_8	270/158	100 %	2 %	+9
			270/43	98 %	12 %	
		DDAC-C_{10}	326/186	118 % [2)]	22 % [2)]	-25
			326/41	117 % [2)]	22 % [2)]	
		DDAC-C_{12}	382/214	98 %	3 %	+17
			382/58	101 %	9 %	
	0.010	BAC-C_8	248/156	102 %	2 %	+6
			248/91	100 %	2 %	
		BAC-C_{10}	279/184	99 %	1 %	+9
			279/91	101 %	2 %	
		BAC-C_{12}	304/212	102 %	4 %	+29
			304/91	101 %	5 %	
		BAC-C_{14}	332/240	95 %	3 %	+35
			332/91	101 %	2 %	
		BAC-C_{16}	360/268	98 %	5 %	+7
			360/91	97 %	4 %	
		BAC-C_{18}	388/296	96 %	5 %	+20
			388/91	96 %	4 %	
		DDAC-C_8	270/158	100 %	2 %	+9
			270/43	99 %	4 %	
		DDAC-C_{10}	326/186	97 %	4 %	-25
			326/41	93 %	11 %	
		DDAC-C_{12}	382/214	95 %	3 %	+17
			382/58	100 %	5 %	

1) Calculated from the mean peak areas of the matrix-matched calibration vs. the mean peak areas of the solvent calibration (120 % calibration level. each) of the quantifier mass trace using the higher concentration level.

2) The blank extract contained DDAC-C_{10} levels >30 % of the spiking level. The validation was therefore considered invalid, as the method criteria of the Document Nº SANTE/11312/2021 were not met.

Exemplary chromatograms of the validation experiments:

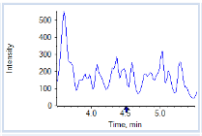
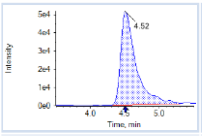
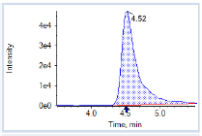
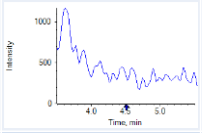
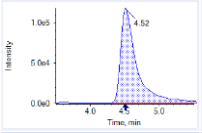
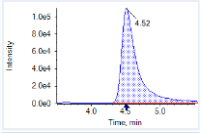
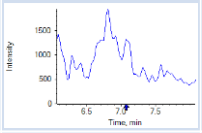
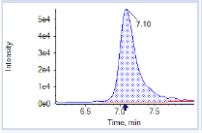
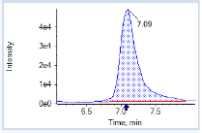
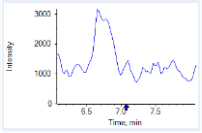
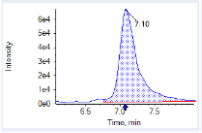
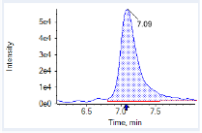
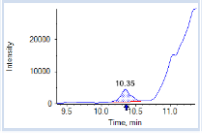
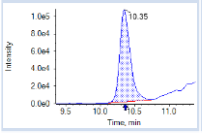
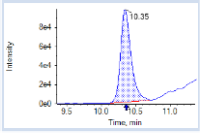
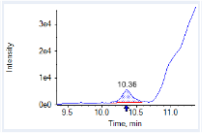
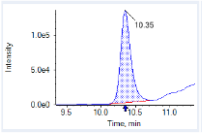
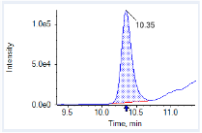
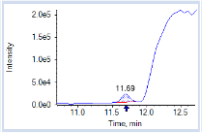
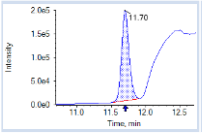
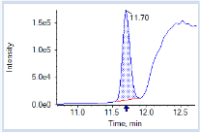
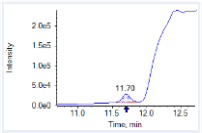
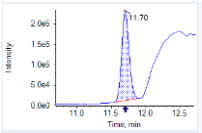
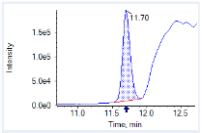
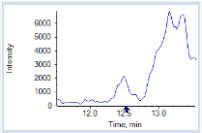
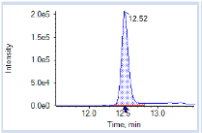
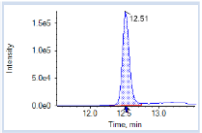
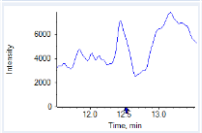
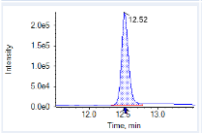
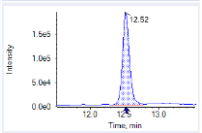
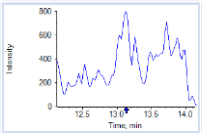
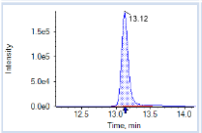
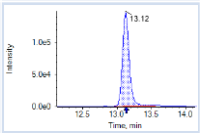
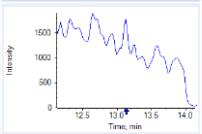
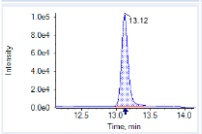
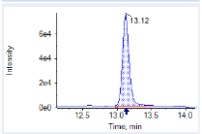
Substance	Mass transition	Matrix blank	Matrix calibration (120 % level)	Recovery
BAC-C8	248/156			
	248/91			
BAC-C10	248/156			
	248/91			
BAC-C12	248/156			
	248/91			
BAC-C14	248/156			
	248/91			
BAC-C16	248/156			
	248/91			
BAC-C18	248/156			
	248/91			

Figure 3*: Exemplary chromatograms for the BACs of the conducted validation in cucumber at 0.005 mg/kg using the trap column approach.*

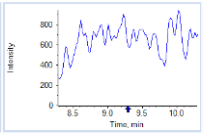
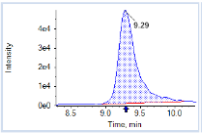
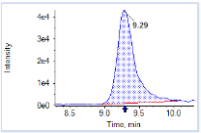
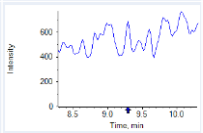
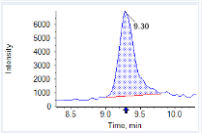
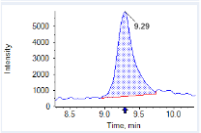
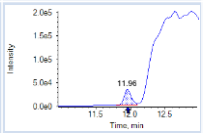
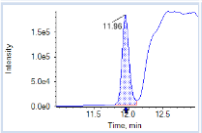
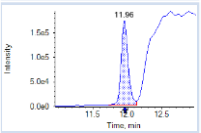
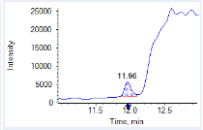
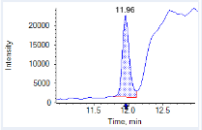
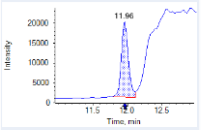
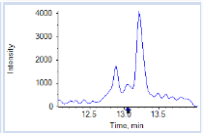
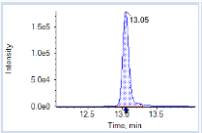
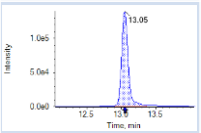
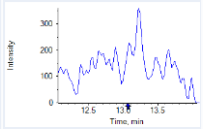
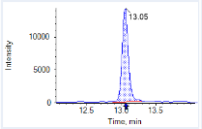
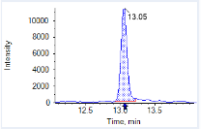
Substance	Mass transition	Matrix blank	Matrix calibration (120 % level)	Recovery
DDAC-C8	270/158			
	270/43			
DDAC-C10	326/186			
	326/41			
DDAC-C12	382/214			
	382/58			

Figure 4: ***Exemplary chromatograms for the DDACs of the conducted validation in cucumber at 0.005 mg/kg using the trap column approach.***

Intermediate Conclusions and Outlook:

A method for the analysis of a number of BAC and DDAC congeners in food of plant origin is presented. The compounds are extracted using the QuEChERS method and analysed by LC-MS/MS in the ESI (pos.) mode using a polar endcapped column and a slightly acidic gradient. Based on the general behavior of the concerned analytes, a sensitive measurement in matrices of animal origin is also deemed possible under the presented conditions.

QAC background contaminations of variable intensity and composition are often reported. These background levels seriously compromise their ability of the labs to analyze those compounds at low levels and to control MRL compliance. Background contaminations deriving from the LC system in front of the autosampler, e.g. from contaminated eluents and tubing appear to be the most crucial to address. A simple and practical way of separating the background contamination deriving from the LC-system is the use of the trap column, between the pump and the autosampler of the LC-system. Any QAVs contaminants eluting from the system in-between chromatographic runs (e.g. during column equilibration), are trapped onto this trap column, and prevented from accumulating at the beginning of the analytical column or pre-column. During the chromatographic run, these contaminants will eventually also elute through the analytical column, but they will experience additional retention (in the trap column), and will thus separate from their analogues introduced through the injection of sample extracts.

Validation experiments according to the Document Nº SANTE/11312/2021 were successful for all analytes at 0.005 mg/kg and 0.01 mg/kg on cucumber, grapes, wheat flour and peanuts. Validations of $DDAC_{10}$ in grapes and peanut at the 0.005 mg/kg level were considered invalid, as the blank extracts contained DDAC-C_{10} levels > 30 % of the spiking level, thus not fulfilling the requirements of Document Nº SANTE/11312/2021. For BAC-C_{18} in wheat flour, the validation was regarded successful, despite the mean recoveries being slightly below the acceptable range of 70 %-120 %, because the relative standard variation was < 5 %, suggesting that the recovery rates a sufficiently consistent.

The presented approach can be easily transferred to other areas where background contaminations deriving from the LC-system may occur, for example in the analysis of other ubiquitous compounds, such as per- and polyfluoroalkyl compounds and plasticizers.

Document History

Action	When	Changes / Actions	Document Version
Publication of V6	24.02.2023	General text revision and layout update Discussion of problems in routine analysis deriving from contaminations with QACs Implementation of the trap column approach to address contamination at the LC-step Update of the LC-MS/MS conditions Update of the standard substance providers Presentation of method validation data at 0.005 and 0.01 mg/kg Presentation of exemplary chromatograms	V6
Publication of V5	24.03.2016	General text revision Update of recovery data Inclusion of BAC-C8, BAC-C18, DDAC-C8, DDAC-C12, and isotope labelled internal standards Update of LC and MS/MS method details Revision of legal background information	V5
Publication of V4	14.09.2012		V4
Publication of V3	06.07.2012	Elimination of errors	V3
Publication of V2	05.07.2012	Addition of detailed LC-Conditions	V2
Publication of V1	29.06.2012		V1