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

Version 5 (last update: 24.03.2016)

Brief description:

A method is presented for the analysis of Quaternary ammonium compounds (QACs) in food of plant origin. 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.

Compound details:

Quaternary ammonium compounds (QACs) are surface-active substances containing a quaternary cationic nitrogen atom, substituted by alkyl chains of varying length. They are used as biocides, pesticides, disinfectants and additives for technical applications.

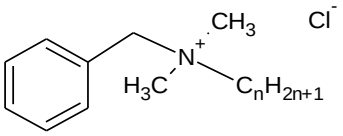
BAC is a mixture of alkylbenzyldimethylammonium chlorides of various even-numbered alkyl chain lengths (C8-C18). The greatest biocide activity is associated with the C12-C14 derivatives, which are the main components of the mixture.

DDAC is a mixture of alkyl-quaternary ammonium salts with typical alkyl chain lengths of C8, C10 and C12. C10 is the main component and makes more than 90 % of the mixture. In most cases the term DDAC is thus used for the Didecyldimethylammonium chloride congener.

DDAC used to be approved in the EU as a plant protection product (for ornamental plants). BAC has never been approved as active substance in plant protection products. Still, as both Both BAC and DDAC have pesticide properties they were until a few years ago contained in various products used for the treatment of various food-related crops in the field within the EU. Some of these products adulterated were even marketed as suitable for organic agriculture (e.g. grapefruit seed extract based products containing >20% BAC) without the QACs contained being declared.
BAC and DDAC are furthermore widely used as **biocides** for the **cleaning/sanitation of surfaces** in the areas of food, feed and medical production sectors as well as domestically. Personal care products such as hand sanitizers, wet wipes and eye-drops may also contain them.
Dairy Industry is probably the most important food-related field of DDAC and BAC use. DDAC and BAC containing products are for example employed for the disinfection of all kind of surfaces milking equipment, milk storage tanks and ice-cream machines. 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.

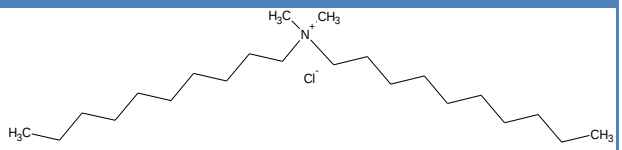
Compound facts at a glance:

Benzalkonium chloride (BAC) CAS 8001-54-5	
Parameter	**Value**
Molecular Formula:	C9H13ClNR (R=C8H17 to C18H37)
Molecular weight:	variable
Residue definition EU	Benzalkonium chloride (mixture of alkylbenzyldimethylammonium chlorides with alkyl chain lengths of C8, C10, C12, C14, C16 and C18)



n=8, 10, 12, 14, 16,1

Didecyldimethylammonium chloride (DDAC-C10) CAS 7173-51-5	
Parameter	**Value**
Molecular Formula:	C22H48ClN
Molecular weight:	362,08
Residue definition EU:	Didecyldimethylammonium chloride (mixture of alkyl-quaternary ammonium salts with alkyl chain lengths of C8, C10 and C12)



Legal Aspects and Monitoring Programs: In October 2012 the Standing Committee on the Food Chain and Animal Health (SCoFCAH) endorsed Guidelines on measures to be taken as regards the presence of DDAC[1] and BAC[2] in or on food and feed. These guidelines contained an agreed temporary enforcement level of 0.5 mg/kg for food and feed. It was further recommended that EU Member States carry out investigations on the possible causes of BAC / DDAC contamination and a monitoring program was put in place to get an overview of the BAC and DDAC levels in food and feed of plant and animal origin.
Based on the monitoring data collected (summarized in an EFSA report[3]) a flat MRL level of 0.1 mg/kg was established in 2014 for both didecyldimethylammonium chloride and benzalkonium chloride in all food products (Reg. 1119/2014/EU). These MRLs apply to the chloride salts. A revision of these MRLs is planned to take place by 31 December 2019 and if necessary they will be amended modified based on newer residue data. Before the agreement on temporary MRLs (0.5 mg/kg) in 2012 and the implementation of specific MRLs in 2014 (0.1 mg/kg), the default MRL of 0.01 mg/kg laid down in Reg. 396/2005/EC used to apply.

[1] European Commission, 2012a. Guidelines as regards measures to be taken as regards the presence of Didecyl Dimethyl Ammonium Chloride (DDAC) in or on food and feed agreed by the Standing Committee of the Food Chain and Animal Health (SCoFCAH) on 13 July 2012 and modified on 5 October 2012
[2] European Commission, 2012b. Guidelines as regards measures to be taken as regards the presence of Benzalkonium Chloride (BAC) in or on food and feed agreed by the Standing Committee of the Food Chain and Animal Health (SCoFCAH) on 25 July 2012 and modified on 5 October 2012
[3] European Food Safety Authority; Evaluation of monitoring data on residues of didecyldimethylammonium chloride (DDAC) and benzalkonium chloride (BAC). EFSA supporting publication 2013:EN-483, 30 pp

Standard Materials Supply:

Table 1: Exemplary Providers of QACs (11.03.2016)[4]

Acronym	Compound	CAS	Company	Order No.
Native standards				
BAC-C8	Benzyldimethyloctylammonium chloride	959-55-7	HPC	675698
BAC-C10	Benzyldimethyldecylammonium chloride	965-32-2	Aldrich	13371
BAC-C12	Benzyldimethyldodecylammonium chloride	139-07-1	Aldrich	13380
BAC-C14	Benzyldimethyltetradecylammonium chloride	139-08-2	Fluka	13401
BAC-C16	Benzyldimethylhexadecylammonium chloride	122-18-9	Sigma	B4136
BAC-C18	Benzyldimethyloctadecylammonium chloride	122-19-0	HPC	674644
DDAC-C8	Dimethyldioctylammonium bromide	3026-69-5	HPC	675696
	Dimethyldioctylammonium chloride	5538-94-3	LGC	DRE-C12726470
DDAC-C10	Didecyldimethylammonium chloride	7173-51-5	LGC	DRE-C12588000
DDAC-C12	Didodecyldimethylammonium chloride	3401-74-9	Chemos	135628
			TRC	D441760
Internal Standards (Options)				
BAC-C10 D7	D7-Benzyldimethyldecylammonium chloride		HPC	674610
BAC-C12 D6	D6-Benzyldimethyldodecylammonium iodide		HPC	674572
BAC-C14 D7	D7-Benzyldimethyltetradecylammonium chloride		HPC	674611
DDAC-C10 D6	D6-Didecyldimethylammonium iodide		HPC	674541

Measurement

Table 2: LC details for QACs (exemplary)

LC	Agilent 1290 Infinity UPLC		
MS/MS	ABSCIEX API 4000 Q-Trap, run in ESI positive mode		
Column	Phenomenex Aqua 5µ C18 125A		
Pre-column	Aqua C18 125A 4mm x 2mm (Phenomenex AJO-7510)		
Mobile Phase	A: 5 mmol NH4-formiate in H2O + 0,01 % formic acid B: 5 mmol NH4-formiate in methanol (MeOH) + 0,01 % formic acid		
Ionisation mode	ESI pos		
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		
Dilution of extract	no		
Column temperature	40°C		

[4] **Disclaimer**: this information is given solely for the convenience and information of the readers and it does not constitute any advertisement, preference or endorsement of the companies and products listed

Table 3: MS/MS details for QACs (ESI-negative mode, Tune-data ABSciex 4000Q) (exemplary)

Compound	Sensitivity Ranking (1= best)	Parent Mass	Daughter Mass	DP	CE	CXP
BAC-C8	1	248	156	36	25	25
	2	248	91	36	41	8
BAC-C10	1	276	184	55	27	10
	2	276	91	55	37	36
BAC-C12	1	304	212	91	29	10
	2	304	91	91	37	16
BAC-C14	1	332	240	83	31	10
	2	332	91	83	59	8
BAC-C16	1	360	268	78	33	12
	2	360	91	78	67	10
BAC-C18	1	388	296	31	33	8
	2	388	91	31	111	26
DDAC-C8	1	270	158	46	35	6
	2	270	43	46	61	2
DDAC-C10	1	326	186	61	39	12
	2	326	41	61	93	6
DDAC-C12	1	382	214	121	43	6
	2	382	58	121	67	4
Internal Standards (Options)						
BAC-C10 D7	-	283	98	81	51	4
BAC-C12 D6	-	310	218	91	37	16
BAC-C14 D7	-	339	98	96	61	4
DDAC-C10 D6	-	332	192	96	41	10
Chlorpyrifos D10		360	199	36	27	4

Validatation Data

Table 4: Compilation of exemplary recovery data for various commodity types and spiking levels

Commodity group	Commodity	Compound	spiking Level	Average Recovery	RSD_R (Reproduciobility)	No. of recoveries
High water content	Asparagus	BAC-C8	0.025	102	13	7
		BAC-C10	0.025	102	12	10
		BAC-C12	0.025	101	13	10
		BAC-C14	0.025	99	11	9
		BAC-C16	0.025	93	14	8
		BAC-C18	0.025	90	18	8
		DDAC-C10	0.025	98	13	10
	Cucumbers	BAC-C8	0.025	100	8	11
		BAC-C10	0.025	100	7	11
		BAC-C12	0.025	100	10	11
		BAC-C14	0.025	97	9	11
		BAC-C16	0.025	95	8	11
		BAC-C18	0.025	91	9	11
		DDAC-C10	0.025	97	8	11
High acid content	Mandarins	BAC-C8	0.025	100	4	5
		BAC-C10	0.025	101	3	5
		BAC-C12	0.025	103	5	5
		BAC-C14	0.025	101	2	5
		BAC-C16	0.025	102	3	5
		BAC-C18	0.025	97	5	5
		DDAC-C10	0.025	99	2	5
	Oranges	BAC-C8	0.025	100	8	8
		BAC-C10	0.025	102	8	8
		BAC-C12	0.025	104	10	8
		BAC-C14	0.025	104	12	8
		BAC-C16	0.025	100	9	8
		BAC-C18	0.025	97	11	8
		DDAC-C10	0.025	103	4	6
Dry (pulses cereals)	Wheat	BAC-C8	0.05	107	7	7
		BAC-C10	0.05	104	7	7
		BAC-C12	0.05	99	10	7
		BAC-C14	0.05	96	9	7
		BAC-C16	0.05	86	10	6
		BAC-C18	0.05	80	16	7
		DDAC-C10	0.05	100	10	7

Commodity group	Commodity	Compound	spiking Level	Average Recovery	RSD_R (Reproduciobility)	No. of recoveries
High sugar Content	Honey	BAC-C8	0.083	103	5	6
		BAC-C10	0.083	104	4	6
		BAC-C12	0.083	105	5	6
		BAC-C14	0.083	104	4	6
		BAC-C16	0.083	103	4	6
		BAC-C18	0.083	103	4	6
		DDAC-C10	0.083	103	4	6

The sensitivity of detection is good so that determinations below 0.01 mg/kg are principally possible. It should be considered, however, that the compounds are ubiquitary and have the tendency to strongly interact with surfaces and thus the potential for cross-contaminations. Background levels mostly concern BAC-C12 and BAC-C14, which are typically the predominant components in formulations. Thus LOQs of 0.02 mg/kg are more realistic.

Recoveries can also be found at **www.eurl-pesticides-datapool.eu**

Document History

Date	Action	Changes
29.06.2012	Publication of V1	
05.07.2012	Publication of V2	Addition of detailed LC-Conditions
06.07.2012	Publication of V3	Elimination of errors
14.09.2012	Publication of V4	
24.03.2016	Publication of V5	General text revision Revision of legal background information Inclusion of BAC-C8, BAC-C18, DDAC-C8, DDAC-C12, and isotope labelled internal standards Update of LC and MS/MS method details Update of recovery data