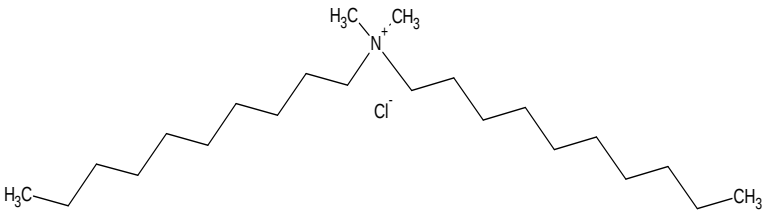
Analysis of BACs and DDAC in Milk using QuEChERS method and LC-MS/MS

Version 2 (last update: 05.05.14)

Short description:
A method is presented for the analysis of BAC and DDAC in milk. The pesticides are analyzed using a modified QuEChERS method and LC-MS/MS.

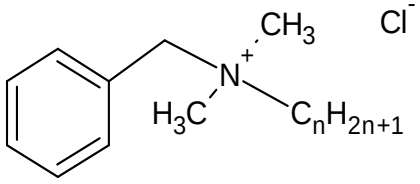
Compound details:
BAC (Benzalkonium Chloride) and DDAC (Dialkyldimethylammonium Chloride) belong the Quaternary Ammonium Compounds (QACs), which constitute a large group of chemicals that are among others used as cationic surfactants, disinfectants, biocides, herbicides/growth regulators, fabric softeners, antistatic agents and phase transfer agents. QACs containing long alkyl chains, such BAC and DDAC are mainly used as antimicrobials and disinfectants.

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 alkyl derivatives, which are the main components.



Dialkyldimethylammonium chloride (DDAC)

Benzalkoniumchloride (BAC)



n=8, 10, 12, 14, 16,18

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

DDAC was registered in the EU as a plant protection product (for ornamental plants only). BAC has never been approved as active substance for plant protection products, and is thus rather classified as a "food and feed area disinfectant" in the sense of Directive 98/8/EC. Still, as both BAC and DDAC have pesticide properties they were until recently contained in various products used for the treatment of food related crops in the field within the EU. Some of these products were declared as “plant strengtheners” and were thus also widely used in the organic sector. BAC and DDAC are furthermore widely used for the cleaning/sanitation of surfaces in the medical and food 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.
According to a recent EFSA evaluation of monitoring data on residues of DDAC and BACs[1] in food EFSA, BACs and DDAC were found in 12% of the tested milk and milk products. This was the highest figure across all food groups. Leafy vegetables and fresh herbs (6 %) and baby food (5 %) followed.

Legal Aspects:
Because no specific maximum limit for residues of DDAC and BAC was established under EU Regulation No. 396/2005, the general residue limit of 0.01 mg/kg applies.
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[2] and BAC[3] in or on food and feed. It was recommended that EU Member States carry out investigations on the possible causes of BAC / DDAC contamination and to put in place a monitoring program to get an overview of the BAC and DDAC levels in all food and feed of plant and animal origin. Considering that the current default MRLs for DDAC and BAC (of 0.01 mg/kg) are not a health standard a **temporary enforcement level of 0.5 mg/kg was agreed upon**. As no specific residue definition was defined, there is still uncertainty as to how residues are to be expressed. Based on the first results of the monitoring program a lower enforcement level for QACs is under discussion.

Sources of Supply (exemplary):
- DDAC: Dr. Ehrenstorfer (C12588000)
- BAC10: Aldrich (13371);
- BAC12: Aldrich (13380);
- BAC14: Fluka (13401);
- BAC16: Sigma (B4136)
- BAC12-D6: HPC (674572);
- BAC14-D7: HPC (674611);
- DDAC10-D6: HPC (674541)

Apparatus and Consumables:
Use materials described in the QuEChERS standard procedure (EN15662). As a mechanical shaker you can use a horizontally or vertically reciprocating shaker or a rotatory shaker (e.g. HS260 by IKA or GenoGrinder by Spex or SSL1 Labscale Orbital Shaker by Stuart). To filter the extract use e.g. polyester disposable syringe filters of 0.45 µm pore size.

[1] **http://www.efsa.europa.eu/de/supporting/doc/483e.pdf**
[2] 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
[3] 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

Extraction and Cleanup Procedure:

Extraction: Weigh 10 g of milk, add 10 mL acetonitrile and internal standard (e.g. 100µL of an appropriately concentrated solution) and shake 15 min using a mechanical shaker. Add QuEChERS citrate-buffer mix, shake 1 min and centrifuge --> Raw Extract.

Cleanup: Transfer e.g. 6-8 mL raw extract into a vial and place it in a freezer for at least 2 h. Take the vial out of the freezer and either a) quickly pass 1mL through a syringe filter into an LC-MS/MS vial, paying attention to avoid heating up the mixture as this would progressively re-dissolve lipids; OR b) quickly decant 1 mL into an LC-MS/MS vial paying attention to leave precipitates behind and to avoid heating up; OR c) quickly pass some milliliters through a cotton-wool-filled funnel into a vessel and transfer 1mL filtrate into an LC-MS/MS vial.

For a more intensive cleanup, optionally, transfer 4 mL of the extract that was cleaned-up via freeze-out into a 15 mL centrifuge tube containing 100 mg PSA and 600 mg $MgSO_4$, shake 1 min, centrifuge, and transfer 1 mL of the supernatant into an LC-MS/MS vial.

Preparation of calibration standards:

Matrix matched calibration standards are prepared using an extract of blank milk (not containing any of the pesticides of interest). The blank extract is produced as described above however without addition of an IS. 1mL final extract will represent approximately 1 g matrix.

Measurement:

LC-Conditions - A	
Instrument	Waters Acquity, ABSciex API 4000 QTrap
Ionization mode	ESI pos.
Column	Phenomenex Synergi 4µm Hydro-RP; 150 x 2 mm
Pre-column	Security Guard AQ C18 4 x 2.0 mm
Eluent A	5 mmol ammonium formate in purified Water,
Eluent B	5 mmol ammonium formate in Methanol
Gradient	time / flow [µL/min] / A% / B% 0 / 100 / 100 / 3 / 100 / 30 / 70 6 / 300 / 15 / 85 9 / 300 / 10 / 90 20 / 300 / 10 / 90 20.5 / 300 / 100 / 30 / 300 / 100 /
Injection volume	3µl, automatically diluted w. 10µl of mobile phase A during injection procedure
Column Temperature	40°C

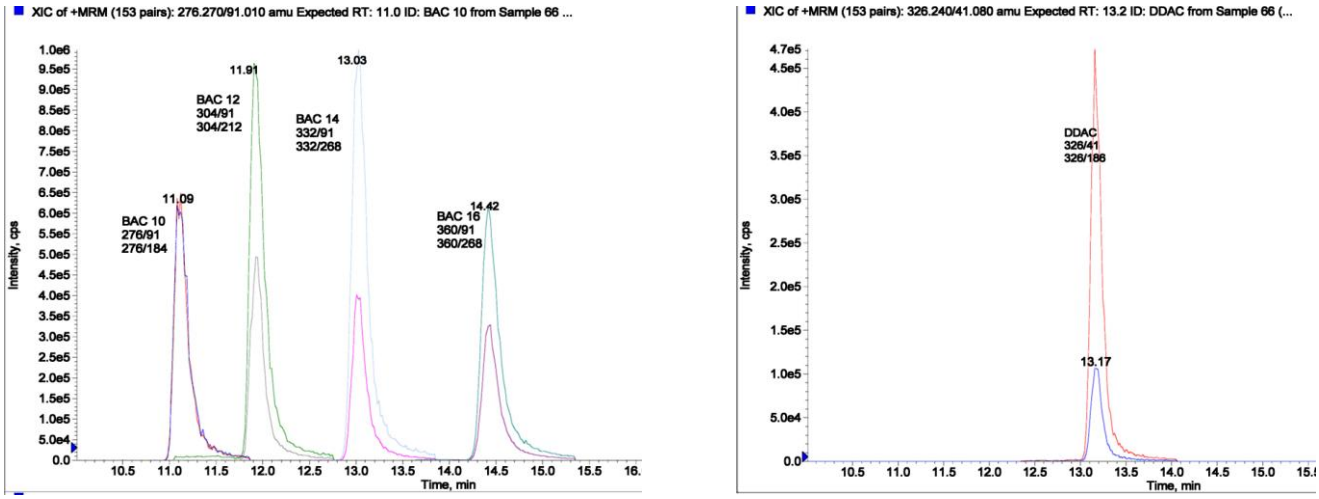


Figure 1: Exemplary chromatograms at 0.12 mg/mL in whole milk extract

OR

LC-Conditions - B	
Instrument	Waters Acquity, ABSciex API 4000 QTrap
Ionization mode	ESI pos.
Column	Phenomenex Aqua 5µm C18; 125 A; 50x2 mm
Pre-column	Aqua C18 125A 4mm x 2mm (Phenomenex AJO-7510)
Eluent A	5 mmol ammonium formate in Water + 0.01 % formic acid
Eluent B	5 mmol ammonium formate in Methanol + 0.01 % formic acid
Gradient	**Time** / **A%** / **B%** 0 / 100 / 2 / 50 / 50 8 / 40 / 60 12 / 10 / 90 16 / 10 / 90 16.5 / 100 / 24 / 100 /
Injection volume	3µl
Column Temperature	40°C

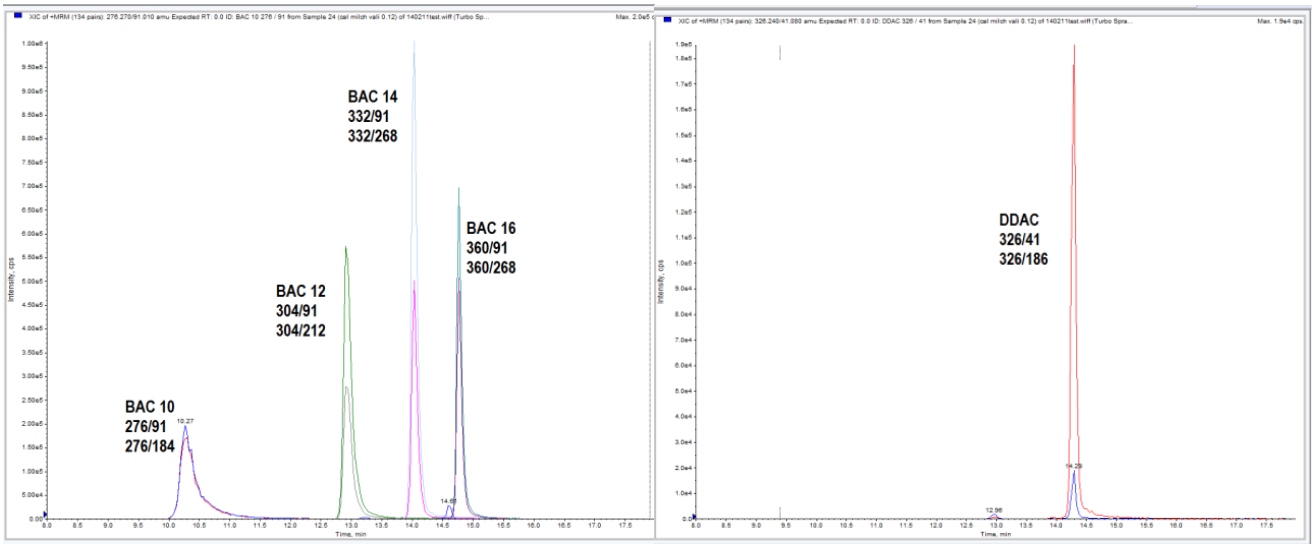


Figure 2: Exemplary chromatograms at 0.12 mg/mL in whole milk extract

Exemplary Instrumentation details for QACs:

Tab. 2: MRM Details (ESI positive mode):

Transition Name	Rel. Sensitivity	Parent Mass	Daughter Mass	DP	CE	CXP
DDAC 326/41	1	326	41	61	93	6
DDAC 326/186	2	326	186	61	39	12
BAC10 276/184	1	276	184	55	27	10
BAC10 276/91	2	276	91	55	37	36
BAC12 304/212	1	304	212	91	29	10
BAC12 304/91	2	304	91	91	37	16
BAC14 332/240	1	332	240	83	31	10
BAC14 332/91	2	332	91	83	59	8
BAC16 360/268	1	360	268	78	33	12
BAC16 360/91	2	360	91	78	67	10
Internal Standards (Options)						
DDAC (D6)		332	192	96	41	10
BAC 10 (D7)		283	98	81	51	4
BAC 12 (D6)		310	218	91	29	10
BAC 14 (D7)		339	98	96	61	4
Chlorpyriphos D10		360	199	36	27	4

Validation data:

Tab. 3: Recoveries of BAC and DDAC from Whole Milk (n=5).

Compound	MRMs	0.1 mg/kg	0.02 mg/kg
BAC10	276>184 276>91	96 (1.5)	98 (5.2)
BAC12	304>212 304>91	96 (3.6)	95 (2.0)
DDAC10	326>41 326>186	103 (2.5)	109 (8.5)

Validation data with modified methods:

Tab. 4: Recovery experiments for BAC/DDAC from Whole Milk (n=3), Cleanup: freeze-out -> filtration ->dilution with water (1:1)

Spiking Level	BAC10	BAC12	DDAC
	Mean recovery rates (RSDs)		
0.05 mg/kg	91% (5.8%)	101% (4.9%)	102% (4.3%)

Tab. 5: Recovery experiments for BAC/DDAC from Whole Milk (n=5), Cleanup: dilution with water (1:1) + ODS 25mg/mL raw extract

Spiking Level	BAC10	BAC12	DDAC
	Mean recovery rates (RSDs)		
0.025 mg/kg	83% (10%)	64% (11%)	39% (10%)

NOTE: This cleanup approach leads to unacceptable losses!!

Tab. 6: Recovery experiments for BAC/DDAC from QuEChERS raw extracts of Whole Milk applying dSPE cleanup with PSA sorbent 25 mg/mL (spiking on raw extract of whole milk at level corresponding to 0.1 mg/kg); (n=2)

Spiking Level	BAC10	BAC12	DDAC
	Mean recovery rates		
0.1 mg/kg	94%	103%	98%

dSPE cleanup using PSA sorbent does not have any significant effect on QAC recoveries:

Impact of cleanup on dry residue of extracts

Cleanup approach	Dry residue %	Notes
None (Raw milk extract)	100%	3,3 mg dry residue/mL (=0,033%), compared to ca. 12% dry residue in whole milk
dSPE w. C18 (25 mg/mL)	64%	w. additional PSA/$MgSO_4$ (25/150 mg/mL) 53%
Freeze-out and decanting though cotton	44%	w. additional PSA/$MgSO_4$ (25/150 mg/mL) 35%
1:1 dilution w. water -> filtration	35%	Calculated on basis of non-diluted extract (x2)
1:1 dilution w. water + simultaneous dSPE w. C18 (25 mg/mL) -> filtration	23%	Calculated on basis of non-diluted extract (x2). **Substantial recovery losses of QACs observed!!**
Freeze-out -> filtration ->dilution with water (1:1) -> filtration	10%	Calculated on basis of non-diluted extract (x2)

Limits of Quantification are well below 0.01 mg/kg. However as small peaks of DDAC, BAC12 and BAC14 seem to be always present, the Reporting Limit is set 0.01 mg/kg for the individual BACs and 0.02 mg/kg for DDAC10.

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

References

[3] The ePesticide Manual, ISBN 1-901396-31-2, The British Crop Protection Council
[4] http://www.alanwood.net/pesticides/class_herbicides.html