Validation of an analytical method for analyzing residues of quaternary ammonium compounds in animal and plant samples by LC-MS/MS

Irene Jiménez, Eduardo Perogordo, Natividad Andrés and Begoña Galíndez.
Spain's National Food Centre. Spanish Agency for Consumer Affairs, Food Safety and Nutrition

Abstract

The aim of this work is to develop and validate a method for analyzing residues of quaternary ammonium compounds. A validation has been carried out and the application has been tested in both animal and plant samples.

The analytical procedure consists of an extraction based on the QuEChERS method and a detection, quantification and confirmation by LC-MS/MS.

Key words

Validation, residues, quaternary ammonium.

Abbreviations

Quaternary ammonium waste compounds:

- BAC: benzalkonium chloride and derivatives (benzyldimethyldodecylammonium chloride -BAC 12-, myristalkonium chloride -BAC 14-, cetalkonium chloride -BAC 16-).
- DDAC: didecyldimethylammonium chloride.

CV: Coefficient of variation.

LC-MS/MS: Liquid chromatography with triple quadrupole mass detector.

LOD: Limit of Determination.

MRL: Maximum Residue Limit.

QuEChERS Method: Quick Easy Cheap Effective Rugged Safe method.

Translated from the original published in the journal: Revista del Comité Científico de la AECOSAN,20, pp: 45-52

1. Introduction

Quaternary ammonium compounds are a chemical group of compounds that contain a cationic quaternary nitrogen atom substituted by alkyl radical chains of variable longitude. Within this group the most characteristic are BAC and DDAC.

In the case of the BAC the substitutes are two methyls and two linear chains, of which one is a phenyl group and the other may contain a variable number of carbon atoms (n). Depending on this number they are called:

- Benzyldimethyldodecylammonium chloride (BAC 12), n=12.
- Myristalkonium chloride (BAC 14), n=14.
- Cetalkonium chloride (BAC 16), n= 16.

When this study began, there was no specific European legislation for these compounds in relation to their maximum residue limits; however, due to their potential use as biocides the German Ministry of Food, Agriculture and Consumer Protection applied, by default, a MRL of 0.01mg/kg in fruits (Eurofins, 2012).

On 13 July 2012, the Standing Committee on the Food Chain and Animal Health (SCFCAH) of the Directorate General of Health and Consumers of the European Commission (DG SANCO) published guidelines (SCFCAH, 2012), indicating that food and feed of animal or plant origin with a DDAC level higher than 0.5 mg/kg (provisional limit) should not be marketed, and should be withdrawn and removed safely, pending a subsequent decision from the SCFCAH.

Subsequently, the European Fresh Products Association (FRESHFEL) warned that in a study carried out in Germany, considerable amounts of residues from these DDAC and BAC compounds were found in food of animal and plant origin, both from countries in the European Union and countries that do not belong to it.

It should be highlighted that after the study, the EU published the Commission Regulation (EU) No 1119/2014 of 16 October 2014, which includes a maximum limit of 0.1 mg/kg for these compounds in food (EU, 2014).

2. Materials and equipment

2.1 Reference Materials

The reference material certified for DDAC with the brand Dr. Ehrenstorfer and a certified purity value of 88 % ± 2.0 % were used, as well as reference materials for BAC 12, 14 and 16 with the brand Aldrich and an uncertified purity of 99 %. From them reserve solutions of 1 mg/ml were prepared in methanol. Using these solutions, working solutions were prepared in acetonitrile of different concentrations.

2.2 Equipment

- Mechanical stirrer.
- Balance scale and gram scale.
- Centrifuge including 2 500 rpm.
- Column: Phenomenex SYNERGI MAX-RP 4m 150 mm x 2.00 mm.
- Guard column: Phenomenex MAX-PP.

- LC-MS/MS chromatography equipment containing the following modules: ALLIANCE equipment (injector, column, column oven) (WATERS), Q-MICRO (WATERS) detector and data processing system.
- Evaporation system with thermostatic bath and nitrogen stream.

2.3 Reagents

- Formic acid (minimum purity of 95 %).
- MilliQ water.
- Sodium citrate dibasic sesquihydrate (minimum purity of 98 %).
- Sodium citrate tribasic dihydrate (minimum purity of 99 %).
- Sodium chloride (minimum purity of 99.5 %).
- LC-MasScan methanol.
- PSA: Primary secondary amine.
- Magnesium sulphate (minimum purity of 98 %) treated in a muffle furnace at 650 ºC over 12 h.
- Mixtures of salts (preparation for the analysis of 10 samples) (Tables 1 and 2):

Table 1. First salt mixture (g)

Matrix	Anhydrous magnesium sulphate	Sodium chloride	Sodium citrate dihydrate	Sodium citrate dibasic sesquihydrate
Animal origin	40.0 ± 2.0	10.0 ± 0.5	10.0 ± 0.5	5.00 ± 0.25
Plant origin	48.0 ± 2.4	12.0 ± 0.6	12.0 ± 0.6	6.0 ± 0.3

Table 2. Second salt mixture (g)

Matrix	PSA	Anhydrous magnesium sulphate
Animal origin	2.1 ± 0.1	12.7 ± 0.6
Plant origin	2.40 ± 0.12	14.5 ± 0.7

3. Method

3.1 Extraction

This was carried out using the QuEChERS method (Annastassiades et al., 2003). Simultaneously to the analysis of a sample, we analysed a reagent blank and a fortified sample with a working solution at the desired concentration (at the determination limit of the compounds analysed).

- For matrices of animal origin: the same procedure as that indicated in the previous paragraph (QuEChERS method) with the following variations: 6.50 g ± 0.05 g of the first salt mixture and 1.50 g ± 0.05 g of the second salt mixture were added.
- For matrices of plant origin: in a centrifuge tube that includes a ceramic homogeniser, 12.00 g ± 0.05 g of homogenised sample were weighed and 12 ml of acetonitrile was added. 7.80 g ± 0.05 g of the first salt mixture was added, stirring manually for 1 min. It was centrifuged for 5 min at 2 500 rpm, collecting

the supernatant and transferring it to another centrifuge tube to which 1.69 g ± 0.05 g was added from the second salt mixture. It was stirred and centrifuged again. 3 ml of the supernatant was taken which evaporated to dryness. The extract was re-dissolved 1 ml of acetonitrile mixture: water (9:1) and was filtered through a PVDF sample filter. 25 µl of this extract was injected in the LC-MS/MS.

3.2 Instrumental analysis

3.2.1 Chromatographic conditions

- Mobile phase A: water with 0.1 % formic acid.
- Mobile phase B: acetonitrile with 0.1 % formic acid.

Table 3. Gradient

Minutes	Mobile phase A (%)	Mobile phase B (%)	Curve
0	97	3	1
20	20	80	6
26	10	97	3
27.5	3	97	3
29	97	3	6
42	97	3	6

3.2.2 MS/MS conditions

Table 4. MS/MS conditions

Compound	Molecular ion	Transition 1	Cone voltage	Collision energy	Transition 2	Cone voltage	Collision energy
DDAC	326.3	186	50	25	57	50	40
BAC 12	304.2	90.6	40	26	212.3	40	20
BAC 14	332	90.5	38	25	240.3	28	22
BAC 16	360.3	90.5	35	50	268.4	35	43

4. Validation

4.1 Matrices used

A complete validation was carried out in four matrices of plant origin (lettuce, orange, pear and tomato), as well as a complementary validation in two matrices (potato and cabbage) and in six matrices of animal origin: chicken meat, pangasius fillets, egg, milk, cream and honey.

4.2 Levels of fortification

Table 5. Levels of fortification (mg/kg)		
Compound	**First level (LOD)**	**Second level**
BAC 12	0.01	0.12
BAC 14	0.01	0.12
DDAC	0.01	0.12
BAC 16	0.01	0.12

4.3 Linearity

This was calculated from the calibration lines for each compound.

In each analytical series a calibration line was made with at least three points obtained by linear regression, adjusting for the method of least squares. In all cases the criteria used for evaluating linearity were met:

- Coefficient of determination of the calibration curve $R^2 \geq 0.990$.
- Coefficient of linearity of the calibration curve $C_m \geq 92$ %.

Four of the calibration points were prepared with all the reference material of the study components and on each of the matrices, with the first point of the curve being the LOD (limit of determination) of each compound.

4.4 Accuracy

This is expressed as the recovery percentage. Acceptance-rejection criteria are established prior to the validation of 60-120 % mean accuracy (See Tables 7 and 8).

4.5 Precision

This is expressed as a coefficient of variation (CV). Acceptance-rejection criteria are established prior to the validation that, depending on the accuracy obtained, are as follows (Table 6) (See Tables 7 and 8):

Table 6. Acceptance-rejection criteria	
Mean accuracy (%)	**Precision CV (%)**
70-120	25
60-70	15

4.6 Results

Table 7. Samples of plant origin. Accuracy and precision study

Compound	LOD (mg/kg)	Pear		Tomato		Lettuce		Orange		Cabbage		Potato	
		Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)
BAC 12	0.01	104.0	10.3	105.0	1.6	100.7	1.3	109.1	8.1	102.5	7.5	112.7	0.9
BAC 14	0.01	109.0	14.0	104.6	1.6	100.7	1.3	107.8	7.3	105.1	7.9	105.5	3.4
DDAC	0.01	107.4	12.7	99.9	5.1	95.2	8.7	109.1	8.1	98.6	10.4	108.0	1.9
BAC 16	0.01	104.0	3.9	104.2	3.8	101.4	8.1	106.2	5.7	100.2	7.4	101.0	9.9

Table 8. Samples of animal origin. Accuracy and precision study

Compound	LOD (mg/kg)	Egg		Milk		Pangasius		Chicken		Cream		Honey	
		Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)	Mean R (%)	CV (%)
BAC 12	0.01	112.6	8.7	107.9	14.2	109.4	4.8	111.2	7.1	93.3	4.9	74.0	16.0
BAC 14	0.01	111.2	7.6	106.3	16.2	109.5	5.5	109.3	5.2	95.2	2.1	79.1	13.7
DDAC	0.01	110.1	11.5	103.8	13.6	108.7	9.9	108.5	9.6	88.2	8.1	105.4	6.1
BAC 16	0.01	105.2	7.6	97.9	9.3	103.1	4.9	103.0	4.7	90.5	6.5	90.8	4.7

4.7 Uncertainty of the results

Under the recommendations in the SANCO/12571/2013 document, uncertainty of 50 % is accepted (SANCO, 2013).

4.8 Confirmation

Two different transitions of each molecular ion were selected and the ion ratio values of the reference materials were compared with those of the samples, with a deviation being accepted for values in accordance with the following table of tolerances of the SANCO (2013) document:

Table 9. Table of tolerances	
Relative intensity (% of base peak)	**Tolerance**
0.50-1.00	±30 %
0.20-0.50	±30 %
0.10-0.20	±30 %
<0.10	±30 %

4.9 Results of the validation

The results obtained for the validation parameters meet the abovementioned required criteria, and as such the validation is considered to be correct and the analytical method is considered as fit for purpose.

5. Applicability

The applicability of the validated analytical method has been confirmed both in samples of animal origin (different types of fish: perch, halibut, trout, panga) and in samples of plant origin (fruit and vegetables), with satisfactory results.

References

Annastassiades, M., Lehotay, S.J., Stajnbaher, D. and Schenck, Fj. (2003). Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) approach for the determination of pesticide residues. Journal of AOAC International, 86, pp: 412-413.

EU (2014). Commission Regulation (EU) No 1119/2014 of 16 October 2014 amending Annex III to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for benzalkonium chloride and didecyldimethylammonium chloride in or on certain products. OJ L 304, 23 October 2014, pp: 43-74.

Eurofins (2012). Global control GMBH. Grossmoobogen 25 Hamburg

SANCO (2013). DG Health & Consumers. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed. SANCO/12571/2013.

SCFCAH (2012). Standing Committee on the Food Chain and Animal Health. Guidelines as regards measures to be taken as regards the presence of DDAC in or on food and feed agreed by the Standing Committee of the Food Chain and Animal Health (SCFCAH) on 13 July 2012.