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Liquid chromatography-tandem mass spectrometry multiresidue method for the analysis of quaternary ammonium compounds in cheese and milk products: Development and validation using the total error approach

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ABSTRACT

Quaternary ammonium compounds (QACs) are both cationic surfactants and biocidal substances widely used as disinfectants in the food industry. A sensitive and reliable method for the analysis of benzalkonium chlorides (BACs) and dialkyldimethylammonium chlorides (DDACs) has been developed that enables the simultaneous quantitative determination of ten quaternary ammonium residues in dairy products below the provisional maximum residue level (MRL), set at 0.1 mg kg^{-1}. To the best of our knowledge, this method could be the one applicable to milk and to three major processed milk products selected, namely processed or hard pressed cheeses, and whole milk powder. The method comprises solvent extraction using a mixture of acetonitrile and ethyl acetate, without any further clean-up. Analyses were performed by liquid chromatography coupled with electrospray tandem mass spectrometry detection (LC-ESI–MS/MS) operating in positive mode. A C18 analytical column was used for chromatographic separation, with a mobile phase composed of acetonitrile and water both containing 0.3% formic acid; and methanol in the gradient mode. Five deuterated internal standards were added to obtain the most accurate quantification. Extraction recoveries were satisfactory and no matrix effects were observed. The method was validated using the total error approach in accordance with the NF V03-110 standard in order to characterize the trueness, repeatability, intermediate precision and analytical limits within the range of 5–150 μg kg^{-1} for all matrices. These performance criteria, calculated by e.noval® 3.0 software, were satisfactory and in full accordance with the proposed provisional MRL and with the recommendations in the European Union SANTE/11945/2015 regulatory guidelines. The limit of detection (LOD) was low (<1.9 μg kg^{-1}) and the limit of quantification (LOQ) ranged from 5 μg kg^{-1} to 35 μg kg^{-1} for all matrices depending on the analytes.

The validation results proved that the method is suitable for quantifying quaternary ammoniums in foodstuffs from dairy industries at residue levels, and could be used for biocide residues monitoring plans and to measure the exposition consumer to biocides products.

1. Introduction

Quaternary ammonium compounds (QACs) are surfactant substances containing a quaternary cationic nitrogen atom. Their structure and properties differ according to the nature of the radicals attached to the nitrogen atom. QACs are widely used for their biocidal properties as plant protection products [1,2] or wood preservatives [3], being both bactericidal and antimicrobial [4–7]. They are also commonly used as disinfectants and detergents in industrial and commercial formulations [8–10].

In the food industry, cleaning-disinfection (CD) processes are essential to eliminate microorganisms, and chemicals such as QACs are typically used. In the event of poor cleaning practices, especially in the event of insufficient rinsing with water, residues of these compounds can persist on food surfaces and thus become a potential source of contamination for food. The presence of biocide residues on surfaces and their subsequent transfer onto and

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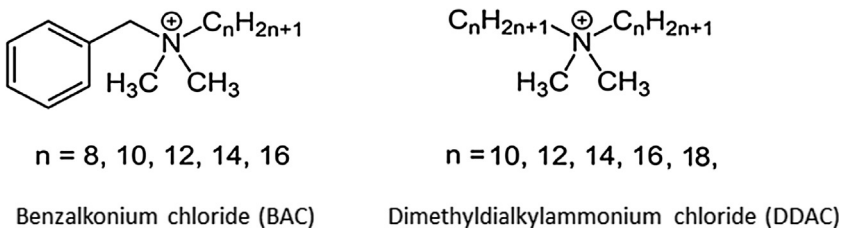

Fig. 1. Molecular structures of the BACs and DDACs analyzed.

into food could be potentially harmful to the consumer. It is therefore important to have a reliable analytical method with which to quantify the concentration level of residues in food. This study was thus designed to develop a sensitive, specific and accurate analytical method using LC–MS/MS to measure QACs in milk products, as these compounds are widely used to disinfect food contact surfaces in the dairy industry.

Among QACs, benzalkonium chloride (BAC) is one of the most commonly used in disinfectant products [7,11,12]. BAC is a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl group has a chain length from C8 to C16 (Fig. 1). In commercial formulations, the most commonly used homologues are BAC-C12, BAC-C14 and BAC-C16 in different proportions. The BAC mixture is advantageous since each homologue has its own biocidal properties [13], for example against fungi and yeast (BAC-C12), gram-positive bacteria (BAC-C14) and gram-negative bacteria (BAC-C16).

Another important class of QACs, very often mixed with BACs is dialkyldimethylammonium chloride compounds (DDACs). DDAC homologues vary in the two *n*-alkyl chain length, *n* representing an even number of carbons from C10 to C18. The most commonly encountered and used homologue, at more than 90%, is didecyldimethylammonium chloride (DDAC C10) [14]. Like BACs, DDACs are used in the food industry for disinfecting surfaces and equipment. Although regulation [15] defines BAC as a mixture of alkylbenzyldimethylammonium chlorides with alkyl chain lengths of C8 to C18, and DDAC in the form of a mixture of alkyl-quaternary ammonium salts with alkyl chain length of C8, C10 and C12; this study focuses on a multiresidue method including BAC C8 to C16 and DDAC C10 to C18 (Fig. 1) since the targeted analytes are those that can be found in commercial disinfectant formulations.

Knowing that QACs are used in various fields, it is legitimate to wonder whether they may affect human health. Occupational and domestic pathologies have been observed due to their use as disinfectants and detergents, which exposes users to their irritant and allergenic effects [16]. The harmful effects encountered are mainly dermatological (such as eczema), ocular (lacrimation, stinging) and/or respiratory (such as asthma) [17,18]. These cases are widespread among hospital, agricultural and maintenance staff.

Under current European legislation, these substances are currently covered by Biocidal Products Regulation (BPR) 528/2012/EU [19], which repeals Biocidal Products Directive 98/8/EC [20] concerning the placing on the market and use of biocidal products, designed to protect both the consumer and the environment. Given the frequent use of this chemical family in the food industry, a risk of contamination into food could not be ruled out, which led Germany to alert the European Commission. Accordingly, the results of a European survey led to the definition of a provisional MRL set at 0.1 mg kg^{-1} for application from August 2015 [14,15]. This provisional MRL is scheduled to be reviewed in December 2019.

Because of the broad use of QACs (mostly BACs and DDACs), and considering that there is a high probability of finding them in food following transfer from food surfaces, we considered it important to focus on the analytical measurement of these compounds in dairy products. Indeed, the application of QACs during the cleaning and disinfection of surfaces throughout the dairy production chain, even if associated with rinsing steps, may lead to residues of these substances being left on industrial surfaces that may in turn contaminate dairy products. To address this issue, a reliable method needs to be developed in order to detect and quantify QACs at low concentration levels in cheese and milk products, in accordance with the provisional MRL (0.1 mg kg^{-1}). This one could allow to determine the contamination level in dairy products in the aim of risk assessment and to measure the consumer exposition to biocides products.

A review of the literature showed that various instrument techniques could be used to analyze QACs depending on the structure of the analytes studied.

Some authors used capillary electrophoresis to analysis QACs and more precisely the BACs [21–25]. A recent study [26] shows the interest of using potentiometric sensor technique for the determination of BACs used as preservatives in pharmaceutical formulations.

Gas chromatography (GC) is used to a lesser extent for the analysis of quaternary ammoniums [27,28]. In the case of analysis by GC, a dequaternization step is necessary and a loss of information is ensured [9].

Liquid chromatography with ultraviolet (UV) detection was commonly used to determine BACs in various fields [3,4,6,7,10]. LC-UV may be used to analyze BACs because they contain a benzene ring [6,9,10]. However, this technique cannot be used for multiresidue analysis of QACs, since DDACs do not contain any chromophore groups. Furthermore, LC-UV analysis lacks specificity and selectivity since interference linked to co-extracted components may affect the analysis of BACs, as noted by Miyauchi et al. [3]. More recently, mass spectrometry (MS) and tandem mass spectrometry (MS/MS) detection have been used for analyzing QACs in commodities as diverse as fruit, vegetables and milk [1,2,11,12,29–35] because of their high selectivity and specificity based on molecular ion information, which offers unequivocal identification of the compound in samples.

Concerning QACs separation, reverse phase chromatography was commonly used with a predominance of C18 phase [1–3,7,8,12,30–32,35]. However, cyano column [4–6,9] have been used mainly for the analysis of BACs, and more recently HILIC column was also used [33,34].

Several of instrumental methods have been developed for the separation of BACs and DDACs using liquid chromatography with binary gradient mode (e.g. Water/ACN or Water/MeOH) [1–6,10–12,29–35]. Most authors add acid solution (acetic or formic) in the eluents, and some add also buffer solution (acetate or formate) [1–3,29,31–34] whose the addition achieve to improve the separation.

The objective of our work was to develop and validate a multiresidue method for the simultaneous quantitative determination of five BACs and five DDACs in complex dairy product extracts, namely raw milk, milk powder, hard pressed cheese and processed cheese. Special attention was paid to the efficiency of the extraction method, i.e. minimizing the number of steps and interactions between the matrix and analytes. Validation was conducted with the concept of total error to determine the performance character-