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Simultaneous determination of aminoglycosides and colistins in food

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ABSTRACT

A novel method for the simultaneous identification and quantification of twelve aminoglycosides (AGs) and two colistins in meat and bovine milk has been developed. The analysis was carried out using liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry (LC-Q-Orbitrap). Among the HILIC (Hydrophilic Interaction Liquid Chromatography) stationary phases tested, the bare silica Poroshell 120 provided the best results. The samples were extracted with an aqueous solution followed by an SPE clean up based on the weak cation exchange mechanism. The validation study was performed carrying out 72 experiments per matrix at six different concentrations in a range encompassing the Maximum Residue Limits. The recoveries were from 72 to 87% in meat (except colistins) and from 82 to 96% in milk. Repeatabilities and intra-lab reproducibilities were lower than 10 and 15%, respectively. Limits of detection were lower than or equal to 33 μg kg^{-1}. Finally, test materials containing AGs prepared for interlaboratory studies were successfully analysed.

1. Introduction

Aminoglycosides (AGs) are multifunctional hydrophilic sugars that possess several amino and hydroxyl functionalities (Fig. 1). They are widely used in veterinary practices as antibiotics against bacteria and parasites in pork, chicken and beef production. Maximum Residue Limits (MRLs) have been set by Regulation 37/2010 in various animal-origin food (European Regulation, 2010). Due to high polarity of their structure with multiple ionization sites, it is well known that AGs are difficult to extract from complex matrices and are poorly retained on reversed-phase columns. As consequence, their determination at trace levels has always been an analytical challenge also considering that, in the past, the lack of chromophores and fluorophores forced to derivatize AGs to allow sensitive and specific detection (Farouk, Azzazy, & Niessen, 2015, McGlinchey, Rafter, Regan, & McMahon, 2008). Fortunately, today the widespread application of mass spectrometric analysers enable their detection without any derivatization step. Reviewing multiresidue procedures to determine AGs in food with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), the oldest published papers are those of Kaufmann and Maden (2005) and Zhu and coworkers (Zhu, Yang, Wei, Liu, & Zhang, 2008). Kaufmann and Maden (2005) described a protocol able to determine eleven AGs in meat with three solid-phase extraction (SPE) steps prior to LC injection. Zhu et al. (2008) carried out two consecutive SPE purification steps to analyze thirteen AGs in various food types. Although these procedures were effective, they were not efficient being complicated, time-consuming and hard to be operate. In the last ten years, simpler sample treatments have been proposed. They were substantially based on the extraction of AGs with an aqueous solution containing trichloroacetic acid and Na_2EDTA with or without other salts such as sodium chloride or ammonium acetate. The extract was then purified applying the SPE technique. Three kinds of SPE sorbents are used: i) weak cation exchange (WCX); ii) reversed-phase (RP); iii) MIP (molecularly imprinted polymer). Ishii, Horie, Chan and MacNeil (2008), Kumar, Rubies, Companyó and Centrich (2012), Tao et al. (2012), Lehotay et al. (2013), Diez et al. (2015) and Asakawa, Uemura, Sakiyama, and Yamano (2017) used SPE clean-up based on weak cation-exchange mechanism, whereas Bohm, Stachel and Gowik (2013), Alechaga, Moyeno and Galceran (2014) and Bazzan Arsand et al. (2016) applied reversed-phase SPE. Finally, a very recent approach introduced MIP SPE to purify AGs from complex matrices (Moreno-Gonzales, Hamed, Gracia-Campaña & Gámiz-Gracia, 2017; Yang, Wang, Chunying, Xixi, & Chengjun, 2017) (Table S1 - Supplementary Material). With regards to the chromatographic approach, the classical way to separate very polar compounds on reversed stationary phases with the addition of ion-pairing reagents has been widely applied (Kaufmann & Maden, 2005; Zhu et al., 2008; Tao et al., 2012; Kaufmann, Butcher, & Maden, 2012; Lehotay et al., 2013; Bazzan Arsand et al., 2016). On the other hand, in

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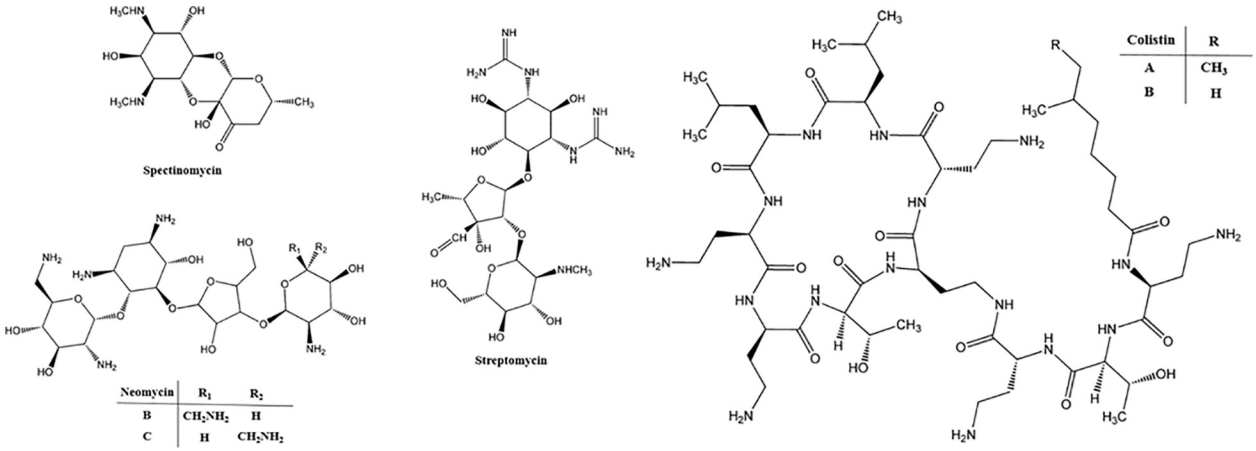

Fig. 1. Chemical structures of aminoglycosides and colistins.

the last ten years the hydrophilic interaction chromatography (HILIC) has been a valuable alternative for the analysis of AGs (Ishii et al. 2008; Kumar et al., 2012; Bohm et al. 2013; Alechaga et al. 2014; Diez et al., 2015; Asakawa et al. 2017; Moreno-Gonzales et al. 2017; Yang et al., 2017). Unlike normal-phase chromatography, HILIC works well with typical RP mobile phases avoiding the use of solvents immiscible with water. The HILIC retention mechanism primarily involves the partitioning of polar analytes between a water-enriched layer of solvent near the sorbent surface and the slightly more hydrophobic bulk eluent (typically acetonitrile). In addition to hydrophilic partitioning, other separation mechanisms are involved, such as hydrogen-bonding, ion exchange or dipole-dipole interactions. The great advantage is that HILIC columns are fully compatible with the MS detectors and, with respect to the traditional reversed-phases, better sensitivity can be obtained since the use of fluorinated ion-pairing reagents can be completely avoided overcoming the well-known problems of ion suppression and frequent maintenance of the LC system. In 2010, Gremilogianni, Megoulaus and Koupparis demonstrated that a method based on HILIC for the determination of streptomycin and dehydrostreptomycin in milk reached lower detection limits when compared to an analogous procedure, which instead used reversed-phase ion pairing chromatography (Gremilogianni, Megoulaus and Koupparis, 2010).

Colistin A (or polymyxin E1) and colistin B (or polymyxin E2) belong to the antibiotic class of polymyxins, a group of cationic polypeptides (Fig. 1). They have been mainly used against the infections caused by *Enterobacteriaceae* in pigs, poultry, cattle, sheep, goats and rabbits and MRLs have been fixed in several matrices. Recently, their administration in farm has been restricted because of the evidence of antibiotic-resistance cases (European Medicines Agency, 2016), further increasing the interest about the development of efficient analytical methods for their determination at trace levels. However, similarly to AGs, colistins are polar drugs and therefore they are not easily extractable from tissues forming bonds with proteins or phospholipids (Kunin, 1970). For this reason, few methods have been developed so far for the determination of polymyxins in food. Before 2000s, these drugs have been mainly analyzed after derivation to introduce chromophore or fluorophore groups enabling the analysis with the traditional LC detectors. The first paper using LC-MS/MS was published by Sin, Ho, Wong, Ho, and Ip (2005). These authors described a method for the determination of colistin A and bacitracin in milk, liver and kidney. Later, the same research group (Wan, Ho, Sin & Wong, 2006) proposed an improved procedure in milk, liver and muscle including colistin B, too. More recently, Xu et al. (2012) developed an analytical protocol for colistin A and B specifically suited for fishery products. Kaufmann and Widmer (2013) published a multiresidue method for five polymyxins in various food, whereas Boison, Lee and Matus (2015) included in the method scope seven polymyxins in chicken muscle. All the aforementioned works shared the same sample treatment strategy, that is an acidic extraction with a mixture of water, methanol or acetonitrile in various proportions followed by a reversed-phase SPE (mainly with polymeric sorbents) to reduce the amount of interfering substances. The reported chromatographic approach to separate polymyxins has been the ion-pair reversed-phase LC (Table S2 - Supplementary Material).

Some structural similarities between AGs and colistins such as high polarity and the presence of several amino-groups suggested that there could be a chance to optimize a common analytical strategy for the both drug classes. Therefore the aim of this work has been the development and validation of a quantitative multiclass method for the simultaneous determination of twelve aminoglycosides (amikacin, apramycin, dihydrostreptomycin, gentamycin C1, gentamycin C1a, gentamycin C2 + C2a, kanamycin A, neomycin B, paromomycin, spectinomycin and streptomycin) and two colistins (A and B) in meat and bovine milk. The determination was performed on an LC system coupled to a hybrid quadrupole-orbitrap analyser (LC-Q-Orbitrap) using a HILIC analytical column to separate the fourteen drugs.

2. Experimental

2.1. Chemical and reagents

Acetonitrile (ACN) (LC-MS CHROMASOLV®) was supplied by Fluka (St. Louis, MO, USA). Formic acid 99% for LC-MS and ammonium formate were obtained from VWR International Ltd (Lutterworth, UK). EDTA sodium salt dihydrate and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Polymeric Oasis WCX (150 mg/6 mL), Sep-Pak® Accell™ Plus CM SPE cartridges (500 mg, 6 mL; 46 μm) with silica support were provided by Waters (Milford, MA, USA). Deionized water was HPLC grade generated by a Milli-Q purification system (Millipore, Molsheim, France). Amikacin (100%), apramycin (> 95%), colistin A (and colistin B (> 75%), dihydrostreptomycin (≥98%), kanamycin A (> 90%), neomycin B (≥70%), paromomycin (≥98%), ribostamycin (≥70%), spectinomycin (≥98%) and streptomycin (≥95%) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gentamycin C1 (≥95%), gentamycin C1a (≥90%) and gentamycin C2 + C2a (≥95%) were obtained from TRC Inc. (Toronto, Canada).

Extraction solution for muscle: 10 mM ammonium acetate, 0.4 mM EDTA, 0.5% NaCl and 2% TCA.

2.2. Apparatus

A refrigerated centrifuge from Eppendorf (Hamburg, Germany, DE), a 12-position SPE manifold from Macherey-Nagel (Düren, Germany, DE), a knife mill from Retsch (Haan, Germany, DE), an ultrasonic bath