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Review

Determination of penicillins in milk of animal origin by capillary electrophoresis: Is sample treatment the bottleneck for routine laboratories?

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ABSTRACT

Capillary electrophoresis (CE) is increasingly being used not only for research purposes but also for routine analyses. The latter, however, are especially difficult when the analytes are present at very low concentrations in complex food samples (e.g. penicillins in milk of animal origin). No study of the difficulties encountered in daily practice in sample treatments for the determination of penicillins (PENs) in milk by CE has to our knowledge been reported. Rather than reviewing the main uses of CE for determining PENs in different types of samples, this paper focuses on the weaknesses of available methods for this purpose, which originate in sample treatment rather than in a lack of robustness of the CE technique. Some problems which, based on our own experience, often confront sample treatment and method development in this context are discussed here. Clearly, the greatest source of error in this context is sample processing, which must provide optimal extraction and preconcentration of analytes, and extracts compatible with the separation technique to be used. In this respect, using time-consuming procedures can cause the loss of variable amounts of analytes in different steps. Interestingly, dramatically simplifying the sample preparation process can detract from sensitivity but lead to increased recoveries. As with any methodological development in routine analysis, acceptable results can only be obtained by considering all potentially influential factors.

Contents

1. Introduction

The accurate determination of antibiotics and their metabolites in food samples is critical not only for their quality control, but also to assure public health. In fact, these substances can cause the development of antibiotic-resistant bacteria—which are more difficult to destroy than the original strains—and allergic reactions or be directly toxic [1]. Although, analytically, antibiotics are usually separated by HPLC, capillary electrophoresis (CE) is being increasingly used for this purpose as confirmed by the more than 1200 papers on this topic published in recent years and several state-of-the-art reviews on the use of CE with antibiotics [1,2].

The most important group of antibiotics for human and veterinary medicine is that of β-lactams, which include penicillins

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(PENs) and cephalosporins, and have been widely used as antimicrobial drugs for more than 80 years [3]. The main use of these antibiotics in the dairy industry is to destroy the pathogens behind mastitis, a disease which causes considerable economic losses [4]. Penicillins account for more than one-third of the total antibiotic production [5] and their widespread use has raised the need for tighter controls. To ensure human food safety, many countries including the United States and those of the European Union (EU) have set definitive maximum residue limits (MRLs) for potentially toxic substances in food products. A need for analytical methods allowing the presence of such substances at levels below their MRLs to be detected therefore obviously exists.

A large number of analytical methods for determining and screening PENs have been developed lately [6]. An interesting review of the monitoring of PENs in food samples by CE describing the potential of the electrophoretic technique for detection and quantitation of PENs was published in 2009 [7]. Few routine applications of CE to real food samples, however, to date have been developed owing to (a) the very small sample volumes used in CE (a few nanoliters), which can have an adverse impact on precision; (b) the low sensitivity of the technique, which is a result of the low volume loadability of capillaries during continuous detection [8]; and (c) incompatibility between some samples and CE methods [9]. These shortcomings have been circumvented by developing new approaches to improving sensitivity, selectivity and robustness in CE (see Fig. 1).

CE is known to have limited sensitivity when used with the UV technique owing to the short optical path length available with in-capillary detection. This has promoted the use of alternative techniques such as laser induced fluorescence, electrochemical, chemiluminescence, electrochemiluminescence and mass spectrometry (MS) detection, which are all more sensitive than classical UV–vis detection. Other, novel techniques including contactless conductivity detection (C^4D) and potential gradient detection (PGD) have also been used for this purpose. Also, in-chip CE has attracted much interest in recent years; for example, chip-based microfluidic systems have been used to determine antibiotics [1]. In any case, PENs are most often determined with a UV or MS detector, which are the best suited to their structure and chemical properties.

Although a number of interesting methods testifying to the analytical usefulness of CE for determining PENs currently exist, few—only five—have been used to extract these analytes from milk samples. This may have contributed to the little acceptance of CE for routine food analyses involving the determination of antibiotics.

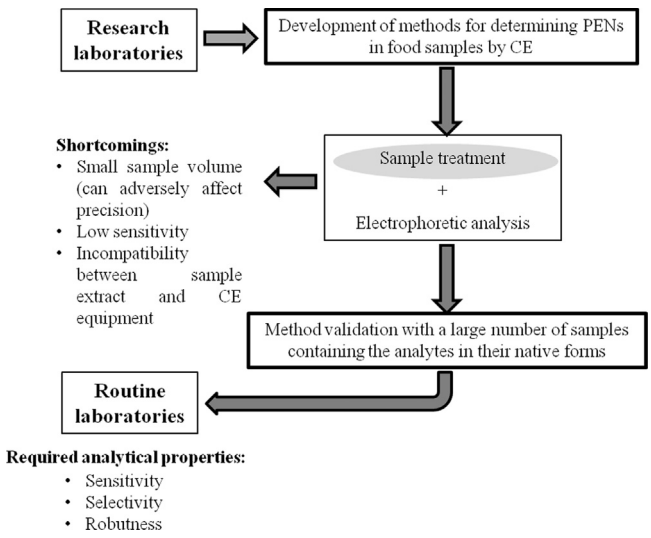

Fig. 1. Shortcomings of sample treatment and required analytical properties for the routine CE analysis of PENs in food samples.

The main difficulty in determining PENs in complex samples such as milk lies in their extraction from the matrix. This step can be the bottleneck of routine analytical methods. Several sample pretreatment steps are required in most cases to extract and preconcentrate the analytes. In fact, some food matrix components such as saline constituents, macromolecules and other major compounds can disturb CE separations. In addition, particulate matter can easily clog a CE system [10]. For these reasons, food samples often require especially complex treatments prior to analysis by CE.

This paper is not a mere review of CE methods for determining PENs in milk; rather, it primarily aims at highlighting the weaknesses of existing methods for this purpose, the greatest of which is sample treatment rather than the characteristics of the CE technique (e.g. its robustness).

2. CE methodologies for the determination of PENs

Research groups worldwide have developed a number methods for determining PENs in food samples. Such methods differ in accuracy, expeditiousness and cost. Most, however, fall into one of these four categories: (a) microbiological methods based on bacterial growth inhibition, (b) biosensing methods; (c) immunochemical methods; and (d) chromatographic or electrophoretic methods. The advantages and drawbacks of these methods, and specific aspects of the determination of PENs with them, are discussed elsewhere [11].

The analytical methods for determining PENs endorsed by the EU (Commission Decision 2002/657/EC) are based on chromatographic techniques and/or analytical molecular spectrometry. However, the EU has stated that regulatory laboratories must find the optimum analytical techniques for determining pharmacological substances, so other methods are expected to be adopted in the future if they prove suitable for the intended purpose [12].

Although PENs are usually separated by HPLC for their subsequent determination, CE is being increasingly used for this purpose by virtue of its high efficiency and simplicity, short analysis times and low consumption of samples and reagents. Also, CE is being increasingly used in routine pharmaceutical and clinical analyses on the grounds of its acceptable analytical performance and good quantitative results. The determination of PENs by CE can be approached in two may ways, namely: (a) by capillary zone electrophoresis (CZE), where a separation buffer with or without additives is used to determine ionic antibiotics by their differences in electrophoretic mobility; and (b) by micellar electrokinetic chromatography (MEKC), where a micellar system (usually a surfactant at a concentration exceeding its critical micelle concentration) is added to the separation buffer to effect the separation of neutral and/or ionic antibiotics by generating a pseudostationary phase for the analytes to partition [13]. CZE (46%) and MEKC (36%) are the preferred separation modes for PENs, but microemulsion electrokinetic capillary chromatography (MEEKC) (11%), cyclodextrin electrokinetic chromatography (CD-EKC) (3.5%) and nonaqueous capillary electrophoresis (NACE) (3.5%) have also been used for this purpose.

Several methods for determining PEN residues by CE have been reported in recent years, [14–39]. Table 1 lists them in chronological order from the most recent to the oldest and shows their experimental conditions (background electrolyte composition, capillary conditioning, temperature, injection pressure and time, voltage, detection system, analysis time and CE instrument used). As can be seen, most PENs were separated by using a borate and/or