Antibiotic analysis using Electro-Filtering Paper Spray Ionization

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ABSTRACT

In this work, we analyzed the performance of the Electro-Filtering Paper Spray Ionization (EFSI) method for detecting compounds in unprocessed samples. A relatively rigid and electrically conductive copper filter was used as a substrate to insure sufficient and efficient sample-solvent extraction and to increase the conductivity for paper spraying. The method was demonstrated as applicable for indirect high-throughput analysis of large-volume unprocessed samples, which is not possible with conventional nanoESI or direct paper spray methods. The new method can generate different desired ion signals for a wide range of compounds by selecting different extraction solvents. Moreover, key parameters related to extraction efficiency were optimized in detail to obtain the most satisfactory extraction efficiency during antibiotic analysis. Finally, under optimal conditions, the EFSI method was successfully used to detect four antibiotics in animal products of egg, chicken, and chicken liver, exhibiting good reproducibility with calibration curves between 81.6% and 96.3%, and R^2 values above 0.99. Recoveries of 75.0%−94.6% were obtained for the four antibiotics. Hence, the proposed EFSI-MS is a successful, economical, rapid, and high-throughput method that is effective for both unknown and targeted extraction of unprocessed samples by mass spectrometric analysis.

1. Introduction

Antibiotic resistance is a major global concern due to their widespread and illegal use as feed additives for livestock [1,2]. The contamination of residue by antibiotics has considerable impacts, including potential antibiotic resistance in many human pathogenic bacteria [3], the risk of growth-promotion in animals [4], and problems of environmental pollution [5]. Moreover, antibiotic contamination in animal products or the environment would accumulate in the human body via the food chain, which represents a potential risk for human health, even at low concentrations [6]. For these reasons, the European Union (EU) has banned antibiotics as veterinary pharmaceuticals for livestock since 1 January 2006 [7]. However, to ensure food safety, a systematic, comprehensive, and high-throughput approach to the quality assessment and control of antibiotic contamination in animal products is essential.

The main analytical methods used for antibiotic determination are based on mass spectrometry (MS), and primarily include gas chromatography-mass spectrometry (GC-MS) [8] and high performance liquid high-performance spectrometry (HPLC-MS) [9,10]. Although they boast wide applicability, good accuracy, high sensitivity and selectivity, and reasonable qualitative and quantitative capabilities, tedious and time-consuming sample pre-treatment is required prior to instrumental analysis. Thus, these methods do not meet the requirements of simple, rapid and high-throughput profiling.

Nevertheless, MS is one of the most powerful techniques for chemical and biological analysis. Therefore, multiple different ionization sources have been developed, such as traditional capillary electrospray ionization (ESI) [11] and ambient ionization [12,13]. ESI is the most commonly used technology for solution samples, in which the sample solution is introduced and ESI processing is performed via a capillary under high voltage conditions. However, due to the typically small bore diameter of the capillary used for sample introduction, particular precautions should be taken to prevent clogging of the capillary with samples. As for complex samples, tedious and multi-step sample pre-treatment or chromatographic separation is also unavoidable.

Consequently, a variety of ambient ionization methods have emerged for direct sample analysis that involve minimal or no sample pre-treatment or chromatographic separation. Dominant ambient ionization methods include atmospheric pressure chemical ionization (APCI) [14], matrix-assisted laser desorption ionization (MALDI) [15], desorption electrospray ionization (DESI) [16], laser ablation electrospray ionization (LAESI) [17], direct analysis in real time (DART) [18], desorption atmospheric pressure chemical ionization (DAPCI) [19],
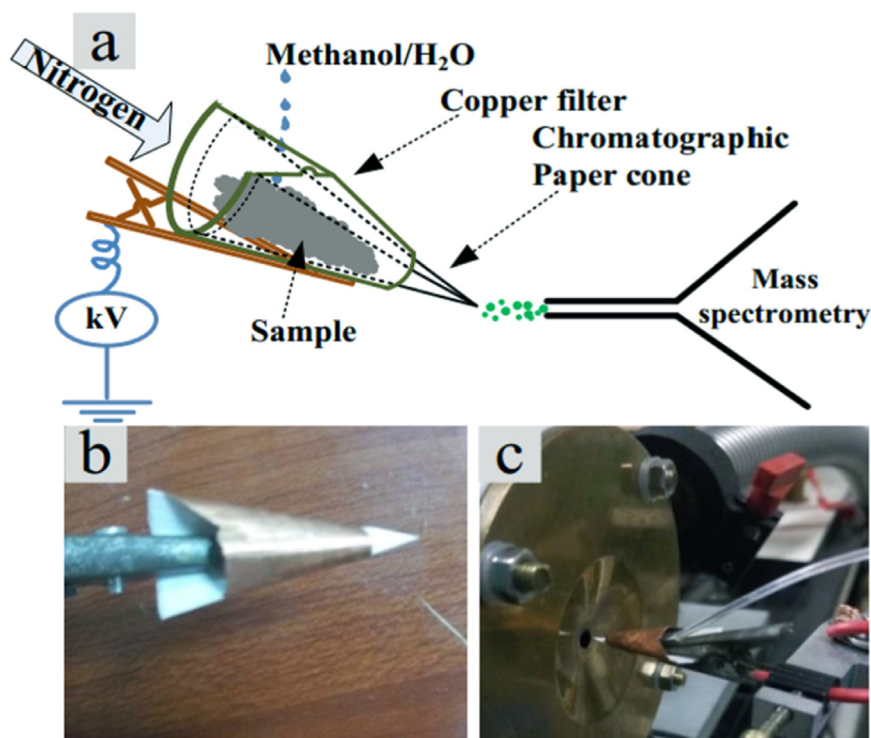

Scheme 1. Copper Electro-filtering spray ionization source: (a) illustration of the ionization source; (b) photograph showing Copper Electro-filtering spray ionization; (c) photograph of the entire ionization process.

internal extractive electrospray ionization (IEESI) [20], wooden-tip electrospray ionization [21], and paper spray electrospray ionization (PS) [22,23].

Among these, PS is an attractive method, first developed by Ouyang et al. [22,23], that possesses the features of both capillary-ESI and ambient ionization methods and allows sample analysis in their native environment; i.e. in the open air external to the mass spectrometer without the need for sample preparation or pre-separation. Samples are directly loaded onto a triangular piece of paper, a high voltage is applied at the base of the paper after wetting with a solvent, and a spray of charged droplets is formed by placing the paper tip in front of the mass spectrometer inlet. Moreover, owning to the specific characteristics of PS-MS, such as simplicity, high-throughput, speed, and cost-effective analysis, it has been widely used and successfully applied for determining a variety of compounds in biological samples, including urine [23], blood [24], and tissue [25]. However, for solid or powder samples, the weight of the sample should be considered to prevent paper deformation.

Unfortunately, for trace analysis, a small amount of sample cannot generate the required signal. Therefore, other ambient ionization methods derived from PS have been developed. For instance, leaf spray [26], paper cone spray [27], aluminum foil spray [28], and electro-filtering spray [29] are not only more convenient for solid sample loading, but also allow large amounts to be loaded and are more tolerant to salts or detergents. Moreover, ESI occurs on the surface, enabling sequential and exhaustive ionization of analytes. Thus, the above methods have attracted increasing attention in the solid sample analysis community in recent years.

In our previous research, metal Electro-Filtering Paper Spray Ionization (EFSI) was developed for the direct analysis of soil samples [29]. In this study, we determine the performance and application of the EFSI-MS method using a relatively rigid metal filter for direct analysis of unprocessed samples for both unknown and targeted monitoring. The rigid metal filter is a desirable substrate for a range of sample-solution extraction processing and increases conductivity

during paper spraying. Copper, aluminum, and iron metal filters are employed to evaluate ionization efficiency. This method is then implemented and evaluated for unknown compound analysis in various solid and powder samples. Moreover, the experimental conditions, including the types of paper spray, extraction solvent composition, solvent volume, and extraction frequency, are optimized to obtain the most satisfactory extraction efficiency when targeting compounds for antibiotic analysis. Finally, under these optimized conditions, we validate the method and analytical parameters by testing animal products (egg, chicken, and chicken liver) for four antibiotics: tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CLOR), and erythromycin (ERY).

2. Experimental

2.1. Materials

Reserpine and cytochrome C were purchased from Aladdin (L.A. USA). Formic acid (analytical reagent) and cesium iodide (CsI) were purchased from Shanghai Chemicals. Methanol and acetone (HPLC grade) were obtained from Merck (Darmstadt, Germany). Tetracycline (TC, M/Z = 445.5), oxytetracycline (OTC, m/z = 461.2), chlortetracycline (CLOR, m/z = 479.9), and erythromycin (ERY, m/z = 734.5) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The filter paper and weigh paper used for the electro-filtering paper spraying were purchased from Hangzhou Special Paper, and chromatography paper was purchased from Whatman International Ltd (Maidstone, England). The filter paper had three different pore sizes: 101 (20–25 µm), 102 (15–20 µm), and 103 (10–15 µm), and the thickness of chromatography paper was 0.18 mm.

2.2. Sample preparation

Standards of reserpine (1.0×10^{-5} mol L^{-1}) and cytochrome C (5.0×10^{-5} mol L^{-1}) were prepared by dissolution in a solvent of