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Analysis of trimethoprim, lincomycin, sulfadoxin and tylosin in swine manure using laser diode thermal desorption-atmospheric pressure chemical ionization-tandem mass spectrometry

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ABSTRACT

A new extraction method coupled to a high throughput sample analysis technique was developed for the determination of four veterinary antibiotics. The analytes belong to different groups of antibiotics such as chemotherapeutics, sulfonamides, lincosamides and macrolides. Trimethoprim (TMP), sulfadoxin (SFX), lincomycin (LCM) and tylosin (TYL) were extracted from lyophilized manure using a sonication extraction. McIlvaine buffer and methanol (MeOH) were used as extraction buffers, followed by cation-exchange solid phase extraction (SPE) for clean-up. Analysis was performed by laser diode thermal desorption-atmospheric pressure chemical-ionization (LDTD-APCI) tandem mass spectrometry (MS/MS) with selected reaction monitoring (SRM) detection. The LDTD is a high throughput sample introduction method that reduces total analysis time to less than 15 s per sample, compared to minutes when using traditional liquid chromatography (LC). Various SPE parameters were optimized after sample extraction: the stationary phase, the extraction solvent composition, the quantity of sample extracted and sample pH. LDTD parameters were also optimized: solvent deposition, carrier gas, laser power and corona discharge. The method limit of detection (MLD) ranged from 2.5 to 8.3 $\mu g\ kg^{-1}$ while the method limit of quantification (MLQ) ranged from 8.3 to 28 $\mu g\ kg^{-1}$. Calibration curves in the manure matrix showed good linearity ($R^2 \geq 0.996$) for all analytes and the interday and intraday coefficients of variation were below 14%. Recoveries of analytes from manure ranged from 53% to 69%. The method was successfully applied to real manure samples.

1. Introduction

In the past few decades, veterinary antibiotics have been widely used in swine breeding [1]. They have been administered routinely at therapeutic doses to prevent diseases, improve feed efficiency and accelerate growth, and as a result, huge quantities have been used for swine husbandry. However, all these antibiotics are not absorbed by the animals and a significant portion is excreted in the feces and urine and end up in the manure. Those antibiotics enter the environment through the land application of manure as organic fertilizer and can potentially contribute to bacterial resistance [2–4]. For several years, researchers have studied the anaerobic digestion of swine manure slurry [5]. They are now trying to understand the biodegradation of veterinary antibiotics in swine manure. Thus, robust analytical methods are necessary in order to quantitate and measure the degradation of these compounds. Various authors have dealt with the analysis of veterinary antibiotics in manure or other matrices (soils, wastewaters, animal meat, etc.) and they almost systematically use liquid chromatography (LC) before analysis by tandem mass spectrometry (MS/MS) [6–11]. This article explores an original analytical approach for the analysis of veterinary antibiotics in manure which switches from using a time consuming method based on LC-MS/MS (measured in minutes) with an ultrafast analytical method based on laser diode thermal desorption (LDTD) coupled to MS/MS (measured in seconds).

Most of the methods proposed in the literature for the analysis of antibiotics use LC and require time-consuming preparation steps such as solid phase extraction (SPE) with solid liquid extraction (SLE), followed by evaporation to dryness [6,12,13], and reconstitution in the solvent selected for analysis. Moreover, for LC techniques using ultraviolet or fluorescent detection, a derivatization step is usually required prior to analysis [12]. Therefore sample preparation and chromatography require several minutes. The global objective of this study is to develop an

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original, simple, sensitive, robust and fast method to extract and quantify veterinary antibiotics from a complex dirty matrix like swine manure. This method requires the use of an LDTD interface to permit high throughput sample introduction. The LDTD is coupled to an atmospheric pressure chemical ionization (APCI) source which precedes a triple-quadrupole MS instrument capable of MS/MS determinations. This method was applied to four different antibiotics: trimethoprim (TMP), sulfadoxin (SFX), lincomycin (LCM) and tylosin (TYL) which are among the most widely used antibiotics in veterinary medicine for swine production. The sample preparation time is minimized to an ultrasonic extraction followed by SPE and only one evaporation step. Ultrasonic extraction using a solution of methanol (MeOH), McIlvaine buffer and ethylenediaminetetraacetic acid (EDTA) was used for the extraction of the target analytes [12,14,15]. Efficient clean-up was required, therefore a cation exchange cartridge was used to strongly retain the target analytes to the sorbent and allow the use of organic solvents to remove a large portion of the interfering matrix. This clean-up is crucial because no chromatographic separation occurs using the LDTD-APCI prior to MS/MS detection. The method was tested and validated with freeze-dried manure from the experimental farm of Agriculture and Agri-food Canada (Lennoxville, QC, Canada). Method performance was evaluated by the determination of extraction recovery, linearity, precision, repeatability and limits of detection and quantification. The determination of targets compounds at micrograms per kilogram in pig manure was performed to confirm the applicability of the method in real environmental samples.

LDTD-APCI is an alternative sample introduction technique without a separation step like LC or gas chromatography (GC) prior to detection. For that reason, LDTD-APCI technology permits the virtual elimination of chromatographic columns and mobile phase, thus drastically reducing analysis time, sample preparation and analysis costs while increasing sample throughput. In fact, the LDTD-APCI is coupled to MS/MS and reduces total analysis time to 15 s compared to minutes with LC coupled to MS/MS. The LDTD technology is based on the volatilization and on the physicochemical properties of the compound. An IR laser diode beam hits the back of the sample well (metal bottom) and the target sample is volatilized by the heat-gradient that is thus generated. In a second step, the compounds are transferred with a gas flow and ionized in the APCI before entering the MS/MS. To the best of our knowledge, there are no published methods using LDTD or similar APCI-based approaches to quantify veterinary antibiotics in swine manure. Some analytical methods have been published on LDTD-APCI-MS/MS and it has so far been applied in toxicology [16,17], pharmaceutical [18], environmental samples such as endocrine disruptors in wastewaters [19], municipal sludge and aquatic sediments [20], and sulfonamides in dairy milk [21]. The schematic and assembly of the LDTD-APCI source apparatus have previously been detailed [22].

2. Materials and methods

2.1. Chemicals, reagents and stock solutions

LCM (purity ≥ 89.1%), SFX (purity ≥ 99.9%), TMP (purity ≥ 99.5%), TYL (purity ≥ 83.8%) and spiramycin (SPI, purity ≥ 90.0%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Isotopically-labeled trimethoprim, [$^{13}C_3$]-trimethoprim ([$^{13}C_3$]-TMP) used as internal standard (IS, purity ≥ 99%), was obtained from ACP Chemical Inc. (Montreal, QC, Canada). All solvents used were of HPLC grade purity from Fisher Scientific (Whitby, ON, Canada) and deionized/distilled water (dd-H_2O) was used for dilutions. Individual stock solutions were prepared in MeOH at a concentration of 1000 mg L^{-1} and kept at −20 °C for a maximum of 6 months. Individual intermediate solutions were prepared by dilution of the 10 mgL^{-1} stock solution in MeOH. Given the potential for degradation of the target analytes [23], working solutions were prepared daily at a concentration of 1 mg L^{-1} by dilution in MeOH from individual intermediate stock solution. Sodium phosphate dibasic (Na_2HPO_4, purity ≥ 99.0%) and citric acid (purity ≥ 99.5%) were purchased from Sigma Aldrich (St Louis, MO, USA).

2.2. Agricultural soil

Swine manure was obtained from the Dairy and Swine Research and Development Center of Agriculture and Agri-Food Canada (Lennoxville, QC, Canada). Manure samples were collected in plastic flasks, homogenized and freeze-dried in Lennoxville. All the samples were kept at 6 °C until analysis. This manure does not contain target analytes and was used for all method validation tests.

2.3. Extraction and cleanup

Approximately 100 mg of freeze-dried manure was weighted into a 15 mL conical-bottom centrifuge tubes from Kimble Chase (Rockwood, TN, USA) and 5 mL of extraction buffer MeOH/McIlvaine/EDTA (50:45:5, v/v/v) at pH 5 was added. McIlvaine buffer (20 mL) was prepared by mixing 9.70 mL of 0.1 M citric acid and 10.30 mL of 0.2 M Na_2HPO_4. The tubes were mixed for 1 min on a vortex and were subsequently placed into an ultrasonic bath for 15 min. They were then centrifuged at approximately 2750 g for 15 min. The supernatant was collected into a 15 mL brown glass tube. These extractions were repeated twice but 50 μL of acetonitrile (MeCN) was added for the second extraction, before the centrifugation step, to help precipitate proteins [11].

SPE was done using a 12-position manifold manufactured by Phenomenex (Torrance, CA, USA). Strong cation mixed mode phase Strata-X-C (surface-modified styrene divinylbenzene polymer) cartridges with a total volume of 6 mL and a 200 mg bed mass from Phenomenex were used to wash the sample extracts. Multiple SPE parameters were optimized: cartridge type, loading step, loading flow rate, washing step and sample pH. The SPE cartridges were conditioned with 2 × 5 mL of MeOH and 2 × 5 mL of distilled-deionized water (dd-H_2O) acidified at pH 4. Samples were loaded on the cartridge column at a flow rate of 2–3 mL min^{-1} by applying negative pressure using a mechanical pump. The SPE cartridges were washed with 2 × 5 mL of MeOH followed by 2 × 5 mL of ethyl acetate (EtAc). The analytes were eluted with 2 × 5 mL of MeOH/NH_4OH (95:5, v/v) at pH 9.0 into conical-bottom centrifuge tubes. Before evaporation, eluates were filtered on 0.45 μm pore size fiberglass membranes from Whatman (Piscataway, NJ, USA) to eliminate particulate materials. The eluates were then evaporated to total dryness under a gentle stream of nitrogen at 40 °C with a nine-port Reacti-vap unit from Pierce (Rockford, IL, USA) and then reconstituted to 200 μL with MeOH/H_2O (90:10, v/v) for LDTD-APCI-MS/MS analysis.

2.4. LDTD-APCI-MS/MS

Desorption and ionization of target veterinary antibiotics were performed with the T-960 LDTD-APCI ionization source controlled by the LazSoft 4 Software, developed and manufactured by Phytronix Technologies (Quebec, Canada) and data integration was performed using the Xcalibur™ 2.0 software (Thermo Fisher Scientific, Watthan, MA). For analyte detection, LDTD-APCI was mounted on a Quantum Ultra AM triple quadrupole mass spectrometer by Thermo Fisher Scientific (Waltham, MA). Ionization was