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Detection of digoxin in urine samples by surface-assisted laser desorption/ionization mass spectrometry with dispersive liquid–liquid microextraction

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ABSTRACT

A novel method for the detection of digoxin using dispersive liquid–liquid microextraction (DLLME) coupled to the surface-assisted laser desorption/ionization mass spectrometric detection (SALDI/MS) was developed. Acetone and chloroform were used as the disperser solvent and extraction solvents, respectively. After the extraction, digoxin was detected using SALDI/MS with colloidal palladium as the matrix. Under optimal extraction and detection conditions, the calibration curve, which ranged from 0.01 to 0.50 μM, was observed to be linear. The limit of detection (LOD) at a signal-to-noise ratio of 3 was 2 nM for digoxin. With a sample-to-extract volume ratio of 400, the enrichment factor for digoxin was calculated to be 252. This novel method was successfully applied for the determination of digoxin in human urine samples.

1. Introduction

Digoxin is a cardiac glycoside that has become the most widely prescribed medication for treating congestive heart failure and arrhythmias [1]. Unfortunately, digoxin has a small therapeutic range; when the concentration of digoxin in plasma exceeds 2 ng mL^{-1} (2 nM), intoxication may occur [2]. Therefore, a simple and sensitive analytical procedure for monitoring the concentration of digoxin in biological fluids is required to ensure optimum efficacy while minimizing the risk of toxicity and other adverse effects.

Traditional digoxin detection has been performed using microbiological assays [3], but these methods are often time-consuming and lack the specificity and reproducibility needed for analytical purposes. High performance liquid chromatography (HPLC) has been proposed for the analysis of digoxin. However, because digoxin does not contain a chromophore or fluorophore in its structure (Fig. 1), the compound is difficult to detect with a UV-based detector, with the exception of low UV wavelengths [4] for which the detection limits are not favorable. Fluorescence detection [5] and mass spectrometric (MS) detection [6–7] have also been reported for HPLC analysis of digoxin. Among these methods, LC/MS is the most popular technique due to its sensitivity and specificity, as well as its ability to identify unknown compounds. However, LC/MS instruments require complicated sample preparation and are expensive. Recently, an optical sensor using a molecularly imprinted polymer (MIP) membrane was developed for the determination of digoxin in serum samples [8].

Despite the high sensitivity achieved by many analytical methods, an extraction/preconcentration step is generally required for the determination of digoxin in biological samples. Liquid–liquid extraction (LLE) [9,10] and solid-phase extraction (SPE) [4,11] using commercial SPE cartridges are the most common procedures for extraction/preconcentration of digoxin from urine and plasma samples. However, these sample pre-treatment procedures are tedious and time-consuming. Recently, dispersive liquid–liquid microextraction (DLLME) has become an important sample preparation technique due to its rapidity, ease of operation, and low cost [12,13]. DLLME is based on a ternary solvent system in which the extraction and disperser solvents are rapidly injected into the aqueous sample to form a cloudy solution. Extraction equilibrium is quickly achieved due to the large amount of surface contact between the droplets of the extraction solvent and the aqueous sample. Therefore, the extraction time is very short. After the centrifugation of the cloudy solution, the extraction solvent generally settles at the bottom of the tube and is aspirated with a microsyringe for instrumental analysis. The DLLME technique, coupled with GC and LC, has been widely applied to the analysis of PAH and pesticides [14–15]. Despite

Abbreviations: surface-assisted laser desorption/ionization mass spectrometry, SALDI/MS; dispersive liquid-liquid microextraction, DLLME; nanoparticles, NPs

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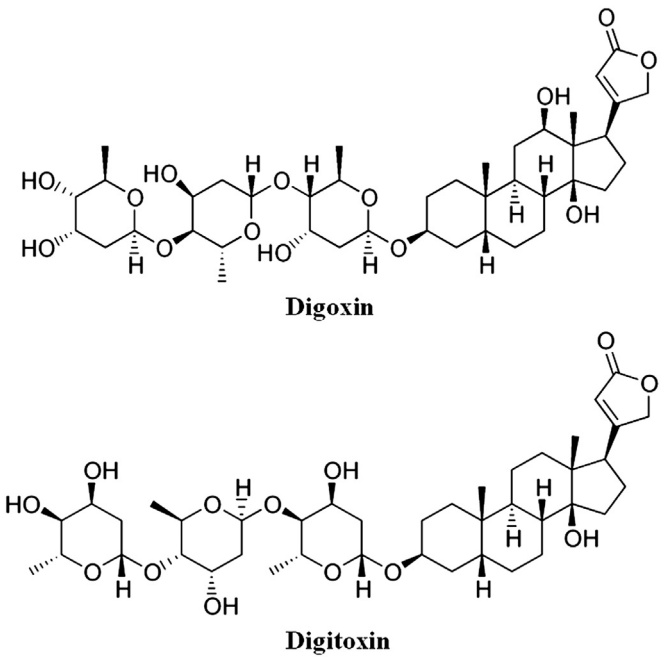

Fig. 1. Structural formulas for digoxin and digitoxin.

the wide applicability of DLLME coupled with GC and LC, there are a few reports on the use of DLLME coupled with the surface-assisted laser desorption/ionization mass spectrometry (SALDI/MS) in the literature [16]. Additionally, DLLME has not yet been applied to the extraction of digoxin.

SALDI/MS has become a popular tool for the analysis of drugs due to its excellent capability for high-throughput, reduced background noise in the low-mass region, and better shot-to-shot reproducibility. Nanomaterials, such as gold nanoparticles (NPs) [17,18], silver NPs [19,20], titanium dioxide NPs [21], colloidal graphite [22], nanostructured silicon [23], and diamond nanowire [24] have been recognized as effective SALDI matrices. Although a variety of NPs have been successfully applied as matrices in SALDI/MS, to date, no application of palladium NPs in SALDI/MS has been reported. The maximum absorption wavelength of Pd NPs is 320 nm [25,26], which is close to the emission wavelength of the most commonly used nitrogen laser (337 nm) in SALDI/MS. Pd NPs may efficiently absorb laser energy, rapidly transfer laser energy to the analytes, and then induce the desorption and ionization of the analytes. Our goal in this study is to examine whether the colloidal Pd can be used as a SALDI matrix for digoxin. In addition, we combined the DLLME technique with SALDI/MS for the analysis of digoxin. The factors that influence the extraction efficiencies and analyte detection were investigated. The applicability of the method for the determination of digoxin in human urine was also demonstrated. To our knowledge, this is the first report that demonstrates the use of SALDI/MS for the analysis of digoxin.

2. Experimental methods

2.1. Chemicals and solutions

Digoxin, digitoxin and carbon tetrachloride were purchased from Sigma (St. Louis, MO, USA). Dichloromethane was obtained from J.T. Baker (Phillipsburg, NJ, USA). Chloroform was obtained from Showa (Tokyo, Japan). Colloidal suspensions of palladium (10 ppm), gold (20 ppm), and silver (10 ppm) were purchased from Purest Colloids (Westhampton, NJ, USA). All chemicals were used as received without further purification. Water that was purified with a Millipore Synergy water purification system (Billerica, MA, USA) was used for all solutions.

Stock standard solutions (1 mM) of digoxin and digitoxin were prepared in methanol and were diluted to the desired concentrations with 5 mM phosphate buffer (pH 7). The digoxin and digitoxin solutions were stored at 4 °C for a month.

2.2. DLLME procedure

An aliquot (1 mL) of buffered solution containing the digoxin was placed in a 1.5 mL sample vial. The disperser solvent acetone (140 μL) and the extraction solvent chloroform (60 μL) were rapidly injected into the sample solution using a 1.0 mL syringe (Hamilton, Reno, NV, USA), and a cloudy solution was formed. The mixture was gently shaken for 5 min. The mixture was then centrifuged at 3500*g* for 5 min, and the dispersed fine droplets of the extraction solvent settled at the bottom of the sample vial. Fifty-five microliters of the sediment phase was transferred to a separate sample vial using a 100 μL HPLC syringe (Hamilton, Reno, NV, USA). The extract was evaporated to dryness. The extract residue was re-dissolved with 2.5 μL of 5 mM phosphate buffer (pH 7) containing I.S. (5 μM).

2.3. SALDI/MS measurements

One microliter of the extract solution was mixed with 1 μL of colloidal Pd solution. Then, 1 μL of the mixture was deposited onto a stainless steel target and was allowed to dry at room temperature.

Mass spectrometry experiments were performed in positive-ion mode on a reflectron-type time-of-flight mass spectrometer (Microflex, Bruker Daltonics) with a flight length of 1.96 m. The samples were irradiated with a 337 nm nitrogen laser at 20 Hz. The generated ions were accelerated at a voltage of 19 kV. To obtain good signal-to-noise ratios, the laser energy settings were adjusted to slightly exceed the threshold, and each spectrum was acquired from an average of 100 laser pulses.

2.4. Preparation of urine samples

Human urine samples were collected from healthy volunteers who were not receiving any pharmaceutical treatment at the time of sampling. The urine samples were stored at −20 °C until analysis. An aliquot (990 μL) of the urine sample was spiked with 10 μL of the digoxin standard. Urine samples of various digoxin concentrations were prepared by spiking the urine with the desired amounts of digoxin. A blank urine sample was prepared by spiking 10 μL of DI water into 990 μL of urine. The digoxin-spiked urine (1 mL) was treated with DLLME following the procedure described above.

3. Results and discussion

3.1. Determination of digoxin by SALDI/MS

Previously, Au and Ag NPs were the most commonly used SALDI matrices. We investigated the use of colloidal Au and Ag as SALDI matrices for the detection of digoxin. Fig. 2A and B shows the mass spectra of digoxin using colloidal Au and Ag as SALDI matrices, respectively. The ion signal at $m/z=803.42$ corresponded to the sodium adduct ion of digoxin. Although colloidal Au and Ag contributed some background ions, the results indicated that colloidal Au and Ag successfully desorbed and ionized digoxin. Because the maximum absorption wavelength of Pd NPs is 320 nm, colloidal Pd may be a more efficient SALDI matrix when