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Determination of lidocaine in urine at low ppm levels using dispersive microextraction and attenuated total reflectance–Fourier transform infrared measurements of dry films

David Pérez-Guaita, Ángel Sánchez-Illana, Salvador Garrigues *, Miguel de la Guardia

Department of Analytical Chemistry, University of Valencia, Research Building, 50 Dr. Moliner Street, Burjassot, Valencia 46100, Spain

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ABSTRACT

IR spectra provide valuable information about biological systems and can be obtained with compactable and affordable instruments, but the lack of sensitivity of this technique hampers its use in the determination of drugs in clinical fluids. Taking lidocaine as a target molecule, in this paper we introduced a methodology for determining drugs in urine samples using infrared spectroscopy. The lack of sensitivity of the IR was compensated with the combination of an effective and straightforward dispersive liquid–liquid microextraction and the measurement of the dry film of the organic extracts through attenuated total reflectance (ATR). The method developed improves the sensitivity by eliminating the solvent and preconcentrating the analyte in the surface of the ATR crystal. Urine samples were taken from 15 volunteers, and 9 samples were spiked at 6 concentration levels ranging from 0 to 33 mg L^{-1}. Multivariate models based on partial least squares regression and science-based calibration were performed and validated with a separated set of spiked urine samples from other patients, obtaining ratio prediction to deviation (RPD) values higher than 3.5. The limit of detection values obtained were 0.5 mg L^{-1} for the univariate calibration and 1.7 mg L^{-1} for a reliable multivariate calibration. Therefore, the procedure is limited to only levels of lidocaine higher than 2 mg L^{-1} but serves as an untargeted, fast, and versatile screening tool, which maintains all the advantages of the widespread application of the IR spectroscopy to the clinical analysis, such as simplicity, compactability, and minimum use of reagents and solvents.

1. Introduction

In the last years, infrared (IR) spectroscopy has been evidenced as a promising technique in the analysis of clinical samples, and literature provides several methodologies for the determination of clinical parameters based on IR measurements of the IR spectra [1,2]. This technique presents several advantages against the classical methods (enzymatic and mass spectrometry (MS)-based methods), such as the large amount of information that the IR spectra provide, the cost-efficiency and compactability of the instrumentation used, and the minimum treatment of sample required [3].

Nevertheless, this technique suffers also from some drawbacks. All the vibrations of inorganic and organic compounds are active in the mid-infrared (IR) range, and although this versatility permits that almost all clinical compounds could be potential target analytes, this fact also implies that several interferences are normally present in the spectra, especially taking into account the complexity of biological systems. Besides the absence of selectivity in complex spectra, which makes mandatory the use of chemometric models, IR lacks also from sensitivity, being hampered the determination of clinical parameters at low concentration levels [4,5]. Methodologies based on IR measurements using transmission measurements of dry films [6,7] or attenuated total reflection (ATR) [8,9] are limited to the determination of main compounds. In contrast, the determination of exogenous compounds (e.g. drugs or ingredients of cosmetic products/formulations and their metabolites), which are normally found at low ppm, ppb, or even ppt levels, especially in clinical samples, is a difficult task. To the best of our knowledge, only few works reports methodologies for the analysis of these compounds in clinical samples via IR, as the detection of cocoa polyphenol metabolites in urine samples using ATR-IR microspectroscopy of dried urine samples [10], the quantification of ibuprofen in urine samples using solid phase microextraction (SPME) and transmission measurements [11], or the determination of cocaine in saliva using IR-ATR [12].

In the past years, several research groups have developed new strategies for overcome the aforementioned lacks of sensitivity and selectivity of the IR determinations in liquid samples, such as the use of new chemometrical algorithms [13], the isolation of low concentrated analytes from proteins using microfluidics [7], or the use of quantum cascade lasers as a powerful IR source [14]. Most recently, our group has determined lipidic parameters in serum by measuring the dry film formed in an ATR crystal after a deposition of the sample organic extracts obtained from a liquid–liquid extraction of serum samples [15]. With a minimum preprocessing of the sample, this simple strategy enhances

* Corresponding author.

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the selectivity of the measurements by eliminating the contribution of solvents and other volatile compounds and improves the sensitivity by concentrating all the lipids in the surface of the ATR crystal.

The aim of this work was to take advantage of the aforementioned strategy for analyzing drugs at low concentration levels in urine samples using IR spectroscopy. The molecule selected for performing this study was lidocaine, a widely used local anesthetic and antiarrhythmic drug, employed in minor surgery, as a dental topical anesthetic or to relieve itching, burning, and pain from skin inflammations [16]. Previous studies propose methodologies for the determination of lidocaine, in urine of human and horses, based on different techniques including enzyme-linked immunosorbent assay [17], tandem MS [17], liquid extraction followed by gas chromatography [16], solid phase extraction (SPE) combined with liquid chromatography (LC)-tandem MS [18], capillary zone electrophoresis (CZE) preceded by SPE [19], or head space-solid phase microextraction (HS-SPME) [20]. The levels found in human urine by those studies differs depending on the treatment applied to the patients, namely, between 0.73 and 4.88 mg L^{-1} after a by-pass surgery [20], 13.9 mg L^{-1} after a cholecystectomy [21], or the exceptional 54 mg L^{-1} value after a death caused by intravenous drip lidocaine [16]. Lombardo-Agüí et al. [19] also estimated that the levels of patients following a recommended treatment varies between 12 and 15 mg L^{-1}.

Our goal was to propose a technique that enhanced the IR determination sensitivity to reach the low ppm levels of lidocaine found in human urine by the aforementioned studies but also simple enough for retaining the advantages of IR analysis. For this propose, dispersive liquid–liquid microextraction (DLLME) was used as an extraction procedure, which minimizes the volume of solvents employed and provides an ecological way for greening analytical determination [22,23]. Using human urine spiked samples from volunteers, multivariate calibration models were performed and validated with spiked urine samples from different volunteers. We also calculated limits of detections from univariate and multivariate calibration, evaluating the use of the new proposed approach as a fast, simple, and in situ screening tool and comparing it with the available methodologies in terms of sensitivity and complexity.

2. Experimental

2.1. Urine Samples

In order to ensure variability within the urine samples, urine samples from 15 volunteers with a wide range of age (23–70 years) were selected and collected in different hours of the day. After centrifugation at 4000 rpm, solid NaCl (Scharlau, Germany) was added to the samples (at a concentration of 8 g/100 mL) in order to keep constant the ionic strength. A 300 mg L^{-1} stock solution of lidocaine was prepared each measurement day by dissolving the calculated amount of lidocaine hydrochloride monohydrate (99.9% p/p purity, obtained from Cofares, Madrid) in milliQ water. Nine urine samples (5 for calibration and 4 for validation in the multivariate analysis) were spiked at 6 levels of concentration (approx. 0, 2, 4, 8, 15, and 30 mg L^{-1}). The rest of urine samples (6) were used as extra blanks.

2.2. Microdispersive extraction procedure

Five hundred microliters of urine sample and 40 μL of an extracting mixture of chloroform and methanol (HPLC grade, from Scharlau, Germany) 2:1 (v/v) were poured into conical glass microvials and dispersed using a vortex mixer (Velp model ZX classic, Usmate, Italy) for 30 s at 2000 rpm. Samples were then centrifuged for 5 min at 7200 rpm. Using a microsyringe, 10 μL of the organic phase was transferred to chromatography vials with inserts until the measurement in order to avoid volatilization of the solvent.

2.3. FTIR analysis and chemometrical treatment

Using a 5 μL Hamilton syringe #7005 (Bonaduz, GR, Switzerland), 3 μL of the sample organic extracts was deposited in the ATR crystal, allowing the evaporation of the solvent during 1 min. Then the spectra were recorded averaging 300 scans against a background of the clean empty cell, in the range between 900 and 3600 cm^{-1} and using a 4 cm^{-1} resolution. ATR crystal cleaning between measurements was made using 5 μL of the extracting solution and dried with cellulose paper.

A Bruker (Bremen, Germany) IFS 66/v Fourier transform IR spectrometer equipped with a liquid nitrogen cooled mercury–cadmium–telluride detector, a vacuum system, and a dry air purged sample compartment was used for the acquisition of the spectra. Measurements were made using an ATR DuraSampleIR accessory from Smiths Detection Inc. (Warrington, UK), equipped with a nine reflections diamond/ZnSe DuraDiskIRE element.

Analysis of spectral data based on principal component analysis (PCA), partial least squares (PLS) were performed using in house written MATLAB 7.7.0 (Mathworks Inc., Natick, MA, USA) functions and PLS Toolbox 7.0 from Eigenvector Research Inc. (Wenatchee, WA, USA). Science-based calibration (SBC) was performed using the Matlab code provided by Marbach [24]. PCA, PLS, and SBC analysis were performed using the fingerprint (950–1800 cm^{-1}) and the C-H stretching (2723–3046 cm^{-1}) spectral regions. A 1st derivate (polynomial order 2 and number of supporting points 15) obtained using the Savitzky-Golay filter was used for the chemometric analysis, except for PCA, where a 2nd derivative (polynomial order 2 and number of supporting points 15) was used. In all cases, spectra matrix and concentrations vector were mean centered.

3. Results

3.1. Selection of the extraction and deposition parameters

In a first step, we studied the best conditions of lidocaine extraction from liquid samples and deposition of the extracts in the ATR crystal. Fig. 1a shows the effect of the volume deposited on the crystal in terms of signal and standard deviation of the integrated area between 1480 and 1530 cm^{-1}. It can be appreciated that the spectra of depositions were reproducible and that the signal to noise ratio increases on increasing the volume deposited. Finally, a volume of 3 μL was selected taking into account that the aspiration of higher volumes from the 20 μL extract volumes was difficult and also increased the drying time.

The extraction yield was also studied by measuring the spectra of three successive extractions of three different samples and a water blank, all of them spiked with 15 mg L^{-1} of lidocaine. The recovery percentages of lidocaine are shown in Fig. 1b, calculated from the integrated area measured between 1480 and 1530 cm^{-1}, obtained for each extraction respect to the sum of the three areas of the three consecutive extractions. The first step was able to extract more than the 90% in all cases, the second step extracted the rest of lidocaine, and the area was undetectable in the third step. A one-step extraction was chosen for simplifying the procedure in terms of time and volume of samples used. Fig. 1b also evidences the high reproducibility of the extraction, being the percentage of the extracted lidocaine similar for the three different samples and also comparable with aqueous standard.

3.2. Spectra obtained from blanks and spiked urine samples: univariate calibration

Our first aim was to determine the limit of detection (LOD) of lidocaine in the urine provided by the proposed methodology itself, i.e., without taking into account the variability of the urine composition. Using the intense band at 1505 cm^{-1} assigned to the aromatic C = C stretching, a univariate calibration curve was built from the spiked samples of one urine using the integrated area between 1480 and