Multi-Walled Carbon Nanotubes as Efficient Sorbent for the Solid Bar Microextraction of non-Steroidal Anti-Inflammatory Drugs from Human Urine Samples

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Abstract: Background: The determination of NSAIDs (ketoprofen, diclofenac, and ibuprofen) in urine samples provides useful information for assessing their safety, therapeutic effect, and their mechanism of action. Urine samples are characterized by their complexity and low concentration of the target analytes, which make their direct analysis difficult. In this work, the potential of multi-walled carbon nanotubes (MWCNTs) as solid bar microextraction (SBME) adsorbents for extraction and preconcentration of selected drugs from urine samples have been investigated.

Method: Five SBME devices (contains 10 mg of MWCNTs) were placed in 30 mL of adjusted pH 2 water samples and stirred for 40 minutes. After finishing the extraction, the devices were ultrasonicated in 250 μL of methanol for 5 min to desorb the selected drugs and analyzed using HPLC-DAD.

Results: The analytical performance of the whole method was evaluated in water samples. Limits of detection and quantitation were in the ranges of (0.36- 0.52) and (1.2- 1.7) μg L\(^{-1}\), respectively, and the relative standard deviation (RSD) < 5.7% over a concentration range of 5–100 μg L\(^{-1}\).

Application: The proposed method was successfully applied to the analysis of selected drugs in spiked human urine samples. The absolute recovery obtained by spiking the urine samples at three concentration levels: 5, 50 and 100 μg L\(^{-1}\) of selected NSAIDs were from 48.8% to 53.5%.

Conclusion: The proposed SBME using MWCNT as a sorbent material can be a useful alternative sample preparation technique for the routine determination of NSAIDs in urine samples at therapeutic levels with very simple sample pretreatment. Furthermore, the application of the developed SBME method might be extended to study the determination of selected non-steroidal anti-inflammatory drugs in veterinary urine samples.

Keywords: Multi-walled carbon nanotubes, Solid bar microextraction, Non-steroidal anti-inflammatory drugs, Urine, Liquid chromatography.

1. INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are compounds that have many pharmaceutical applications in both human health care and in veterinary fields [1]. These drugs, which inhibit cyclooxygenase, are normally used to relieve pain, fever, menstrual pain, swelling, and for the treatment of acute athletic injuries [2]. As customarily prescribed NSAIDs, ketoprofen, diclofenac, and ibuprofen (Fig. 1) are over-the-counter drugs [3] and some of them are accessible without prescriptions and control [4]. Although there are no reports of toxic effects of NSAIDs used at prescribed therapeutic levels, ulcers and bleeding may occur in the stomach or intestine as a side effects if taken in overdose [5-8]. That is why over the last years, specific attention has been paid in observing biological NSAIDs drugs in order to minimize their adverse effects and to enhance their safety.

The determination of NSAIDs in urine samples provides useful information for assessing their safety, therapeutic effect, and their mechanism of action [9]. Urine samples are characterized by their complexity and low concentration of the target analytes, which make their direct analysis difficult [8, 10, 11]. Therefore, several instrumental configurations, extraction and preconcentration procedures and/or devices have been developed to obtain highly selective and sensitive
methods for the determination of NSAIDs in urine samples [12-14]. Among these, solid phase extraction (SPE) using several kinds of sorbent materials offers unquestionable advantages, such as greater extraction efficiencies and minimal consumption of organic solvents [15, 16]. However, these procedures hardly reduce the long time spent in sample preparation and the large volumes of samples required for analysis [17].

To overcome these difficulties solvent-minimized microextraction techniques such as the solid phase microextraction (SPME) [18], stir bar sorptive extraction [19], microextraction by packed sorbent (MEPS) [20] and in-tube solid-phase microextraction [21] were proposed. SPME allows the integration of sampling and sample extraction in a unique step which minimizes the amount of organic solvents. However, the main disadvantages of this technique are expensive, sample carry-over and decline in performance with time [22]. Another solvent-minimized microextraction technique known as hollow fiber liquid-phase microextraction (HF-LPME) [23, 24], has also been reported for the analysis of selected drugs in urine samples. HF-LPME is simple, low cost, uses minimum solvent (μL) and provides a high level of enrichment [25]. Nevertheless, HF-LPME is an equilibrium extraction procedure and required long time of extraction [26]. Lengthy extractions may cause the loss of the extraction phase and this may adversely affect the method performance [27, 28].

An appropriate alternative miniaturized micro-solid phase extraction, solid bar microextraction (SBME) technique [29], inherits the advantages of both SPME and HF-LPME techniques at the same time. SBME devices in the previous reports [22, 29] are very simple and are based mainly on using only a few milligrams of a sorbent wrapped in a hollow fiber micro-tube. The porous membrane works as a filter to exclude particles from the sample matrix while allowing the molecules of the analytes to diffuse through and adsorb onto the sorbent. Afterward, the analytes are desorbed by ultrasonication in a micro-volume of suitable organic solvent.

Multi-walled Carbon Nanotubes (MWCNTs) are a kind of carbon-based nano-materials that have drawn great attention in many application fields [30, 31]. The potential MWCNTs as SPME adsorbents for the preconcentration of environmental pollutants have been investigated in recent years. MWCNTs with characteristics of high porosity and large adsorption capacity make it a suitable SPME coating material for the analysis of different analytes over a wide range of concentration [32-34].

This study aims to investigate the feasibility of MWCNTs as sorbent materials for SBME technique to extract and preconcentrate low concentration levels of ketoprofen (KET), diclofenac (DCF), and ibuprofen (IBU) from human urine samples prior to analysis using commonly used instrument, High Performance Liquid Chromatography connected with diode array detector (HPLC-DAD).

2. EXPERIMENTAL

2.1. Chemicals and Materials

Ketoprofen, diclofenac sodium salt and ibuprofen were obtained from Sigma–Aldrich (all purity grades > 98%). Organic solvents with HPLC-grade and hydrochloric acid (37%) were purchased from Merck (Darmstadt, Germany). Methanol and acetonitrile (HPLC-grade) were obtained from Carlo Erba Reagents (Milan, Italy). Potassium hydrogen phosphate, potassium dihydrogenphosphate, and orthophosphoric acid were of analytical grade and were purchased by Fluka-Riedel-de Haën (Buchs, Switzerland). Multi-walled carbon nanotube (MWCNTs) with outer diameter of 20-40 nm and the length of 5-15 μm was obtained from Shenzhen Nanotech Port Co. (China). Chromabond C18 (C18) was purchased from Macherey-Nagel (Düren, Germany). The Q3/2 Accurel polypropylene hollow fiber membrane (600 μm i.d., 200 μm wall thickness, and 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany).

2.2. Characterization of MWCNTs

The determination of oxygen-containing functionalities on the surface of MWCNTs was performed as previously described elsewhere [35, 36]. The pH value at the point of zero charge (pH\text{pzc}) of MWCNTs was determined by the mass titration procedure as indicated in [37]. The surface area was estimated by methylene blue method [38, 39] as follows: 25 mL of aqueous solutions of 10, 20, 30, 40, 50, 60, 70, 90, 110 mg L\textsuperscript{-1} methylene blue were separately added to 50 mL conical flasks each containing 25 mg of MWCNTs. The flasks were stoppered and left for 1 week in the dark, with shaking twice daily by hand. The remaining concentrations were analyzed spectrophotometrically using Cary 100Bio UV–vis spectrophotometer at 614 nm.

2.3. SBME Preparation

The SBME device was prepared as previously reported [22, 29]. The SBME device consisted of the 2 mg of sorbent materials packed within a hollow fiber polypropylene micro-tube (HF-PPMT) length 2.5 cm (Fig. 2). The two ends were heat sealed and each device was cleaned by ultrasonication in methanol for 3 min then stored in methanol until use.

**Fig. (1).** Molecular structure and pKa values of selected NSAIDs [23].
1-250 mg L\(^{-1}\) were obtained by diluting aliquot of the appro-

2.4. SBME Procedure

A clean SBME device was placed in 30 mL sample solu-

2.5. Chromatography

Stock solutions of ketoprofen, diclofenac and ibuprofen (1 mg mL\(^{-1}\)) were prepared in methanol and stored at 4 °C. For the calibration, a series of standard solutions in the range 1-250 mg L\(^{-1}\) were obtained by diluting aliquot of the appro-

2.6. Pretreatment of Urine Sample

The pretreatment of urine sample was performed as previously reported [40] and was with modifications. The human urine specimens were collected from healthy volunteers (age 25-45). All volunteers were completed a questionnaire including their gender and age. The urine samples were di-

3. RESULTS AND DISCUSSION

3.1. Optimization of SBME Conditions

SBME is an equilibrium-driven procedure where effi-

3.1.1. Comparison of Different SBME Adsorbents

The choice of the most suitable sorbents is very impor-

3.1.2. Sample pH

The pH value plays a significant role in determin-

Due to the difference in performance between the sorbents, the pH value was selected as an anionic form [47]. Thus, at pH 5 it would be expected to have the highest sorption efficiency due to electrostatic interaction between anionic forms of the

Fig. (2). Schematic diagram of the (SBME) system used.
drugs and positively charged surface of MWCNTs. Interestingly, the optimum pH extraction efficiency for selected analytes was at pH 2 (as demonstrated in Fig. 3), indicating that electrostatic interaction is not the predominant mechanism of interaction between MWCNTs and studied drugs. Therefore, further experiments were held at pH 2.

3.1.3. Number of SBME Devices

The extraction efficiency when multiple SBME devices (1-10 pieces) were evaluated with 30 mL sample solutions that had been spiked with 3 µg L⁻¹ of selected analytes. As expected, as the number of SBME devices increased, higher extraction efficiency was observed (Fig. 4). However, when more than five SBME devices were used, no additional enhancement was observed. Consequently, five SBME devices were used for the remaining studies.

Table 1. The effect of sorbent materials on SBME efficiency.

<table>
<thead>
<tr>
<th>Sorbent Material</th>
<th>KET (µg L⁻¹)</th>
<th>DCF (µg L⁻¹)</th>
<th>IBU (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>46.4 (1.2)</td>
<td>43.6 (1.7)</td>
<td>45.3 (1.5)</td>
</tr>
<tr>
<td>MWCNTs</td>
<td>57.6 (1.1)</td>
<td>54.1 (1.7)</td>
<td>54.7 (1.7)</td>
</tr>
</tbody>
</table>

*Extraction efficiency (Ee) for the selected drugs (at concentration 3 µg L⁻¹) were calculated as the ratio of the peak area of KET, DCF and IBU obtained by SBME to that obtained by a direct injection of standard drugs solutions [29].

Table 2. The chemical and physical properties of MWCNTs including surface oxides, surface area and pH value at zero point charge.

<table>
<thead>
<tr>
<th>Sorbent</th>
<th>Surface Areaa (m² g⁻¹)</th>
<th>pHZPCb</th>
<th>Total Basic Groupsc (mmol g⁻¹)</th>
<th>Total Acidic Groupsd (mmol g⁻¹)</th>
<th>Phenolic Groupsd (mmol g⁻¹)</th>
<th>Lactonic Groupsd (mmol g⁻¹)</th>
<th>Carboxylic Groupsd (mmol g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWCNTs</td>
<td>79.9</td>
<td>6.9</td>
<td>0.64</td>
<td>0.64</td>
<td>0.16</td>
<td>0.16</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*a Surface area was estimated by methylene blue method [46].

*b pH value at the point of zero charge, pHZPC, was determined by mass titration procedure as described elsewhere [37].

c Determination of surface oxides was performed as described elsewhere [35,36].

3.1.4. Extraction Time

SBME consists of a two-phase extraction system with a liquid-membrane interface; hence, the analytes need time to diffuse through the liquid phase and cross the interface to reach the sorbent phase. Spiked sample solutions containing 2 µg L⁻¹ of selected drugs were extracted by varying the exposure time from 10 to 60 min at room temperature with ultrasonication. As illustrated in Fig. (5), the extraction efficiency gradually increased with increasing exposure times. The maximum extraction efficiency was found after 40 min. Thus, 40-min extraction time was chosen in the following studies.

3.1.5. Effect of Desorption Solvent, Volume and Time

Three desorption solvents including methanol, water and acetonitrile were investigated. Methanol exhibited the best desorption efficiency and chromatographic peaks for the
three selected NSAIDs. In comparison with the other used solvents, methanol as a polar solvent with the lower value of dielectric constant [48] has the capability to break the interactions between MWCNTs material and the reported drugs. Hence it was adopted as the desorption solvent for the subsequent experiments.

Desorption experiments were investigated using different volumes (300–100 μL) of methanol. As expected, the lower volume of solvent gave the higher peak areas. Desorption volume less than 150 μL was not sufficient to immerse the SBME devices during the ultrasonication but higher volume of methanol (>250 μL) caused a decrease in the peak area resulting from the dilution of selected analytes. Thus, 250 μL was chosen for subsequent desorptions.

Desorption time was varied from 5 to 20 min by using methanol as desorption solvent. It was found that 5 min was suitable. After 5 min, there was a slight decrease in the desorption perhaps due to the analytes being readsorbed by the sorbent material. Under the above condition, no carryover of analytes was observed.

### 3.2. Method Validation

Under the optimum conditions, the analytical performance of the SBME-HPLC-DAD method was evaluated, and the results are summarized in Table 3. Calibration curves of SBME for KET, DCF, and IBU were constructed by diluting appropriate volumes of the working standard solution with water into different concentrations (5-100 μg L^{-1}). All samples were run in triplicate using the optimized method. Good linearity was obtained for all the analytes (R≥0.998). Limit of detection (LOD) and limit of quantification (LOQ) were determined according to the equations:

\[
LOD = \frac{3s_a}{b} \quad \text{and} \quad LOQ = \frac{10s_a}{b}
\]

, where \(s_a\) is the intercept standard deviation, and \(b\) is the slope of the regression line calculated from the calibration graph. The LOD values for KET, DCF, and IBU were found to be 0.52, 0.41, and 0.36 μg L^{-1} respectively, whereas LOQ were varied from 1.73 μg L^{-1} (KET) to 1.22 μg L^{-1} (IBU). Repeatability was evaluated by performing spiked replicates (n=5) at the same analyte concentrations (5-100 μg L^{-1}). The relative standard deviation (RSDs %) were at acceptable levels, ranging from 4.1%, to 5.7 %.

### 3.3. Application of the Method for Urine Sample

The optimized method was further applied to the analysis of real urine samples. The absolute recovery obtained by spiking the urine samples at three concentration levels: low (5 μg L^{-1}), medium (50 μg L^{-1}) and high (100 μg L^{-1}) of selected NSAIDs in urine samples (see 2.6) were from 48.8% to 53.5% (Table 4). Furthermore, the HPLC-DAD chromatogram (Fig. 6) shows the availability of the developed method for the determination of selected NSAIDs without interference of endogenous compounds.

### 3.4. Comparison with Previously Reported Methods

Table 5 lists the comparison of analytical performance obtained by SBME-HPLC-DAD method and other microextraction-based methods for the determination of target NSAIDs in urine samples. The comparatively low recovery was expected; mainly due to the SBME is a non-exhaustive extraction technique. However, in comparison with the gas chromatography-flame ionization detector (GC-FID) method [49], the proposed method offers higher recovery. Moreover, the LODs of SBME-HPLC-DAD method are lower compared to an earlier HPLC-UV [23, 50, 51] and capillary electrophoresis (CE) works [20] that were developed for urine using different microextraction techniques as sample preparation. It is also interesting to notice that the sensitivity of the proposed method (reflected in LOD and LOQ) is better than the more expensive LC–MS/MS method [52].

### Table 3. SBME validation parameters for selected NSAIDs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Linear Range (μg L^{-1})</th>
<th>Regression Equation</th>
<th>Determination Coefficient (R^2)</th>
<th>LOD (μg L^{-1})</th>
<th>LOQ (μg L^{-1})</th>
<th>RSD % (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KET</td>
<td>1.73-100</td>
<td>y = 9.7816x + 2.5662</td>
<td>0.999</td>
<td>0.52</td>
<td>1.73</td>
<td>4.1</td>
</tr>
<tr>
<td>DCF</td>
<td>1.39-100</td>
<td>y = 12.164x + 2.2607</td>
<td>0.999</td>
<td>0.41</td>
<td>1.39</td>
<td>5.2</td>
</tr>
<tr>
<td>IBU</td>
<td>1.22-100</td>
<td>y = 13.911x - 1.4304</td>
<td>0.998</td>
<td>0.36</td>
<td>1.22</td>
<td>5.7</td>
</tr>
</tbody>
</table>
CONCLUSION

This study presents solid bar microextraction (SBME) method combined with an HPLC-DAD determination using a 2 mg of MWCNTs as sorbent material that allows a simple, low-cost, and sensitive methodology for the determination of three active pharmaceutical ingredients (non-steroidal anti-inflammatory drugs) in spiked human urine samples. In view of its simplicity, reusability and minimal usage of solvent, the proposed SBME method can be a useful alternative sample preparation technique for the routine determination of NSAIDs in urine samples at therapeutic levels with very simple sample pretreatment. Furthermore, the application of the developed SBME method might be extended to study the determination of selected non-steroidal anti-inflammatory drugs in veterinary urine samples.

Table 4. Recoveries of spiked urine samples by SBME (n=3).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Added (μg L⁻¹)</th>
<th>Found (μg L⁻¹)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KET</td>
<td>5</td>
<td>2.67</td>
<td>53.5</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>26.45</td>
<td>52.9</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>53.1</td>
<td>53.1</td>
<td>2.2</td>
</tr>
<tr>
<td>DCF</td>
<td>5</td>
<td>2.55</td>
<td>51.1</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16.86</td>
<td>50.6</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>49.8</td>
<td>49.8</td>
<td>1.8</td>
</tr>
<tr>
<td>IBU</td>
<td>5</td>
<td>2.45</td>
<td>49.1</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>24.35</td>
<td>48.7</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>48.3</td>
<td>48.3</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Fig. (6). Chromatogram obtained after the SBME procedure of (A) a blank urine sample and (B) another spiked with the selected NSAIDs at a concentration level of 2 μg L⁻¹.
Table 5. Comparison of the developed method with the previous study for the determination of KET, DCF and IBU in urine samples.

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Drugs</th>
<th>Sample preparation</th>
<th>LOD (µg L⁻¹)</th>
<th>Recovery (%)</th>
<th>Repeatability (% RSD)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC–DAD</td>
<td>KET, DCF and IBU</td>
<td>SBME</td>
<td>0.36-0.52</td>
<td>48.3-53.5</td>
<td>&lt; 5.7 (n=5)</td>
<td>(current work)</td>
</tr>
<tr>
<td>GC–FID</td>
<td>DCF and IBU</td>
<td>HFLM–SPME</td>
<td>0.03–0.07</td>
<td>8.1–12.1</td>
<td>&lt; 7.5 (n=5)</td>
<td>[49]</td>
</tr>
<tr>
<td>UHPLC–UV</td>
<td>KET, DCF and IBU</td>
<td>MEPS</td>
<td>1.07–16.2</td>
<td>99.5–105</td>
<td>&lt; 9.15 (n=6)</td>
<td>[50]</td>
</tr>
<tr>
<td>HPLC–UV</td>
<td>KET, DCF and IBU</td>
<td>HF–LPME</td>
<td>5–15</td>
<td>58–136</td>
<td>&lt; 15.7 (n=3)</td>
<td>[23]</td>
</tr>
<tr>
<td>HPLC–UV</td>
<td>KET and DCF</td>
<td>DLLME</td>
<td>3.4–5.2</td>
<td>95.7–115.6</td>
<td>&lt; 1.1 (n=3)</td>
<td>[51]</td>
</tr>
<tr>
<td>CE</td>
<td>KET and DCF</td>
<td>SBSE</td>
<td>35–41</td>
<td>83.5–98.9</td>
<td>&lt; 6 (n=3)</td>
<td>[20]</td>
</tr>
<tr>
<td>LC–MS/MS</td>
<td>KET and IBU</td>
<td>SPE</td>
<td>8–13</td>
<td>90.4–94.4</td>
<td>&lt; 6.6 (n=3)</td>
<td>[52]</td>
</tr>
</tbody>
</table>

*Hollow-fiber liquid membrane-protected solid-phase microextraction.

Dispersive liquid–liquid microextraction.

Stir bar sorptive extraction.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflict of interest.

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