Determination of morphine in the plasma of addicts in using Zeolite Y extraction following high-performance liquid chromatography

Mahmoud Ghazi-Khansari a,*, Rezvan Zendehdel a,b, Morteza Pirali-Hamedani b, Mohammad Amini b

a Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P. O. Box 13145-784, Tehran, Iran
b Department of Medicinal Chemistry, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Received 21 May 2005; received in revised form 27 June 2005; accepted 7 July 2005
Available online 8 September 2005

Abstract

Background: The measurement of morphine in biological samples has become a routine in many clinical and forensic toxicology laboratories. A high-performance liquid chromatography (HPLC) method was developed for the determination of morphine in plasma.

Methods: Samples were extracted using Zeolite Y column followed by reversed phase HPLC with fluorescence detection. This method was based on an ex-calibration procedure and was linear between 20 and 200 ng/ml of morphine. Blood from 10 male opiate addicts were obtained from Rosbeh Hospital. All of the male smoked opiate (heroin, opium) and cigarettes.

Results: The mean total level 5 h after the last abuse was 152.4 ng/ml and 37.6 ng/ml at 10–15 h. The method was reliable for morphine determination in blood even after 5 half-lives after the last abuse.

Conclusions: This method is simple and rapid and may be useful for routine monitoring of plasma morphine concentration.

© 2005 Elsevier B.V. All rights reserved.

Keywords: Morphine; Plasma; Addict; Zeolite; HPLC

1. Introduction

Morphine exhibits well known analgesic properties which render the drug useful for the treatment of chronic pain such as cancer pain [1,2]. Urine is the preferred biological fluid for the analysis of morphine. The disadvantages of urine testing include concentration variations attributable to changing fluid intake and a greater possibility for adulteration or substitution and another limitation is the range of detection in urine which is <300 ng/ml [3]. The analysis of morphine in plasma are of interest due to human and animal pharmacokinetic studies, investigation of heroin abuse for epidemiological purpose, drug abuse control, the cause of intoxication and death in cases of clinical, pathological or forensic interest [4].

Radioimmunoassays (RIA) have frequently been used to monitor serum concentrations of morphine and its metabolites, but cross-reactivity of the antisera rendered these procedures problematic [5,6]. HPLC has also been used for the measurement of morphine-6-glucuronide and morphine-3-glucuronide in human serum using amperometric, fluorimetric or UV detection [7–10]. The major disadvantages are the expensive equipment needed, turnaround time, and the need for derivatization.

In recent years, HPLC combined with mass spectrometry has become the method of choice for the simultaneous measurement of morphine and its glucuronides using electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [11–14]. Unconjugated morphine is used when pharmacological or toxic effects are to be correlated with morphine or heroin. Its concentration in
plasma from subjects on morphine therapy is in the range of 8–80 ng/ml and in tolerated person could be as low as 50 ng/ml [4]. One of the major problems associated with HPLC is the need of complicated extraction procedure to separate the compound of interest from a biological matrix prior to analysis, such as liquid–liquid [15,16] or solid-phase methods [17,18].

Zeolites are microporous crystalline solids with well-defined structures. Generally they contain silicon, aluminum and oxygen in their framework, and cations, and water or other molecules within their pores. Zeolite Y has a 3-dimensional pore structure with pores running perpendicular to each other in the \(x, y,\) and \(z\) planes. The pore diameter of Zeolite Y is known as 7.6 Å [19]. Because of their unique porous properties, zeolites are used in a variety of applications. Recently zeolite has been used as purification, size exclusion chromatography, molecular absorbing and catalyses effects. In some circumstance zeolites were used as size exclusion while not affecting the target analyzed, which are excluded from the zeolite pores, removes most of the interfering material [20]. Zeolite Y has been used for catalyses effects and its molecular absorbing properties [21] which resulted in using Zeolite Y for size-exclusion chromatography.

2. Materials and methods

Ten addicts chosen from psychiatric department of Rosbeh Hospital who smoke either heroin and opium were used in this study. All were also cigarette smokers. They were between 26 and 68 years old and were addicted for at least 5 years. Patients were explained about the study protocol and those who volunteered were enrolled. The written informed consent was obtained from all patients. The ethics of this study conformed to the ethics committee of the Vice Chancelior for Research of Tehran University of medical sciences.

Four milliliters of blood were collected in heparinized tube. Blood were centrifuged and 1.0 ml of plasma was obtained. Each plasma sample was coded and refrigerated for later analysis. 0.5 ml of human plasma were added to 0.25 ml of zinc sulfate 0.7 mol/l, 2.5 ml of bicarbonate buffer (pH = 10.5) and 6 ml of THF. The mixture was vortex and centrifuged and the upper organic layer was transferred to Zeolite Y column. The HPLC system consisted of a model 6000A solvent delivery pump (Waters Assoc., Milford, MA); a Model 7125 injector equipped with a \(100-\mu l\) loop (Rheodyne, Cotati, CA), a \(\mu Bondapak\ C_{18}\) Column (300 mm \(\times\) 4 mm I.D., 10 µm), a model 474 fluorescence detector with an excitation wavelength of 235 nm and a 349 nm emission. The chromatography was

![Fig. 1. Regression plot used to determine concentration of morphine in plasma.](image)

![Fig. 2. Chromatogram showing extracted plasma blank spiked with 1 µg/ml of morphine by THF.](image)

![Fig. 3. Chromatogram showing extracted plasma blank spiked with 500 ng/ml of morphine by THF followed by Zeolite Y column.](image)

<table>
<thead>
<tr>
<th>Number</th>
<th>Agent abused</th>
<th>Time after the last abuse (h)</th>
<th>Age (years)</th>
<th>Concentration of morphine (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Opium</td>
<td>14</td>
<td>27</td>
<td>47.2</td>
</tr>
<tr>
<td>2</td>
<td>Opium</td>
<td>11</td>
<td>37</td>
<td>13.6</td>
</tr>
<tr>
<td>3</td>
<td>Opium</td>
<td>15</td>
<td>35</td>
<td>51.5</td>
</tr>
<tr>
<td>4</td>
<td>Opium</td>
<td>5</td>
<td>37</td>
<td>66.9</td>
</tr>
<tr>
<td>5</td>
<td>Heroin</td>
<td>4</td>
<td>32</td>
<td>74.6</td>
</tr>
<tr>
<td>6</td>
<td>Opium</td>
<td>3</td>
<td>36</td>
<td>155.4</td>
</tr>
<tr>
<td>7</td>
<td>Opium</td>
<td>3</td>
<td>38</td>
<td>193.8</td>
</tr>
<tr>
<td>8</td>
<td>Opium</td>
<td>2</td>
<td>35</td>
<td>190.8</td>
</tr>
<tr>
<td>9</td>
<td>Opium</td>
<td>2</td>
<td>68</td>
<td>233</td>
</tr>
<tr>
<td>10</td>
<td>Opium</td>
<td>48</td>
<td>26</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number</th>
<th>The time after abusing (h)</th>
<th>Number of abusing</th>
<th>Mean of morphine concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1–3</td>
<td>4</td>
<td>193.25</td>
</tr>
<tr>
<td>2</td>
<td>4–6</td>
<td>2</td>
<td>70.75</td>
</tr>
<tr>
<td>3</td>
<td>13–15</td>
<td>2</td>
<td>49.6</td>
</tr>
</tbody>
</table>

Table 1
Plasma morphine concentration among addicts

Table 2
Mean of morphine concentration in plasma hours after abuse
performed at ambient temperature using a mobile phase consisting of 5% methanol, 3% acetonitrile, 0.5 mmol/l sodium acetate, 0.012 mol/l potassium dihydrogen orthophosphate and 0.148 mmol/l phosphoric acid in distilled water. The flow rate was 1.2 ml/min.

3. Results

The calibration curves showed good linearity between peak-height and concentration from 20 to 200 ng/ml ($y = 0.026X + 0.46; r^2 = 0.99$). Table 1 shows the morphine concentrations in 10 addicted people. Morphine concentration in plasma was not detectable in an individual who abused opiate 48 h before sampling. The regression analysis of linear calibration curves for plasma morphine is shown in Fig. 1. The within-run coefficient of variance (%CV) of morphine at analyte concentrations of 20, 50, 100 and 200 ng/ml ($n=4$ for each) and between-run CVs obtained from independently prepared standards dilutions during 4 days ranged from 7% to 15%. The mean blood morphine concentration with respect to the time of abusing before analysis is shown in Table 2.

Fig. 2 shows typical chromatogram of plasma sample via extraction of morphine by THF using a mobile phase consisting of 8% methanol, 5% acetonitrile, 0.5 mmol/l sodium edetate and 0.012 mol/l potassium dihydrogen orthophosphate in distilled water. The flow rate was 1.2 ml/min with an excitation wavelength of 210 nm and a 340 nm emission as described by others [15]. There is an unknown peak at 5.63 min that interferes with morphine at interest concentrations.

To overcome this interference, several stationary and mobile phases were investigated to establish the optimum separation of morphine and interference peaks but an efficient separation was not achieved. Therefore, we used a column loaded with the Zeolite Y to achieve an improve separation of morphine in plasma (Fig. 3). After collecting the extract solution (THF phase), the zeolite column was washed with 10 ml methanol to show that no morphine remained in the column.

Fig. 4 shows chromatogram of methanol extraction from zeolite column. The Zeolite Y retained the unwanted interference of unknown compounds. The chromatographic condition was the same as in Fig. 2. Our study showed that there was not any specific interaction between morphine and Zeolite Y; therefore, this could be attributing to the other morphine analogues. Morphine was detected in plasma as late as 15 h after smoking, with a maximum mean concentration of 49.6 ng/ml (range 13.6–51.5) measured 13–15 h after the subjects smoked opiates. Detection limit of this method was 10 ng/ml and can be detected as late as 48 h after the subjects smoked opiates. With this method, morphine concentration can be analyzed after 5 half-times of morphine. A typical HPLC chromatogram obtained after analysis of plasma addicts sample of morphine is shown in Fig. 5. The peak of morphine was resolved from the interference peak.

References


