Determination of Fentanyl in Canine Plasma using HPLC-MS Detection

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ABSTRACT

A simple, easy, and accurate high-performance liquid chromatographic method for the determination of fentanyl concentrations in plasma samples has been developed and validated. Following a liquid extraction with acetonitrile, samples were separated by reversed-phase high-performance liquid chromatography (HPLC) on an XBridge C18 column (2.1 x 50 mm, 3.5 µm) preceded by a XBridge C18 guard column. The mobile phase was a mixture of water with 0.1% formic acid, and acetonitrile with 0.1% formic acid (90:10), with a flow rate of 0.6 ml/min. The procedure produced a linear curve over the concentration range of 0.1-25 ng/ml for fentanyl in canine plasma with a LOQ of 0.1 ng/ml. Intra-assay variability ranged from 2.6% to 8.2% and the average recovery for fentanyl was 100%.

INTRODUCTION

Fentanyl, (1R)-N-(phenyl-4-piperidyl) propanilide, is a synthetic opioid which has a high affinity for µ-opioid receptors. Fentanyl injection and interdural patches are used primarily in dogs and cats and have been shown to be useful for the adjunctive control of postoperative pain and in the control of severe pain associated with chronic pain, dull pain, and non-specific, widespread pain associated with cancer, pancreatitis, acute rheumatoid and osteoarthritis. Perioperative injectable fentanyl may also reduce the requirements for inhalational anesthetics during surgery, which could be particularly advantageous in patients compromised cardiac function. Fentanyl levels have been determined using mass spec and ultraspecific detection in plasma, blood, urine, and saliva. Many extraction methods have been reported including solid phase extraction, and liquid-liquid extractions. This paper describes a simple, sensitive, and accurate method for extracting fentanyl from plasma samples using HPLC.

MATERIALS and METHODS

Reagents and Standards

Fentanyl was purchased from USP. Flurazepam, the internal standard, was purchased from Fisher Scientific. Water (18.2 megaohm) was obtained from a Barnstead Micropure Infinity ultrapure water system. Stock solutions of fentanyl and flurazepam (100 µg/ml) were prepared in methanol. Dilutions were prepared to produce working stock standards of 0.01, 0.1, and 1 µg/ml. The standards were stored at 4°C and were stable for a minimum of 6 months. Standard curves for plasma were prepared by spiking untreated plasma with fentanyl which produced a linear concentration range of 0.1 to 25 ng/ml. Spiked standards were treated exactly as plasma samples. For stability testing, canine plasma (400 µl) was spiked with fentanyl at concentrations 0.3, 0.75, and 17.5 ng/ml. A 100 µl sample was pulled from this spiked plasma and analyzed immediately. The remaining spiked plasma was then frozen in an -80°C freezer, thawed, and another 100 µl sample was analyzed. This process was repeated for a total of three freeze-thaw cycles. The results were then compared to the initial analysis.

Chromatographic Conditions

The analysis of fentanyl in plasma was conducted using reverse phase HPLC. The chromatography system consisted of a 2695 separation module and an Acuity QDa quadrupole mass detector (Waters). The separation was achieved on a Waters XBridge C18 column (2.1 x 50 mm, 3.5 µm) preceded by a XBridge C18 guard column.

RESULTS and CONCLUSIONS

Testing of blank and spiked canine plasma revealed no interferences for fentanyl or flurazepam. Figs. 1 & 2 show chromatograms of a (A) blank plasma sample, (B) a 2.5 ng/ml spiked dog plasma standard and (C) a dog plasma sample after fentanyl administration. Retention times were 3.22 min for fentanyl and 3.28 min for flurazepam. The plasma peak ratio (area of fentanyl divided by the internal standard area) produced a linear curve for the concentration range 0.1-25 ng/ml with a correlation coefficient of >0.99 (Table 1), and having a LOQ of 0.05 ng/ml and a LOQ of 0.1 ng/ml. Intra- and inter-day assay relative standard deviation (RSD) for plasma spiked with fentanyl was used to determine accuracy and precision which ranged from 2.6% to 8.2% (Table 2). The precision was found to be below ±15% for all quality control samples. The average recovery for fentanyl was 100%. Stability of fentanyl under freeze-thaw conditions was tested and resulted in an average 2% loss after three cycles (Table 3). In conclusion, this HPLC method quantifies fentanyl from plasma by combining a liquid extraction procedure with mass spectrometry. This analytical procedure was validated in terms of recovery, linearity, LOQ, precision, and accuracy. This method utilizes a small sample size (400 µl), making it potentially useful for small dogs, cats or other small animals. Fig 3 is a representative concentration-time profile of a fentanyl pharmacokinetic study.

Table 1. Fentanyl assay linearity

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Mean ± SD</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 ng/ml</td>
<td>0.3 ng/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.1749 ± 0.0042</td>
<td>3.5</td>
</tr>
<tr>
<td>Y-intercept</td>
<td>0.0047 ± 0.0003</td>
<td>0.19</td>
</tr>
<tr>
<td>PP</td>
<td>0.9995 ± 0.0002</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 2. Intra-assay and inter-assay accuracy and precision for fentanyl in plasma.

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Concentration measured (ng/ml)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Intra assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>RSD (%)</td>
<td>3.5</td>
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<tr>
<td>Inter assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td>RSD (%)</td>
<td>7</td>
</tr>
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</table>