Determination of Fentanyl in Canine Plasma using HPLC-MS Detection

Joan Bailey  
*University of Tennessee, Knoxville*

Molly White  
*University of Tennessee, Knoxville*

Kristen Gordon  
*University of Tennessee, Knoxville*

Reza Seddighi  
*University of Tennessee, Knoxville*

Sherry Cox  
*University of Tennessee, Knoxville*

Follow this and additional works at: [https://trace.tennessee.edu/utk_compmedpubs](https://trace.tennessee.edu/utk_compmedpubs)

Recommended Citation
Bailey, Joan; White, Molly; Gordon, Kristen; Seddighi, Reza; and Cox, Sherry, "Determination of Fentanyl in Canine Plasma using HPLC-MS Detection" (2016). *Faculty Publications and Other Works -- Biomedical and Diagnostic Sciences*.  
[https://trace.tennessee.edu/utk_compmedpubs/153](https://trace.tennessee.edu/utk_compmedpubs/153)

This Poster is brought to you for free and open access by the Veterinary Medicine -- Faculty Publications and Other Works at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Faculty Publications and Other Works -- Biomedical and Diagnostic Sciences by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.
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 reverse-phase high-performance liquid chromatography (HPLC) on an XBridge C₁₈ column (2.1 x 50 mm, 3.5µm) and detected by mass spectroscopy. This analytical procedure was liquid extraction procedure with mass spectroscopy. This analytical procedure was developed and validated. Following a liquid extraction with acetonitrile, samples were separated by reverse-phase high-performance liquid chromatography (HPLC) on an XBridge C₁₈ column (2.1 x 50 mm, 3.5µm) and detected by mass spectroscopy.

INTRODUCTION

Fentanyl, (N-phenethyl-4-piperidyl) propionicanilide, is a synthetic opioid which has a high affinity for µ-opioid receptors. Fentanyl injection and transdermal patches are used primarily in dogs and cats and have been shown to be useful for the adjunctive treatment of chronic pain, dull pain, and non-specific, widespread pain associated with cancer, pancreatitis, asthma, thromboemboli, and paraparitis. Perioperative injectable fentanyl may also reduce the requirements for inhalational anesthetics during surgery, which could be particularly advantageous in patients with compromised cardiac function. Fentanyl levels have been determined using mass spectrometry and ultraviolet detection in plasma samples for stability for a minimum of 6 months. Standard curves for plasma were prepared by spiking canine plasma with fentanyl at concentrations of 0.1 ng/ml. Intra- and inter-assay variability ranged from 2.6% to 8.2% and the average recovery for fentanyl was 100%.

MATERIALS and METHODS

Reagents and Standards

Fentanyl was purchased from USP. Flurazepam, the internal standard, was obtained from Fisher Scientific. Water (18.2 megaohm) was obtained from a Barnstead N-50 water purification system. 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- and inter-assay variability ranged from 2.6% to 8.2% and the average recovery for fentanyl was 100%.

Chromatographic Conditions

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- and inter-assay variability ranged from 2.6% to 8.2% and the average recovery for fentanyl was 100%.

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 canine 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 3.35 min for flurazepam. The plasma peak ratio (area of fentanyl divided by the internal standard area) produced a linear curve for the concentration range of 0.1-25 ng/ml with a correlation coefficient of >0.99 (Table 3), and having a LOD of 0.05 ng/mL and LOQ of 0.1 ng/ml. Intra- and inter-day assay relative standard deviation (RSD) for plasma spiked with fentanyl were 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 can be used for the determination of fentanyl in plasma samples using a liquid extraction procedure with mass spectroscopy. This analytical procedure was validated and found to be suitable for small dogs, cats or other small animals. Figs 3 is a representative concentration-time profile from a fentanyl pharmacokinetic study.

Table 1. Fentanyl assay linearity

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>0.3 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>0.75 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>1.5 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>2.5 ng/ml</td>
<td>17.5</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>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>0.3 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>0.75 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>1.5 ng/ml</td>
<td>17.5</td>
</tr>
<tr>
<td>2.5 ng/ml</td>
<td>17.5</td>
</tr>
</tbody>
</table>

Table 3. Fentanyl freeze-thaw stability

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st Cycle</th>
<th>2nd Cycle</th>
<th>3rd Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>91</td>
<td>91</td>
<td>91</td>
</tr>
<tr>
<td>Sample</td>
<td>87</td>
<td>97</td>
<td>103</td>
</tr>
</tbody>
</table>

Figure 1: Chromatograms for Fentanyl in K9 plasma

A: Blank K9 plasma sample
B: Spiked 2.5 ng/ml K9 plasma standard
C: Canine plasma sample Sherman 90 min after fentanyl administration

Figure 2: Chromatograms for Flurazepam (I.S.) in K9 plasma

A: Blank K9 plasma sample
B: Spiked 2.5 ng/ml K9 plasma standard
C: Canine plasma sample Sherman 90 min after fentanyl administration

Figure 3: Pharmacokinetic profile of fentanyl in canine plasma