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**Determination of Fentanyl in Canine Plasma using HPLC-MS Detection**

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INTRODUCTION

Fentanyl, (3R,4S)-N-(2-phenylethyl)-4-(4-hydroxyphenyl)propionanilide, is a synthetic opioid which has a high affinity for μ-opioid receptors. Fentanyl injection and transdermal patches are particularly advantageous in patients with compromised cardiac function. Fentanyl can be used to reduce the requirements for inhalational anesthetics during surgery, which could be potentially useful for small dogs, cats or other small animals. Fig 3 is a representative concentration - 325 µL of fentanyl 2.5 ng/ml was spiked into 7 mL glass screw top tube and the supernatant was removed and placed in a clean glass tube and evaporated to dryness with nitrogen gas. Samples were reconstituted in 200 µL of mobile phase and 50 µL injected into the HPLC system.

MATERIALS and METHODS

Reagents and Standards

Fentanyl was purchased from USP. Flurazepam, the internal standard, was purchased from Cerilliant. All reagent grade chemicals and solvents were purchased from Fisher Scientific. Water (18.2 MΩ cm) 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, 1 and 10 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 the supernatant was removed and placed in a clean glass tube and evaporated to dryness with nitrogen gas. Samples were reconstituted in 200 µL of mobile phase and 50 µL injected into the HPLC system.

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 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 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 spectroscopy. This analytical procedure was validated in terms of recovery, linearity, LOQ, precision, and accuracy. This method utilizes a small sample size (100 µL) making it potentially useful for small dogs, cats or other small animals. Fig 3 is a representative concentration-time profile from a fentanyl pharmacokinetic study.