

### Introduction

Fentanyl is a synthetic opioid analgesic with a potency 80 times higher than morphine. The drug acts in the central nervous system (CNS) by agonising mu ( $\mu$ ) receptors, which are highly concentrated in the areas of the brain involved in nociception.

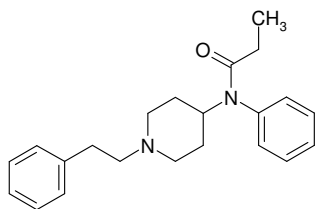


Figure 1. The chemical structure of fentanyl.

Fentanyl is extensively used in anaesthesia and analgesia. It is usually administered by intravenous infusion, but can also be administered transdermally as a patch and sublingually (Figure 2). It has a short pharmacokinetic half-life (1-6 hours) and a high fat solubility, which allows rapid crossing of the blood brain barrier leading to quick onset.

Administration of large or repeated doses will result in accumulation, which causes prolonged therapeutic and adverse effects. The main adverse effects are similar to morphine and include: sedation, respiratory depression, constipation, nausea and vomiting, dependence and euphoria. Overdose levels can cause severe breathing problems, unconsciousness and death.

The analysis of fentanyl is problematic due to:

- rapid metabolism
- low blood concentrations
- narrow window between therapeutic (1-2ng/mL) and toxic concentrations (2-20ng/mL).

### Experimental

Blood taken during the autopsy of a person who had been prescribed "Actiq" 1600 $\mu$ g lozenges was received by the Forensic Toxicology Service. A method was developed to confirm the presence of fentanyl.

"Actiq" is a solid formulation of fentanyl citrate on a stick, which dissolves slowly in the mouth. This form of fentanyl is intended for opiate-tolerant patients with cancer pain. Transmucosal absorption into the blood stream is rapid with peak plasma concentration achieved at 20 min. "Actiq" is available in 6 dosages, from 200 $\mu$ g to 1600 $\mu$ g.



Figure 2. "Perc-O-Pop's" or "Lollipop's" are street terms for "Actiq" lozenges.

### Chemicals and Reagents

Fentanyl citrate (purity >99%) and Fentanyl-d5 (0.1mg/mL in methanol) were obtained from Sigma and LGC Promochem respectively. HPLC grade methanol and methyl-tert-butyl-ether (MTBE) were purchased from Rathburns Chemicals Limited (Walkerburn, Scotland). Sodium hydroxide (40% solution) was obtained from BDH (Poole, Dorset, England). Deionised water was prepared on site (ELGA Limited).

### Extraction

100 $\mu$ L of whole blood calibrator/sample, 25 $\mu$ L of 1mg/L fentanyl-d5 (internal standard), 100 $\mu$ L of 1 molar sodium hydroxide and 1mL of MTBE were added to 2mL tubes and mixed for 15 minutes. Following centrifugation the organic phase was transferred to a 4.5mL polypropylene tube and evaporated to dryness. The residue was reconstituted in 250 $\mu$ L of 80% methanol and injected onto the LC/MS/MS system.

### Method

The HPLC system consisted of Perkin Elmer PE200 series autosampler, pump and column oven. Chromatography was achieved using a silica column (Supercosil LC-Si, 10cm x 4.6mm, 5 $\mu$ m) maintained at 50°C. The mobile phase consisting of acetonitrile/de-ionised water/formic acid (50/50/0.2, v/v/v) was pumped at 1mL/min. The output from the column was split 10:1 before entering the mass spectrometer. The volume of injection was 10 $\mu$ L.

Detection was by tandem mass spectrometry (LC/MS/MS), using a Sciex API2000 triple quadrupole equipped with a turbo-ion spray interface. The method was run in positive ionisation mode and was monitoring precursor and product ions of fentanyl (m/z: 337.1/105.1) and fentanyl-d5 (m/z: 342.1/105.1).

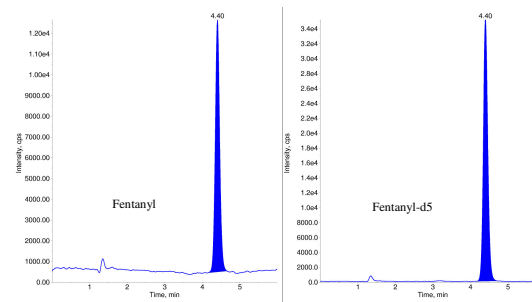


Figure 3. Chromatogram of the case sample. Fentanyl concentration of 59.5ng/mL.

Fentanyl and fentanyl-d5 eluted after 4.4 min (Figure 3). The wide range of standards used for the assay was from 10ng/mL to 500ng/mL (Figure 4). The concentration of fentanyl in the sample was 59.5ng/mL, which was indicative of an overdose. The lower limit of quantification (LLOQ) of fentanyl was 10ng/mL. A lower limit of detection (LLOD) of 1ng/mL can be achieved.

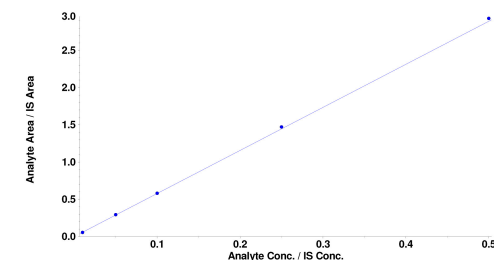


Figure 4. Calibration curve of fentanyl; regression linear through zero, weighting  $1/(x^2)$ ,  $y=5.78x$ ,  $r=0.9997$

### Conclusion

The use of fentanyl is steadily increasing as the biological effects of fentanyl are identical to heroin; with the exception that fentanyl is more potent. The drug is very "attractive" within the narcotic market and with its analogues is sold under the names; synthetic heroin or "China white". The drug is also occasionally abused by medical personnel who have easy access to the drug.

The increased use of fentanyl requires an easy and reproducible method for quantification. Fentanyl was measured using LC/MS/MS, which offers rapid and sensitive analysis with simple mobile phase composition and small sample volume. The assay can be used to measure the more potent analogues: alfentanil, sufentanil, lofentanil, carfentanil and remifentanil.

### References:

1. O.H.Drummer "The forensic pharmacology of drugs of abuse" 2001
2. [www.dea.gov](http://www.dea.gov)
3. A.Skulaska *et al* "Fentanyl and its analogues in clinical and forensic toxicology"
4. Hata T *et al* "Opioids"