The Absence of Methadone in Fly Larvae Found Feeding on a Putrefied Cadaver, Following Methadone Fatality

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Introduction:

Methadone is a synthetic opiate that is frequently prescribed by doctors to help overcome heroin addictions. It is available in syrup, tablet or injectable form. Methadone was originally prescribed as a pain killer and marketed as *Dolophine*®, a white round shaped tablet available in 5mg and 10mg dosages. *Lilly*®, (Figure 1) is formulated as pink, rounded-square-shaped tablets containing 40mg of methadone (the typical concentration given to heroin addicts).

Methadone is designated chemically as 6-dimethylamino-4,4-diphenyl-3-heptanone (Figure 2). It is a white to off-white crystalline powder with a solubility of 3.5mg/mL in water and a molecular weight of 666.7amu. When methadone is administered orally, it is rapidly metabolised to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidene (EDDP) (Figure 2) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP). Distribution around the body results in larger concentrations of methadone in the liver, kidneys and lungs as compared to those found in the blood.

Case History:

On the 11th May 2004, the police were called to the residence of the deceased by neighbours complaining of an unpleasant smell. Upon entry to the residence, the police found the body of a 44 year old woman in an advanced state of decomposition in bed with a number of empty methadone bottles and ventolin inhalers surrounding her. The flat was secure and therefore the police decided the death was non-suspicious. The post mortem examination was carried out on the 13th May 2004 and both liver and fly larvae samples were sent to the Forensic Toxicology Service on the 14th May 2004 for illicit and general drug screens. The medical history included with the request form described the deceased as having been asthmatic – explaining the presence of the ventolin inhalers - and having had a history of IV drug abuse. The deceased had been a previous heroin addict – explaining the presence of the methadone bottles.

Figure 1. 40mg methadone tablets (*Lilly*®).

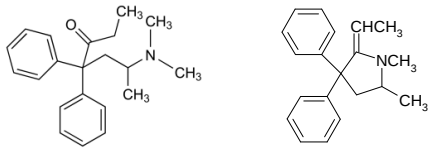

Figure 2. The chemical structures of methadone (left) and its metabolite EDDP (right).

Method & Materials:

The methodology developed involves the detection of methadone and its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), in both liver samples and a pooled of sample fly larvae collected from the cadaver. The method used was gas chromatography - mass spectrometry (GC-MS). The fly larvae were identified as *Calliphora Vicina* and as being in their 3rd instar life stage, by microscopy. Prior to homogenisation, the fly larvae were washed with copious amounts of deionised water and dried on filter paper. Deionised water was used to make 1 in 10 dilutions of the liver and fly larvae. The samples were initially run in full scan mode with three internal standards (quinoline, pyribenzamine and flurazepam), which showed a presence of both methadone and EDDP (Figures 3 & 4).

GCMS – Methadone quantitation: 1mL of both homogenised liver and fly larvae was added to 500µL 1M sodium hydroxide, 4mL of methyl tert-butyl ether (MTBE) and 50µL of internal standard (10mg/L pyribenzamine), mixed (15mins) and centrifuged (5mins, 3500rpm). The organic phase was transferred to 250µL phosphoric acid, then mixed (10mins) and centrifuged (5mins, 3500rpm). The organic phase was removed and 100µL of 1M sodium hydroxide and 200µL MTBE were added to the subnatent, mixed (10mins) and centrifuged (5mins at 3500rpm). The resulting organic phase was transferred to autosampler vials and 1µL was injected onto a GC-MS (HP 5890 GC coupled to an HP 5973 MS). The column used for separation was Solgel (30m x 0.25mm i.d., 0.25µm film thickness). The injector was maintained at 250°C and the detector was maintained at 280°C. The initial column temperature was set at 70°C and held for 6 minutes. It was then ramped at 40°C/min to 280°C and held for 2 minutes, giving a total run time of 13.25 minutes. The liver and fly larvae homogenates were run together with a range of calibrators (extracted using the same method) and screened in SIM mode. Methadone and the internal standard, pyribenzamine, were identified by their retention times and principle ions of 72 (Figure 5), and 91, respectively.

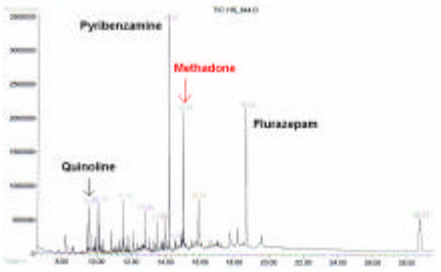

Figure 3. Chromatogram from full scan mode showing the presence of methadone in the liver sample.

Figure 4. Chromatogram from full scan mode showing the absence of methadone in the fly larvae samples.

Results:

Figures 3 & 4 show the chromatograms from the basic drug screens run on both the liver and fly larvae samples (extracted as described previously previously with an additional wash stage). Figure 3 shows the chromatogram of the liver with a clear peak at a retention time of 15.07 mins. This peak was identified as methadone from both its principle ion 72 using the NIST library and its retention time (Figure 5). Figure 4 shows the chromatogram of the fly larvae which lacks the peak at a retention time of 15.07 mins. Quantitative analysis carried out on the liver using the same extraction method and the GC-MS, gave a concentration of 5.5mg/Kg. No methadone was detected in the fly larvae. A small peak of EDDP was detected in both the liver and the fly larvae (RT 14.55 mins) - Figure 6). The liver concentration of methadone in the case study was consistent with those found in other fatalities.

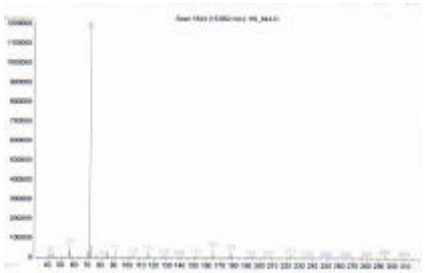

Figure 5. Spectra of methadone in liver sample.

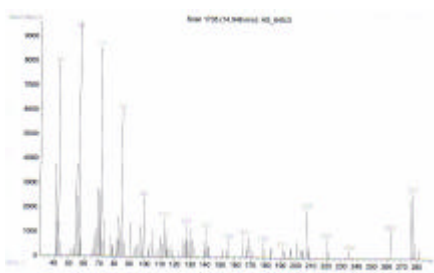

Figure 6. Spectra of EDDP in fly larvae sample.

Conclusions:

Despite the absence of methadone in the fly larvae, the presence of its metabolite, EDDP, suggests that fly larvae are able to metabolise methadone. This case study has raised a number of questions concerning the analysis for methadone in fly larvae such as: Is the absence of methadone in the fly larvae due to the rapid metabolism of methadone? Was the concentration of methadone below the detection limit of the assay? In this instance a pooled sample of fly larvae was used, however, it is likely that different sampling locations would have yielded different results.

This study contributes to the forensic community by raising interesting questions as to the analysis of entomological samples in toxicology cases where methadone overdose is the suspected cause of death. Research is currently being carried out on fly larvae reared on methadone spiked liver.