The Measurement of Methadone and its Principle Metabolite (EDDP) in *Calliphora Vicina* Fly Larvae using GC-MS

Analytical Unit, St George's - University of London, London, UK

Heidi Sanderson, Terry D Lee and David W Holt

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. The typical dosage of methadone given to heroin addicts is 40mg (Figure 1).

Methadone, designated chemically as 6-dimethylamino-4,4-diphenyl-3-heptanone (Figure 2), is a white to off-white crystalline powder with a solubility of 3.5mg/mL in water and a molecular weight of 309.5amu. When methadone is administered orally, it is rapidly metabolised to its principle metabolite 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, compared to those found in blood[1].

Figure 1. 40mg methadone tablets (manufactured by *Lilly*®)[2].

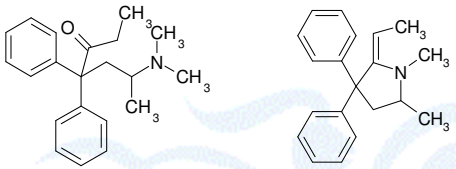

Figure 2. The chemical structures of methadone (left) and its metabolite EDDP (right).

Case History:

This research stems from a case of a 44 year old woman found dead in her bed with a number of empty methadone bottles and ventolin inhalers surrounding her. Her body was partially decomposed and contained a number of live maggots. 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.

Despite a high level of methadone (5.5mg/Kg) found in the liver, there was no methadone detected in the fly larvae. There was, however, a trace of EDDP (the principle metabolite of methadone) found in both the liver and fly larvae samples. Following the results of the analysis, the cause of death was given as methadone overdose. The aim of this research was to determine whether methadone could be detected in fly larvae reared on methadone spiked liver.

Method & Materials:

The methodology developed involves the detection of methadone and its principle metabolite, EDDP, in fly larvae samples reared on spiked liver (average concentration of 4mg/Kg). The method used was gas chromatography - mass spectrometry (GC-MS). One batch of fly larvae were reared on spiked liver throughout, one batch was reared on spiked liver until day six (after hatching) when the liver was substituted for blank liver and a third batch was reared solely on blank liver. The fly larvae were sampled everyday between days five and twelve. They were sacrificed by dropping them into a beaker of recently boiled water and then stored in 70% methanol until analysis. 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 single fly larvae. The samples were run in SIM mode with pyribenzamine as the internal standard.

Some of the fly larvae from all three batches were allowed to develop into adult flies. The flies were identified as *Calliphora Vicina* (Figure 3) by microscopy.

Materials

HPLC grade methanol and methyl tert-butyl ether (MTBE) were purchased from Rathburns Chemicals Limited (Walkerburn, Scotland). Sodium hydroxide (40% solution) and phosphoric acid were obtained from BDH (Poole, Dorset, England). Deionised water was prepared on site (ELGA Limited). EDDP and pyribenzamine were obtained from Sigma Aldridge (Poole, Dorset, England).

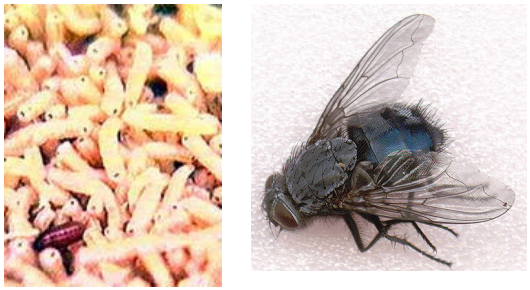

Figure 3. *Calliphora Vicina*: Fly larvae[3] and adult fly[4].

GC-MS – Methadone and EDDP quantitation: 1mL of fly larvae homogenate, 500µL 1M sodium hydroxide, 4mL of MTBE and 50µL of internal standard (10mg/L pyribenzamine) were mixed (15mins) and centrifuged (5mins, 3500rpm). The organic phase was re-extracted with 250µL phosphoric acid. The pH was of the aqueous phase was adjusted using 100µL of 1M sodium hydroxide and then extracted with 200µL MTBE. The resulting organic layer 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 HP-5MS (30m x 0.25mm i.d., 0.5µm 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 fly larvae homogenates were ran with a range of calibrators (extracted using the same method) and screened in SIM mode. Methadone, EDDP and pyribenzamine, were identified by their retention times and principle ions of 72, 277 and 91, respectively.

www.forensic-toxicology.sgul.ac.uk

Results:

Figure 4 shows the concentrations of methadone and EDDP found in the fly larvae sampled from the first batch reared solely on methadone spiked liver. The results show that EDDP was not detected until day seven; indicating that the methadone was not being metabolised until then. There was no methadone detected on days nine and ten but it was detected on days eleven and twelve. Figure 5 shows the concentrations of methadone and EDDP found in the fly larvae reared initially on methadone spiked liver until day six (after hatching) and then substituted for blank liver. The results show that EDDP was detected from day six (with the exception of day seven). With the exception of day nine, there was no methadone detected in the fly larvae from day eight. For up to three days after the spiked liver was substituted for blank, methadone could still be detected in the fly larvae. All of the fly larvae reared solely on blank liver tested negative for both methadone and EDDP.

The adult flies from the larvae reared on methadone spiked liver appeared a paler shade of blue than those from the larvae reared on blank liver. They were also smaller in size (average length was 7.93mm compared to 8.36mm) and had a rounded shape abdomen compared to the more pointed shape of those from the larvae reared on blank liver.

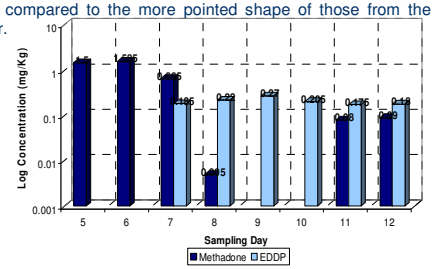

Figure 4. Average concentrations of methadone and EDDP from fly larvae reared solely on methadone spiked liver.

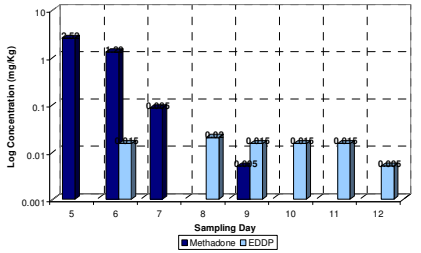

Figure 5. Average concentrations of methadone and EDDP from fly larvae reared on methadone spiked liver, substituted for blank liver on day six (after hatching).

Conclusions:

This study has shown that fly larvae are able to metabolise methadone (confirmed by the presence of EDDP) and that both these analytes can be detected in a single fly larva by GC-MS. It has been shown that fly larvae take approximately seven days to begin to metabolise methadone to EDDP. In fly larvae that have been removed from the methadone-spiked food source, methadone can still be detected for up to three days where as EDDP can be detected for at least five days. The negative results for methadone and EDDP in the fly larvae reared on blank liver indicate that fly larvae are unable to bioaccumulate methadone and EDDP. The appearance of the adult flies suggests that methadone slows the development rate of fly larvae. The flies from the larvae reared on blank liver were bigger and appeared to be at a more advanced stage of development.

References:

1. Ed.Moffat, A C, (1986) "Clarke's Isolation and Identification of Drugs", London: The Pharmaceutical Press
2. http://members.tripod.com/~jbabs714/meth.htm
3. Copyright Malcolm Storey, www.bioimages.org.uk
4. http://www.childrenfirst.nhs.uk