Heroin addiction is known to be a major problem all over the world and abused heroin is known as the main cause of drug related deaths. 6-monoacetylmorphine (6-MAM) is the main metabolite of heroin that is detected in forensic toxicology screening, however due to their few studies, little is known about the stability of 6-MAM. Presence of 6-MAM in analysed sample is important to differentiate between heroin and morphine related deaths. We investigated the stability of 6-MAM in conditions likely to be encountered in any forensic laboratories, in two common organs heart and lung at pH (6-7.5). We analysed blood from heart and lung, because the heart is the main organ that distribute the blood all over the body, which was not analysed before for 6-MAM stability. Heroin has a pKa of 7.95 and generally weak lipophilic basic drugs with pKa more than 8 tend to accumulate in the lung so we use lung tissue for 6-MAM stability investigation. We recommend that 6-MAM should be stored at -18ºC or less in order to reduce its degradation.

Methods
The method and validation are based on a previous study by Ritva Karinen et al.(6).
1. 100 µl of human whole blood or blood plus heart or lung sample was prepared in polypropylene tube.
2. Added 50 µl morphine D3 as internal standard (morphine D3 0.5 mg/ml in methanol), to all samples then followed by immediate agitation on a Multi tube vortex mixer.
3. 500 µl of ice-cold acetonitrile/methanol (85:15) was added to each tube, followed, by shaking on a Whirl mixer.
4. The tubes were covered and put in the deep-freezer at –20°C for a minimum of 10 minutes,
5. Then centrifuged at 5000 rpm (3900 × g) at 5°C for 10 minutes.
6. 500 µl of upper layer of all samples was transferred to a glass tube.
7. Evaporated to dryness at 40°C under a gentle stream of nitrogen.
8. The dry residue was then reconstituted with 100 µl of cold mobile phase.
9. The samples were analysed by LC-MS-MS.

Results
- 6-MAM disappeared in all samples.
- At room temperature preserved heart blood was stable for 5 days.
- At 5°C 6-MAM was stable for 3 days in all samples.
- 6-MAM was found to be stable at least for one month at temperatures of -18ºC.

Conclusion
Stability of 6-MAM decreased by 30% after 3 days in blood and after 10 days 6-MAM disappeared in all samples.
At room temperature preserved heart blood was stable for 5 days.
At 5°C 6-MAM was stable for 3 days in all samples.
6-MAM was found to be stable at least for one month at temperatures of -18ºC.
Our results showed that heart tissue is better than lung tissue to detect the stability of 6-MAM.
We recommend that 6-MAM stored at temperature of -18°C or less in order to reduce its degradation.

References

The Stability of 6-MAM in synthetic cardiac and femoral human blood
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Abstract
Heroin addiction is known to be a major problem all over the world and abused heroin is known as the main cause of drug related deaths. 6-monoacetylmorphine (6-MAM) is the main metabolite of heroin that is detected in forensic toxicology screening, however due to few studies, little is known about the stability of 6-MAM. Presence of 6-MAM in analysed sample is important to differentiate between heroin and morphine related deaths. We investigated the stability of 6-MAM in conditions likely to be encountered in any forensic laboratories, in two common organs heart and lung at pH (6-7.5). We analysed blood from heart and lung, because the heart is the main organ that distribute the blood all over the body, which was not analysed before for 6-MAM stability. Heroin has a pKa of 7.95 and generally weak lipophilic basic drugs with pKa more than 8 tend to accumulate in the lung so we use lung tissue for 6-MAM stability investigation. We recommend that 6-MAM should be stored at -18ºC or less in order to reduce its degradation.

Introduction
Heroin was first used medically in 1898 as an analgesic(1,2) Heroin is rapidly converted to 6-MAM within half-life (t 1/2 -15 minutes).(3) Heroin was first used medically in 1898 as an analgesic(1,2) Heroin addiction is known to be a major problem all over the world and abused heroin is known as the main cause of drug related deaths. 6-monoacetylmorphine (6-MAM) is the main metabolite of heroin that is detected in forensic toxicology screening, however due to few studies, little is known about the stability of 6-MAM. Presence of 6-MAM in analysed sample is important to differentiate between heroin and morphine related deaths. We investigated the stability of 6-MAM in conditions likely to be encountered in any forensic laboratories, in two common organs heart and lung at pH (6-7.5). We analysed blood from heart and lung, because the heart is the main organ that distribute the blood all over the body, which was not analysed before for 6-MAM stability. Heroin has a pKa of 7.95 and generally weak lipophilic basic drugs with pKa more than 8 tend to accumulate in the lung so we use lung tissue for 6-MAM stability investigation. Vitreous humour is not always possible to find in a deceased, as it’s well protected sample from post-mortem bacterial contamination and degradation, but not from drying.

We spiked all samples with the 6-MAM (15µl/ml). The amount of 6-MAM in all samples was quantitated using LC-MS-MS. 6-MAM stability was initially investigated at room temperature (RT), 5ºC and -18ºC. It was found to be unstable at RT. At 5ºC, 6-MAM was stable for 3 days in all samples. 6-MAM was found to be stable at least for a month at -18ºC. Morphine ratio on all samples has increased whilst ratio of 6-MAM decreased. We recommend that 6-MAM should be stored at -18ºC or less in order to reduce its degradation.