Int J Legal Med (1998) 111 : 165–167

SHORT COMMUNICATION

K. A. Hadidi · J. S. Oliver

Stability of morphine and buprenorphine in whole blood

Received: 20 January 1997 / Received in revised form: 17 October 1997

Abstract Delays between time of sampling and time of toxicological analysis are common, therefore the length of time that postmortem blood can be stored at various temperatures (e.g. 4°, 25°, –20° C) was evaluated for the effect on the stability of morphine and buprenorphine from day one up to one year. Solid phase extraction and GC-MS were used for the isolation and quantification of the drugs. Morphine and buprenorphine were found to be very stable for up to 6 months under these storage conditions, where at least 85% and 77% of morphine and buprenorphine respectively, were recovered. The study showed that a reasonable amount of the drugs (not less than 70%) was still detectable after one year of storage regardless of the temperature when blood samples were stored in silanized glass vessels.

Key words Morphine · Buprenorphine · Stability · Whole blood

Introduction

As a result of the increase in the abuse of drugs, both morphine and buprenorphine are frequently encountered in forensic cases [1–4] however the stability in blood has not been thoroughly investigated. Morphine may be converted to pseudomorphine in cadavers and found as such after exhumation [5]. Morphine was found to be stable in macerated liver when stored in sealed containers for a short period of time at ambient temperature [6]. A false positive morphine in putrefied tissue due to the presence of putrefactive products has been reported [7]. A recent report on morphine conjugate stability showed that morphine glucuronides are stable for a few days [8] and controversial data exist concerning the stability of morphine in urine samples [9, 10]. Buprenorphine is a recently marketed opiate and most of the published work relates either to the pharmacokinetics [11, 12] or analysis [13–16]. No data are available regarding its stability in blood, but some reports have evaluated the stability of other drugs in biological fluids stored for 1 year at various temperatures [17, 18]. In this study blood morphine and buprenorphine were prepared at levels likely to be encountered in forensic toxicology cases.

K. A. Hadidi (✉)
Forensic Medicine and Toxicology Division,
Faculty of Medicine, University of Jordan, Amman 11942, Jordan

J. S. Oliver
Department of Forensic Medicine and Science,
University of Glasgow, Glasgow, UK

Materials and methods

Materials

The solvents used were HPLC grade and other chemicals of analytical grade were supplied by BDH. Ion exchange columns SCX (Benzenesulfonylpropyl) were supplied by Analytichem International a division of Varian. Diatomaceous earth (Extrelut) was supplied by Merck (Germany) and free base drug standards were supplied by Sigma.

GC – MS

The separation of derivatized morphine, buprenorphine and their standards was achieved using Hewlett Packard model HP 5980 gas chromatography fitted with a capillary column (Chrompack CP-SIL5, 25 m × 0.32 mm internal diameter with 0.4 μm film thickness). Helium was used as a carrier gas, the linear velocity was approximately 60 cm through the column achieved by maintaining a column head pressure 5 psi. The injector temperature was 300° C, the initial oven temperature was 180° C maintained for 1 min and then increased at 10° C/min to 300° C and the final temperature was maintained for 10 min. The mass spectrometer used was a VG model 70-250S and high resolution selective ion recording was used to reduce interference in the analysis. The SIM channel were calibrated and tuned using perfluorokerosene (PFK) reference mass at (m/z 454) at a resolution of 1000.

Sample preparation

Samples of morphine (500 ng/ml) and buprenorphine (250 ng/ml) in blank postmortem blood were prepared from a stock methanolic standard solution of morphine (5 mg/ml) and buprenorphine (2.5 mg/ml). From the prepared samples 5 ml aliquots were transferred to pre-silanised hypo-vials then sealed with butyl rubber septa. All the glass vials were silanised with dimethylchlorosilane

[$H(CH_3)2SiCl$] and then washed with methanol to remove excess dimethylchlorosilane and left to dry before use.

Postmortem samples

As part of the standard autopsy procedure two blood samples were obtained from known morphine addicts. The morphine concentration was determined and the samples were then stored at –20° C for a few weeks before re-analysis for morphine concentration using the same analytical procedure.

Sample storage

For each measurement two vials each of spiked blood and blank blood were stored at different temperatures and for designated periods of time before re-analysis to investigate the effect of temperature and time interval on the drug concentration. The time intervals of storage between each analysis were 1, 2, 3, 6, 9 and 12 months at 4°, 25° and –20° C and two samples each of spiked blood and blank blood were removed from storage, opened and analysed at each designated period of time. The storage temperatures were monitored regularly through out the course of the experiment.

Extraction procedure

In a 6 ml screw-capped vial 1 ml of sample (blood/blank blood) and 0.1 ml of opiates internal standard (5 mg/ml D3-morphine, 2.5 mg/ml D2-buprenorphine) were added individually, then the samples were buffered with 1 ml of 0.1 M ammonia solution, mixed and allowed to stand for 2 min before being applied to the Extrelut (Merck) column containing 2 g of Extrelut washed with methanol/ethanol (1 : 1 v/v) and allowed to dry out completely before being introduced into a 10-ml plastic syringe.

The buffered specimens were applied to the Extrelut column and allowed to stand for 5–10 min and the column was then washed with 5 ml hexane. The Extrelut columns were attached to a 1 ml capacity SCX-column via adaptors. The SCX column was preconditioned with 2 × 1 ml methanol followed by 2 × 1 ml de-ionised water and 2 × 1 ml of ethyl acetate/isopropanol (9 : 1 v/v). Morphine and buprenorphine were then eluted from the Extrelut column with 12 ml of ethylacetate/isopropanol (9 : 1 v/v). The SCX column was washed with 5 ml acetonitrile/methanol (1 : 1 v/v). Morphine and buprenorphine were eluted from SCX column with 3 × 1 ml of 10% ammonia in acetonitrile/methanol (1 : 1 v/v) into screw-capped glass vials. The eluate was evaporated to dryness at 60° C under nitrogen gas stream then derivatized with 100 ml diethyltetramethyldisilazane as described [19]. A volume of 1 µl was injected into the GC-MS system.

Analysis

The separation of derivatized morphine, buprenorphine and their internal standards D3-morphine and D2-buprenorphine was achieved using GC-MS and two mass qualifiers were recorded for each drug. The derivatized morphine has the molecular ions (m/z 457) and (m/z 442) as the most abundant ions on the mass spectrum with a retention time of 10 : 43 and 10 : 42 for internal standard D3-morphine molecular ions (m/z 460) and (m/z 445). Derivatized buprenorphine has the most abundent molecular ions (m/z 464) and (m/z 422) with a retention time of 18 : 47 and 18 : 46 minutes for the internal standard D2-buprenorphine molecular ions (m/z 466) and (m/z 424). Other masses of the derivatized morphine and buprenorphine are (m/z 385, 301, 250, 210) and (m/z 553, 506, 207, 84) respectively.

Results and discussion

The procedure as described [19] has been found both sensitive and specific for the analyses of morphine and buprenorphine in blood. The sensitivity of the method for morphine and buprenorphine derivatives were 200 pg and 1 pg on-column, respectively. The specificity of the method was improved by using two mass qualifiers for each drug. The results were linear over the range 35–1140 ng/ml and 0.5–16.5 ng/ml with correlation coefficients (r = 0.99 and r = 0.98) for morphine and buprenorphine respectively. In calculating the recovered drugs, freshly prepared standard drug samples were used to calculate the concentration of the drug in the spiked samples where the initial quantitation (day one) was assigned a value of 100%.

Successive quantitations were made in duplicate and the percentage of the original amount present was calculated. The standard for significant breakdown of the drug to have occurred was a level measured which had decreased by greater than three standard deviations (S.D.) from the concentration at day 1 (measured concentration of 500 ± 60 ng/ml and 250 ± 45 ng/ml for morphine and buprenorphine respectively). The standard deviations for blood morphine and buprenorphine analysis were 4.0% and 6.2% respectively.

A comparison between the recoveries of blood morphine stored at the three storage temperatures (4°, 25°,

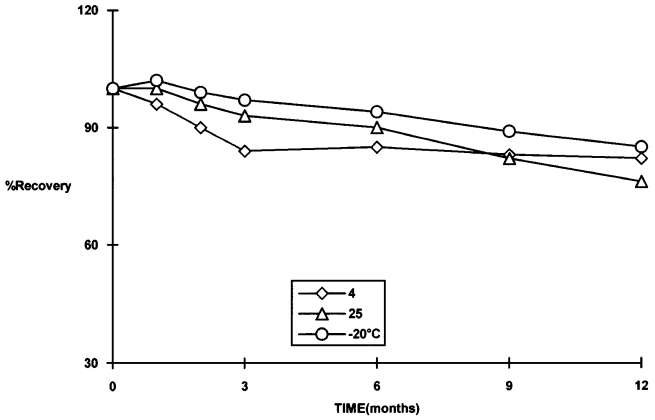

Fig. 1 Changes in blood morphine recovery with time (spiked samples)

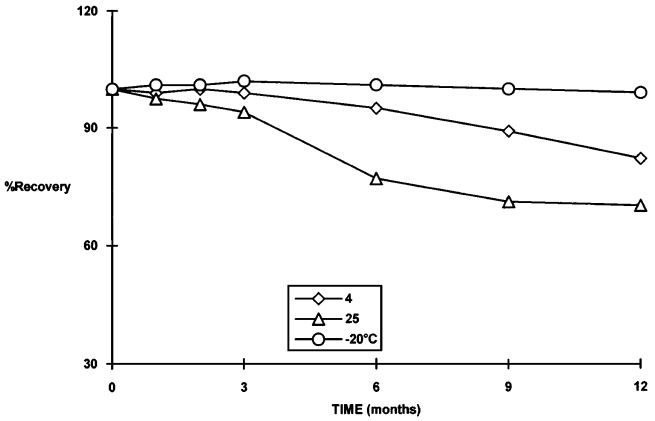

Fig. 2 Changes in blood buprenorphine recovery with time (spiked samples)

Table 1 Recovery of morphine from authentic postmortem samples after storage

Case	Measured concentration	Storage time and temperature	Concentration after storage	% Recovery after storage
1	340 ng/ml	4.5 months at –20° C	320 ng/ml	94%
2	175 ng/ml	3 months at –20° C	170 ng/ml	97%

–20° C) are displayed in Fig. 1. The first significant decrease (measured concentration of less than 440 ng/ml or recovery of less than 88%) in blood morphine concentrations was found after 3 months of storage at 4° C with a recovery of 84%, while samples stored at 25° and –20° C showed a significant decrease after 9 months with recoveries of 82% and 89% respectively. However, it was found that morphine in blood was relatively stable up to 1 year regardless of the storage temperatures and the recoveries were found to be in the range of 76–85%.

It is known that the quality of glass affects the stability of morphine solutions [20] as non-silanised glass allows the release of caustic materials increasing the pH and thus the rate of degradation. In this experiment, all the glass containers were silanised with dimethylchlorosilane before use to minimise the effect of this phenomena.

A comparison between the recoveries of blood buprenorphine kept at the three storage temperatures 4°, 25° and –20° C for up to one year, are shown in Fig. 2. A significant decrease (measured concentration of less than 205 ng/ml or recovery of less than 82%) in blood buprenorphine concentration was found only after 6 months of storage at 25° C with a recovery of 77%. However, samples stored at 4° C showed a significant decrease after only 12 months were 82% of the drug was recovered, while samples stored at –20° C for the same period of time showed recoveries of 99%. After 1 year a reasonable amount of buprenorphine was recovered from blood at the three storage temperatures in the range of 70–99%.

The recovery rates of morphine from the authentic postmortem samples are presented at Table 1. The samples showed no changes in the morphine concentration after 4.4 and 3 months (cases 1 and 2) with recoveries of 94% and 97% respectively. The authentic postmortem morphine samples were found to be stable and consistent with our findings on the spiked whole blood morphine.

In conclusion, morphine in blood was found to be reasonably stable regardless of the storage time and temperature with an expected recovery ranging from 85–94% and 76–80% after 6 months and 1 year of storage respectively. Storage temperatures have an effect on buprenorphine stability regardless of the storage time and as the temperature increased less buprenorphine was recovered. However, buprenorphine in blood was found to be reasonably stable at the three storage temperature with a recovery ranged from 77–101% and 70–99% after 6 months and 1 year regardless of the storage temperature. The drugs studied showed a steady and slow decline in concentration with time and both were found to be easily detected in blood even after 1 year of storage regardless of the storage temperatures. This finding provides important information for forensic toxicologists in cases which have been re-opened after a period of time and meaningful analyses for these drugs can be carried out after up to 12 months of sample storage if the blood samples have been stored in silanized glass vessels.

References

1. Musshof F, Daldrup T (1993) Evaluation of a method for simultaneous quantification of codeine, dihydrocodeine, morphine, and 6-monoacetylmorphine in serum, blood and postmortem blood. Int J Legal Med 106 (2): 107–109
2. Kaa E, Teige B (1996) Drug-related deaths during the 1980s. Int J Legal Med 106 (1): 5–9
3. Kauert G, Rohrich J (1996) Concentration of delta 9-tetrahydrocannabinol, cocaine and 6-nonacetylmorphine in hair of drug abuser. Int J Legal Med 108 (6): 294–299
4. Hand CW, Ryan KE, Dutt SK, Moore RA (1989) Radioimmunoassay of buprenorphine in urine: study in patients and in drug clinic. J Anal Toxicol 13: 100–104
5. Moffat AC (ed) (1986) Clark's isolation and identification of drugs, 2nd edn. The Pharmaceutical Press, London, pp 790–791
6. Stevens HM (1984) The stability of some drugs and poison in putrefying human liver tissues. J Forensic Sci Soc 24: 577–589
7. Christopoulos GN, Chen NW, Toman TJ (1975) Separation of barbiturates and morphine analysis from putrefied post-mortem tissue. J Chromatogr 106: 446–453
8. Skopp G, Lutz R, Ganssmann B, Mattern R, Aderjan R (1996) Postmortem distribution pattern of morphine and morphine glucuronides in heroin overdose. Int J Legal Med 109 (3): 118–124
9. Dugan S, Bogema S, Schwartz KW, Lapps NT (1994) Stability of drugs of abuse in urine samples stored at –20 degrees. J Anal Toxicol 18 (7): 391–396
10. Lin DL, Liu H, Chen CY (1995) Storage temperature effect on the stability of morphine and codeine in urine. J Anal Toxicol 19 (5): 275–280
11. Bullingham, RES, McQuay HJ, Moore A, Bennet MRD (1980) Buprenorphine kinetics. Clin Pharmacol Ther 28 (5) 667: 672
12. Garrett ER, Chandran VR (1990) Pharmacokinetics of morphine and its surrogates X: analysis and pharmacokinetics of buprenorphine in dogs. Biopharm Drug Dispos 11: 311–350
13. Hand CXW, Baldwin D, Moore RA, Allen MC, McQuay HJ (1986) Radioimmunoassay of buprenorphine with iodine label; analysis of buprenorphine and metabolites in human plasma. Ann Clin Biochem 23: 47–53
14. Mello NK, Mendelson JH (1980) Buprenorphine suppresses heroin use in heroin addict. Science 207: 567–569
15. Kay B (1978) A double-blind comparison of morphine and buprenorphine in the prevention of pain after operation. Br J Anaesth 50 (6): 605–609
16. Bartlett AJ, Lloyd-Jones JG, Rance MJ et al, (1980) The radioimmunoassay of Buprenorphine. Eur J Clin Pharmacol 18: 339–345
17. Al-Hadidi KA, Oliver JS (1995) Stability of temazepam in blood. Sci Justice 35 (2): 105–109
18. Howard PJ (1978) The stability of diazepam in plasma samples when stored under vary conditions. J Pharm Pharmacol 30: 136
19. Battah AH (1989) The analysis of drugs and solvent in forensic toxicology by combined GC and LC-MS. Thesis, University of Glasgow
20. Yeh S, Lach JL (1961) Stability of morphine in aqueous solution III. Kinetics of morphine degradation in aqueous solution. J Pharmaceut Sci 50 (1): 35–42