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ORIGINAL ARTICLE

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Stability of trihexyphenidyl in stored blood and urine specimens

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Abstract Trihexyphenidyl (THP) is an anticholinergic agent with forensic toxicological interest. The stability of THP was studied in postmortem blood and urine samples at a concentration of 0.25 μg/ml under different storage temperatures. After solid phase extraction (SFE), THP was measured by gas chromatography. On day zero and at intervals over a 6 months period, there was no significant loss of THP at the storage temperatures –20° C and 4° C in the spiked and authentic samples. Blood and urine samples stored at 25° C showed a maximum recovery loss (about 14%) of THP after 3 months of storage. This loss was considered a significant change and corresponded to a P value < 0.046. The study demonstrates that the analysis of blood and urine samples containing THP would produce consistent results when they are stored for 6 months at –20 or 4° C and for 3 months at 25° C.

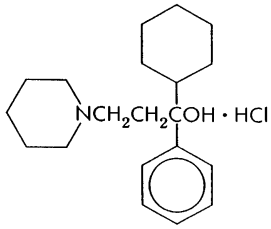
Key words Trihexyphenidyl · Stability · Blood · Urine

Introduction

The knowledge of the fate of a drug in biological samples stored under different conditions is of prime importance for the correct interpretation of the result. There are many studies in the literature on the issue of sample storage and putrefaction on substances of forensic interest [1–10]. Also such literature is continuously enriched with the emergence of new drugs.

Trihexyphenidyl (THP, benzhexol) is an anticholinergic drug used in the treatment of parkinsonism, particularly the tremor that is characteristic of the disease [11]. The chemical structure of THP is shown in Fig. 1. The pharmacokinetics of THP in humans have been evaluated [12–14] and it is rapidly absorbed after oral administration, producing peak plasma levels at a mean of 1.3 ± 0.2 h [13]. The half life ($t^{1}/_{2}$) of THP in plasma in normal volunteers was reported to be 10.1 ± 4.5 h after a 5 mg oral dose [12]. Hydroxy-THP was reported as the major metabolite present in plasma and urine and accounted for two-thirds of the THP present in urine [15]. The drug is of forensic toxicology interest due its frequent abuse [16–21] and a reported overdose [22]. In Jordan, the abuse of benzhexol has also been noticed with increasing frequency in recent years amongst Jordanian youths. In general, the abuse potential of THP is attributed to its euphoric and hallocinogenic properties [11, 23]. Symptoms of an over dose are manifested as drug-induced toxic psychosis and include euphoria, disorientation, hallocination and paranoid ideation, mydriasis, warm skin, dry mouth, tachycardia, ataxia, constipation and absent bowel sound [23]. There are no available data regarding the stability of THP in biological fluids. This article presents a study on the effect of different storage temperatures on the stability of THP in postmortem blood and urine. Solid phase extraction procedures were applied during sample preparations [24–30].

Fig. 1 The chemical structure of trihexyphenidyl hydrochloride



Material and methods

Blood and urine samples

An aliquot of a methanolic standard of THP was added to a glass container and evaporated to dryness at 40° C under a gentle stream of nitrogen. No breakdown products occurred during this process. The residue was reconstituted with blood obtained from cadavers within 24 h after death. The blood was tested for THP before reconstitution and after an initial quantification the THP concentra-

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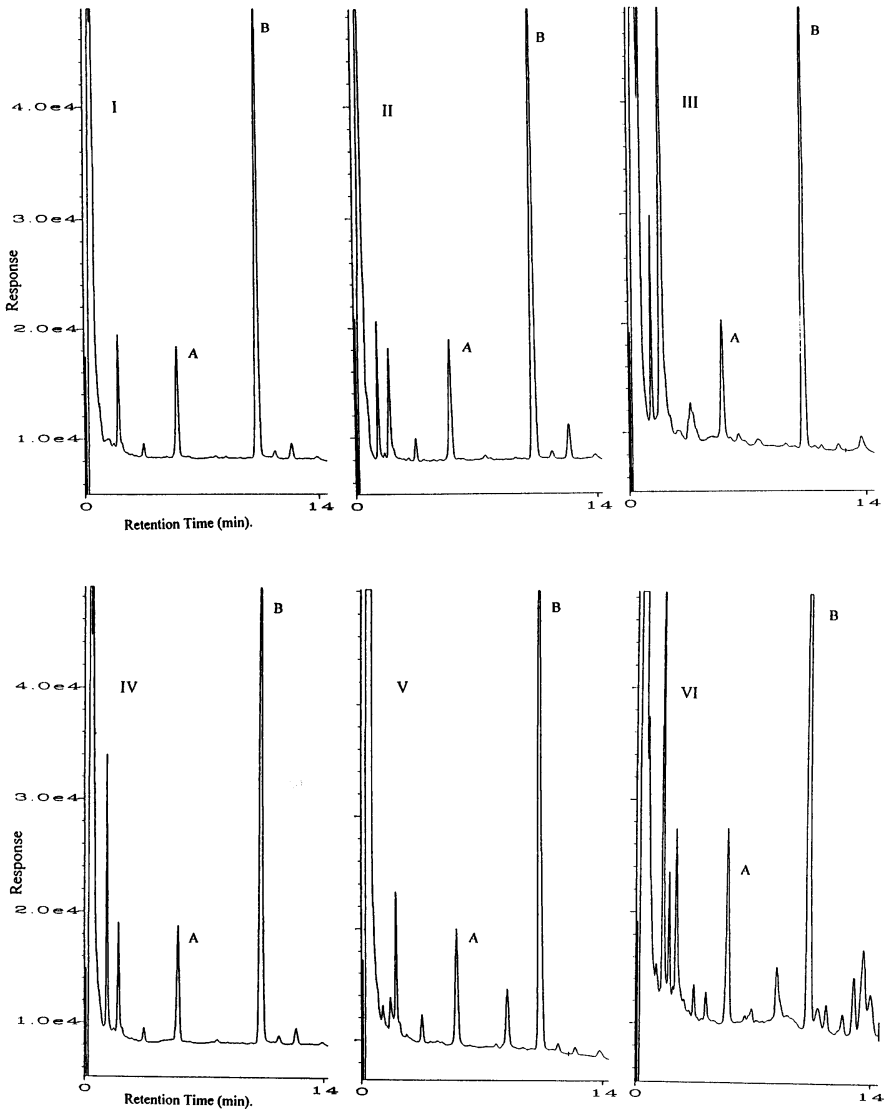

Fig. 2 Gas chromatographic chromatograms of extracted trihexyphenidyl (A) and internal standard (Papaverine) (B) from blood (I, II, III) and urine (IV, V, VI) specimens which were stored for 3 months at –20° C, 4° C and 25° C respectively

tion was 0.25 µg/ml. The blood was then divided into three portions: the first portion was stored in a glass stopper flask at room temperature, the second portion was stored in a glass stopper flask at 4° C, the third portion was divided into aliquots of 3 ml portions in glass vials and stored at –20° C.

Materials and reagents

Solvents were of HPLC grade and reagents were of analytical grade. The extraction columns Isolute (confirm HCN 130 mg columns) were obtained from International Sorbent Technology (IST).

Instrumentation

The gas chromatograph (GC) used was a Hewlett-Packard HP 5890 series II plus model, equipped with a nitrogen phosphorus detector and an HP1 5 m × 0.53 mm with a 2.65 µm film thickness column. The carrier gas (nitrogen) flow rate was set at 10 ml/min through an electronic pressure controller. The GC conditions were set at injector port 260° C, detector 290° C and the oven temperature was programmed from 180° C for 2 min and increased by 8° C/min to 280° C and held for 3 min. The chromatogram was monitored and integrated on HP-chemstation.

Blood extraction

The SPE column Confirm HCX was placed onto a vacuum manifold system and was conditioned with 2 ml methanol, 2 ml de-ionized water and 1 ml phosphate buffer (0.1 M, pH 6.0). Blood samples (1 ml) were extracted by adding 0.1 ml internal standard (Papaverine 5 µg/ml) and 5 ml phosphate buffer (0.1 M, pH 6.0) to an extraction glass tube and vortexing for 1 min. The mixture was poured onto a previously conditioned SPE column. The flow rate of the eluate was controlled at 1–2 ml/min. The column was rinsed with 1 ml of acetic acid (0.1 M) followed by drying the column under vacuum for 5 min. The column was rinsed with 5 ml acetonitrile followed by vacuum drying for 2 min. Trihexyphenidyl and the internal standard were eluted with 2 ml 2% ammonium hydroxide (28%) in ethyl acetate. The eluate was evaporated to dryness under a gentle stream of nitrogen at 40° C on a heating mantle. Each sample was dissolved with 50 µl of ethyl acetate and 2 µl was used for GC analysis.

Urine extraction

The extraction procedure for urine samples was conducted similarly to that described for blood with the exception that urine was mixed with 1 ml of phosphate buffer (0.1 M, pH 6.0) instead of 5 ml.

Schedule of analysis

Samples of blood and urine were analysed weekly for the first month, followed by every two weeks for the second and third months and monthly for the fourth, fifth and sixth months. The results were compared with freshly prepared standards and reported as the relative recovery to that at day zero.

Table 1 Relative recoveries of trihexyphenidyl from spiked blood samples at a concentration of 0.25 μg/ml and stored at different storage temperatures

Time of analysis	Relative recovery (%) of blood stored at		
	4° C	25° C	–20° C
Day zero	100.0	100.0	100.0
1st week	97.0	103.2	93.1
2nd week	100.2	103.6	93.4
3rd week	95.5	99.1	97.8
4th week	90.8	97.9	93.3
6th week	93.2	99.1	95.6
8th week	92.5	95.1	101.1
10th week	89.3	91.2	97.7
3rd month	91.1	86.3	96.6
4th month	90.8	88.7	92.3
5th month	90.3	86.5	94.7
6th month	91.5	87.2	93.1

Table 2 Relative recoveries of trihexyphenidyl from spiked urine samples at a concentration of 0.25 μg/ml and stored at different storage temperatures

Time of analysis	Relative recovery (%) of urine stored at		
	4° C	25° C	–20° C
Day zero	100.0	100.0	100.0
1st week	94.1	107.1	99.6
2nd week	96.3	105.1	103.1
3rd week	97.4	103.4	98.6
4th week	100.2	100.2	101.5
6th week	94.4	97.4	105.2
8th week	95.6	98.4	99.7
10th week	93.8	91.6	96.5
3rd month	93.2	91.2	97.8
4th month	92.2	88.9	95.2
5th month	91.5	86.4	97.1
6th month	93.5	87.7	94.8

Results and discussion

Since the time from sample collection and analysis or repeat analysis might vary, factors affecting the analyte in the sample should be considered before interpreting the results as storage conditions could seriously affect the level of the analyte. Factors which lead to these changes are bacterial contamination and putrefaction [2, 18, 31, 32], temperature [6], the analyte medium and its pH [23, 33, 34] and light [35]. In this experiment temperature conditions were used which resemble the conditions under which authentic samples are usually kept. The experiments were conducted on spiked blood and urine samples since no authentic postmortem specimens containing THP were available. Trihexaphenidyl was selected to be monitored due to its long half life in blood [12] and that it is one of the THP constituents excreted in urine [15]. The method of analysis of THP has been described elsewhere [36] and the extraction recovery was more than 90%. The intra- and interassay coefficient of variation of the method was found to be 5.5 and 6.2% respectively. Figure 2 shows GC chromatograms of extracted THP and the internal standard from blood and urine specimens which were stored for 3 months at the three temperature conditions of the experiment. There were no decomposition products in the GC chromatogram which interfered with trihexyphenidyl or its internal standard in the spiked blood and urine samples over the storage period.

The results of analysis of blood and urine samples spiked with THP at concentration of 0.25 μg/ml and stored over the 6-month period at three temperatures are presented in Tables 1 and 2. The results were considered to be significant if there was a loss of the THP recovery > 1.96 of the coefficient of variation where the probability (*P* value) would be < 0.05 [37]. The data showed that there was no significant loss of THP recoveries in blood and urine samples stored at either 4° C or –20° C. Samples stored at –20° C showed the most apparent stability of THP. Blood and urine samples stored at 25° C started to show significant THP loss after 3 and 4 months of storage with a P value < 0.046. It is concluded that THP was stable over the 6-month period at the cold storage temperatures tested. During the experimental time period there was an opportunity to test blood and urine samples which were taken from patients receiving THP as medication. After the first analysis, fractions of the samples were kept at 4° C. The samples were reanalysed after 3 and 6 months, and the results of the analysis were consistent at all times (Table 3).

Table 3 The results of analysis of blood and urine samples taken from patients receiving trihexyphenidyl, stored at 4° C and analyzed at different intervals

Case no.	Sex	Age (years)	Dose	Blood conc. ng/ml			Urine conc. ng/ml		
				Day zero	Three months	Six months	Day zero	Three months	Six months
1	M	55	2 mg × 3	7	8	8	85	87	89
2	M	45	5 mg × 3	18	16	17	59	57	60
3	F	47	5 mg × 3	13	13	14	89	92	90
4	F	35	5 mg × 3	18	17	18	173	167	172

In conclusion this study demonstrates that THP present in blood or urine samples stored at –20 or 4° C is stable up to 6 months and up to three months at 25° C.

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References

1. Kojina T, Nishiyama Y, Yashiki M, Une I (1982) Postmortem formation of carbon monoxide. Forensic Sci Int 19 : 243–248
2. Tsunenari S, Yonemitsu K, Uchimura Y, Kanda M (1981) The influence of putrefactive changes on the determination of paraquat in autopsy material. Forensic Sci Int 1981 17 : 51–56
3. Lin DL, Liu H, Chen CY (1995) Storage temperature effect on the stability of morphine and codeine in urine. J Anal Toxicol 19 : 275–280
4. Dugan S, Bogema S, Schwartz RW, Lappas NT (1994) Stability of drugs of abuse in urine samples stored at –20 degrees C. J Anal Toxicol 18 : 391–396
5. Hippenstiel M, Gerson B (1994) Optimization of storage condition for cocaine and benzylecogenine in urine: a review. J Anal Toxicol 18 : 104–109
6. Al-Hadidi KA, Oliver JS (1995) Stability of temazepam in blood. Sci Justice 35 : 105–108
7. Chetty M (1994) The stability of anticonvulsant drugs in whole blood. Ther Drug Monit 16 : 491–494
8. Baselt RC, Yoshikawa D, Chang J, Li J (1993) Improved long term stability of blood cocaine in evacuated collection tube. J Forensic Sci 38 : 935–937
9. Bright JE, Inns RH, Tuckwell NJ, Marrs TC (1990) The effect of storage upon cyanide in blood samples. Hum Exp Toxicol 9 : 125–129
10. Skopp G, Lutz R, Ganssmann B, Mattern R, Aderjan R (1996). Post mortem distribution pattern of morphine and morphine glucuronides in heroin overdose. Int J Legal Med 109 : 118–124
11. Reynolds JEF (ed) (1993) Martindale. The extra pharmacopeia. The Pharmaceutical Press, London, p 421
12. Cheung WK, Stravinski SS, Engel S, Aia LL, Yacobi A, Silber M (1988) Pharmacokinetic evaluation of a sustained-release formulation of trihexyphenidyl in healthy volunteers. J Pharmaceut Sci 77 : 748–750
13. Burke RE, Fahn S (1985) Pharmacokinetic of trihexyphenidyl after short term and long term administration to dystonic patients. Ann Neurol 18 : 35–40
14. He H, McKay G, Wirshing B, Midha K (1995) Development and application of specific radioimmunoassy for trihexyphenidyl to pharmacokinetic study in human. J Pharmaceut Sci 84 : 561–567
15. Nation RL, Triggs EJ, Vine J (1978) Metabolism and urinary excretion of benzhexol in human. Xenobiotica 8 : 165–169
16. Cranshaw JA, Mullen PE (1984) A study of benzhexol abuse. Br J Psychiatr 145 : 300–303
17. Pullen GP, Best NR, Maguire J (1984) Anticholinergic drug abuse: a common problem? B M J 289 : 612–613
18. Bachrich PR (1964) New drugs of addiction? B M J 1 : 834–835
19. Harrison G (1980) The abuse of anti-cholinergic drugs in adolescents. Br J Psychiatr 137 : 495
20. Lowry TP (1977) Trihexyphenidyl abuse. Am J Psychiatr 134 : 1315
21. Goggin DA, Solomon GF (1979) Trihexyphenidyl abuse for euphorigenic effect. Am J Psychiatr 136 : 459–460
22. Gall JA, Drummer OH, Landgrem AJ (1995) Death due to benzhexol toxicity. Forensic Sci Int 71 : 9–14
23. Crabtree BL (1989) Substance use disorders. In: DiPiro JT, Talbert RT, Hayes PE, Yee GC, Posey LM (eds) Pharmacotherapy. A pathophysiologic approach. Elsevier, New York, pp 697–713
24. Platoff GE Jr, Gere JA (1991) Solid phase extraction of abused drugs from urine. Forensic Sci Rev 3 : 117–132
25. Scheurer J, Moore CM (1992) Solid-phase extraction of drugs from biological tissue – A review. J Anal Toxicol 16 : 264–269
26. Chen X-H, Frank JP, deZeeuw RA (1992) Solid phase extraction for systemic toxicological analysis. Forensic Sci Rev 4 : 147–159
27. Musshoff 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 : 107–109
28. Kojima T, Taniguchi T, Yashiki M, Miyazaki T, Iwasaki Y, Mikami T, Ohtani M (1994) A rapid method for detecting barbiturates in serum using EI-SIM. In J Legal Med 107 : 21–24
29. Ishii A, Kumazawa T, Nishikawa M, Watanabe K, Suzuki O (1996) Simple and sensitive detection of phenylcyclidine in body by gas chromatography with surface ionization detection. Int J Legal Med 108 : 244–247
30. Geier A, Bergemann L, Meyer LV (1996) Evaluation of a solid phase extraction procedure for the simultaneous determination of morphine, 6-monoacetyl morphine, codeine and dihydrocodeinein plasma and whole blood by GC/MS. Int J Legal Med 109 : 80–83
31. Levine BS, Blanke RV, Valentour JC (1983) Postmortem stability of benzodiazepine in blood and tissue. J Forensic Sci 28 : 102–115
32. Levine BS, Blanke RV, Valentour JC (1984) Postmortem stability of barbiturates in blood and tissue. J Forensic Sci 2 : 131–138
33. Winek CL, Esposito FM, Cinicola DP (1990) The stability of several compounds in formalin fixed tissue and formalin-blood solutions. Forensic Sci Int 44 : 159–168
34. Mendez-Alvarez E, Soto-Otero R, Sierra-Marcuno G (1986) The effect of storage conditions on stability of carbamazepine and carbamazepine-10, 11-epoxide in plasma. Clin Chim Acta 154 : 243–246
35. Levine B, Smith ML (1990) Stability of drugs of abuse in biological specimens. Forensic Sci Rev 2 : 147–156
36. Hadidi KH (1997) Solid-phase extraction of benzhexol from blood and urine. Sci Justice 37 : 265–268
37. Swinscow TDV (1976) Statistics at square one. VII-Statements of probability. B M J 2 : 33–34