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Studies on Different Hydrolysis Procedures for Drugs of Abuse in Hair

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Abstract: Different hydrolysis and extraction procedures have been studied for the determination of drugs of abuse in hair samples. The results showed that acid and enzymatic hydrolysis gave high extraction recoveries for 6-monoacetylmorphine, morphine, codeine and heroin. Enzymatic hydrolysis was more expensive. After alkaline hydrolysis, 6-monoacetylmorphine, a unique proof of heroin abuse, could not be detected and after methanolic extraction, the extract was dirty and the obtained chromatogram showed an interfering background. It is concluded that acid hydrolysis is the extraction method of choice.

Keywords: Drugs of abuse, hydrolysis, hair.

1. Introduction

Drugs of abuse have become a major problem in our society in recent years and have resulted in widespread abuse. The detection of drugs of abuse in biological samples is one of the primary functions of a clinical or forensic toxicology laboratory. Particularly in cases where the results are relevant to legal proceedings, confirmatory tests are essential to ensure the accuracy of results. Hair analysis for drugs of abuse has been proposed as an alternative to urine drug testing because hair analysis may be considered less invasive and more difficult to evade than urine testing. In addition, proponents of hair analysis claim that it provides a calendar or time line of drug use, as well as a means of evaluating the extent of drug use. With the increased marketing of hair analysis for drug testing, a number of questions have been raised regarding the reliability of hair analysis to accurately detect the use of drugs. It is necessary to develop a practical method for extracting drugs of abuse in hair. The technique must accurately determine what drugs are present and at what level. Morphine (MO), codeine (CO), 6-monoacetylmorphine (6-MAM) and heroin can be extracted after hydrolysis by acid[1-2], alkaline[3], enzyme[4] or directly by methanol[5]. Although many papers have been published about these extraction procedures, only a few have compared their efficiencies[6]. Therefore, the present studies were undertaken to develop a method for extraction and analysis. The samples obtained from drug abusers were tested by various analytical approaches to compare extraction efficiencies for morphine, codeine, 6-MAM and heroin.

2. Methods

2.1 Materials

Methanol was of HPLC grade and all other reagents used in sample extraction and analysis were of analytical grade and obtained from Beijing Chemical Plant. Codeine, morphine, 6-monoacetylmorphine and heroin were used as standards and purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R.China).

0.1mol/L acetate buffer (pH 4.0) was prepared by adding 570μl of glacial acetic acid and 80ml of deionized water into a 100ml volumetric flask. Mix and add 1.6ml of 1.0mol/L potassium hydroxide. Make up to volume with deionized water and mix well.

75mmol/L phosphate buffer (pH 6.8) was prepared by using 1.0349g NaH_2PO_4 and 70ml deionized water, the pH was adjusted to 6.8 with 1mol/L NaOH and the solution was made up to 100ml with deionized water.

Solid phase extraction (SPE) column, Bond Elut SPE 3cc/130mg, was obtained from Varian (Harbor City, CA, USA)

2.2 Extraction

Approximately 400mg of hair samples was cut into about 1-mm segments and decontaminated twice in 1ml of methanol for 1min at room temperature. After the methanolic wash procedure, the hair samples were dried and divided into 4 parts and 100mg of each was incubated under the following conditions in the presence of 50μg of internal standard ethylmorphine: (1) Acid hydrolysis: 1ml of 0.1mol/L HCl for 24h at 40℃; (2) Alkaline hydrolysis: 1ml of 1mol/L NaOH for 10min at 100℃; (3) Enzymatic hydrolysis: 1ml of 75mmol/L phosphate buffer (pH 6.8) containing β-glucuronidase at 2500 units/ml for 1h at 37℃; (4) Direct methanolic extraction: 5ml of methanol, sonication for 5h.

After incubation, for direct methanolic extraction, it was necessary to evaporate the methanol to dryness before the extraction and dissolution with 100μl of 0.1mol/L HCl, all other samples were extracted according to the following procedure: Two milliliters of methanol and 2ml of 75mmol phosphate buffer (pH 6.8) were passed through a small Bond Elut SPE column sequentially without allowing the sorbent to dry. The pH value of extracts should be between 8.0 and 9.0. If not, adjust the pH with 10mol/L KOH or 1.0mol/L HCl. The extracts of hydrolyzed samples were added to the column and allowed to pass through by gravity. The column was washed with 1ml of 0.1mol/L acetate buffer (pH 4.0), 2ml deionized water and 2ml methanol and then allowed to dry for 5min at full vacuum. Finally, the drugs were eluted with 3ml of elution solvent—methylene chloride: isopropyl alcohol (8:2) with 2% ammonium hydroxide. The eluate was collected in screw-cap tubes and evaporated to dryness. The residue was finally dissolved in 100μl of HPLC mobile phase and a 20μl aliquot was injected into the chromatographic system.

2.3 HPLC

This work was performed on a Shimadzu LC-4A HPLC with SPD-2AS UV detector. A Zorbax C8 column (10 μm, 25 cm×4.6 mm ID) was obtained from DuPont, USA. Data manipulation was achieved with a Shimadzu C-R2AX Chromatopac. The mobile phase consisted of 0.05 mol/L potassium dihydrogen phosphate buffer-methanol-diethylamine-orthophosphoric acid (73:27:0.5:0.5 V/V). The flow rate was 0.8 ml/min. The eluate was monitored at 220nm[7].

3. Results and Discussion

For reproducible results, it is desirable to quantitatively extract all of the drugs or metabolites from the hair. Complete dissolution of the hair would be preferential in order to ensure that all of the bound analytes are released from the hair and measured. Studies have shown that the amount of drug extracted from the hair with time with this technique reaches a plateau. Reextracting the sample a second time showed only negligible amounts of the analytes, suggesting that the first extraction was efficient.

Because of heroin's inherent pharmacological and chemical characteristics, it is known to have an extremely short half-life (approximately 5 min) and rapidly metabolized to 6-acetylmorphine and morphine[5], especially at elevated pH. 6-MAM is a unique proof of heroin abuse. The identification of 6-MAM in biological specimens indicates use of heroin. 6-MAM has also a short half-life (approximately 45 min). **Table 1** shows that extraction recoveries are dependent on the methods.

Table 1. Recoveries of drugs by different hydrolysis procedures(%)

		Heroin	6-MAM	Morphine	Codeine
Acid hydrolysis	Recovery	79.8	71.7	113.8	92.6
	RSD	5.7	7.4	6.8	7.8
Alkaline hydrolysis	Recovery	—	56.8	134.2	86.5
	RSD	—	6.8	6.3	7.4
Enzymatic hydrolysis	Recovery	75.5	85.4	106.4	93.8
	RSD	7.8	7.3	5.9	6.8
Methanol extraction	Recovery	63.1	63.7	72.9	78.9
	RSD	7.6	6.9	6.7	7.3

Alkaline hydrolysis can dissolve most hair samples. But at these high pHs, 6-MAM, a unique proof of heroin abuse, is hydrolysed to morphine, making measurement of heroin impossible. It is therefore impossible to differentiate between medical intake of CO and MO from heroin abuse. Therefore the alkaline sample preparation procedure could not be recommended .

The acid method and enzymatic hydrolysis gave higher recoveries than the direct methanolic extraction for CO, MO, 6-MAM and heroin. Acid preparation took more time compared with enzymatic preparation. However, the latter is more expensive. Direct methanol extraction was less time consuming compared with acid preparation; it is also less expensive; and the extracts can be injected directly into a HPLC column after

evaporation. But the extracts were dirty and there was background noise on the chromatograms. Therefore, it was necessary to purify the dried extracts by the same extraction procedure before injection. Because alkaline hydrolysis could not detect the presence of 6-MAM, direct methanolic extraction produced dirty chromatograms, and enzymatic hydrolysis was more expensive, acid hydrolysis was preferred as an effective and accurate extraction method.

References

1. M. J. Welch, L. T. Sniegoski, C. C. Allgood, and M. Habram, *J Anal. Toxicol*., **1993**, *17*, 389.
2. S. D. Ferrara, L. Tedeschl, G. Frison, and F. Castagna, *J Anal. Toxicol*., **1992**, *16*, 217.
3. H. Sachs, R. Denk, and I. Raffi, *Int. J Leg. Med*., **1993**, *105*, 247.
4. M. R. Moeller, and P.Fey, *J Anal. Toxicol*., **1992**, *16*, 291.
5. B. A. Goldberger, E. J. Cone, T. M. Grant, et.al, *J Anal. Toxicol*., **1994**, *18*, 22.
6. V. Cirimele, P. Kintz, and P. Mangin, *Biomed. Chromatogr*., **1996**, *10*, 179.
7. C. Ma, H. J. Duan, Y. Q. Xu, and T. H. Zhou, *Chin. Chem. Lett*., **1996**, *7*,1027.

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