Screening of Psychotropic Drugs in Human Hair Based on High-Performance Thin-Layer Chromatography and Microliquid Extraction

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Abstract

The monitoring of the physiological and functional state of personnel from dangerous industries is very important for the ensuring of ecological security. Usually, this involves testing for the consumption of alcohol and drugs of abuse. During the last several years the use of legal psychotropic drugs has increased and the control over these drugs has became an actual problem. As is well known, the long-term history of drugs present in the body is accessible through hair analysis. This is why the aim of our research is to create a screening procedure based on coupling high-performance thin-layer chromatography with microliquid extraction as a psychotropic drug testing method for hair. Some widely distributed antidepressants, neuroleptics, and sedative drugs are chosen for research. The optimal experimental conditions for all of the consequent steps for the screening detection of the model samples are determined. The visual and densitometric detection limits allow for the employment of the proposed technique for a fast and cost-effective analysis of the drugs of abuse.

Introduction

The monitoring of the physiological and functional state of personnel from dangerous industries is very important not only in the health protection sphere. The effectiveness of such control is closely connected with the problem of ecological safety.

In spite of the automation scale of the role of personnel quality, the so-called “human factor”, the providing of a high degree of safety in modern industry still remains very important. Certainly, the constant monitoring of personnel qualification and their training reduces the range of risk when the responsible decision is adopted. It seems likely that this side of the problem is the most methodologically developed. However, the practice points out the fact that these procedures alone are not obviously sufficient in order to avoid unexpected and extreme situations. However, the problem concerning the current monitoring of the psychological state of personnel employed in the most dangerous ventures has to be paid more attention (2).

A usual approach to the monitoring of individual personnel physiological states is based on drugs of abuse and alcohol control. At the moment, society has become increasingly aware of the impact of drug use on public safety and its financial impact on industry because of losses in time and productivity. An expert has estimated that the annual loss of productivity of employees is $100 billion for alcohol and drug abuse. However, during the last several years the use of legal psychotropic drugs has grown and the control of these drugs has also become an actual problem (3).

The conventional procedure for drug abuse detection has some fundamental limitations: (*a*) it is directed only towards illicit drugs but not tranquilizers, barbiturates, and antidepressants; (*b*) a low regularity of personal control (not more than three times per year); (*c*) the dominant samples are urine and blood, and such samples as hair are practically not in use; (*d*) a low sensitivity of the conventional high-performance thin-layer chromatographic (HPTLC) technique; (*e*) a low productivity of confirmative methods for semivolatiles based on gas chromatography–mass spectrometry (MS), liquid chromatography–MS, and time-consuming sample preparation (4–6); and (*f*) a high cost and low reliably of screening based on immunoassay (7–9).

According to this list, the new approach should be developed to include a fast, cost-effective, and reliable individual monitoring of drugs of abuse and related compounds for staff involved in the most dangerous ventures. Our work deals with the study of a required approach based on the screening of psychotropic drugs in human hair.

Experimental

As a model, sample hair from nonaddicted subjects were chosen.

The model compounds of this investigation were amitriptyline (AMI), levomepromazine (LEV), and diphenhydramine (DIF) (structures listed in Table I). These are widely distributed species of antidepressants, neuroleptics, and sedative drugs. The standards were received in medicine form as water solutions of AMI hydrochloride (Lechiva, Prague, Czech Republic), LEV hydro-

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chloride (EGIS, Budapest, Hungary), and DIF hydrochloride (Biostimulator, Odessa, Ukraine). Stock solutions were prepared by the dilution of standard solutions.

For the creation of an appropriate pH, 0.1M HCl and 0.1M NaOH were used.

Analytical-grade hexane was used for normal and microliquid extraction. Microliquid extraction was carried out in a special device (Figure 1).

Precoated silica-gel layers on glass sheets (10 × 10 cm) type Silica Gel F-254 with a preconcentration zone were purchased from Merck (Darmstadt, Germany). Chromatograms were developed in a horizontal tank (Desaga, Germany).

Marqui's and Dragendorff's reagents were used for the visual detection of HPTLC plates (10).

Different solvents were used during the selection of the mobile phase for HPTLC separation. All of them were analytical grade. Densitometric measurements were performed with the Camag (Muttenz, Switzerland) TLC Scanner 3.

Spectra of water and organic solutions of AMI, LEV, and DIF were registered on the spectrophotometer 'SF-46' (LOMO, St. Petersburg, Russia). This device was used for the investigation of the extraction process from water into hexane for every substance individually. Spectrophotometric data are presented in Table I.

Table I. Model Compounds and Their Characteristics for Spectrophotometric Determination

Structure	Name	λ_{max} (nm)	ε ($mole^{-1}cm^{-1}$)
C, CH, $N(CH_3)_2$	AMI	245	$(1.5 \pm 0.1) \times 10^4$
S, N, OCH_3, $N(CH_3)_2$, CH_2, CH_3	LEV	255	$(2.4 \pm 0.2) \times 10^4$
O, CH, $N(CH_3)_2$	DIF	215	$(8.8 \pm 0.6) \times 10^3$

Table II. hR_F Values and LODs of Model Compounds with Densitometric and Visual Detection*

	Color of chromatographic zone			c_{min} (g) (visual)		
Substance	c_{min} (g) (densitometric)	Marqui's reagent	Dragendorff's reagent	Marqui's reagent	Dragendorff's reagent	hR_F
AMI	2.1×10^{-7}	2×10^{-7}	1×10^{-6}	brown	orange	39.5
LEV	8.2×10^{-8}	8×10^{-7}	1×10^{-6}	violet	orange	56.1
DIF	5.4×10^{-8}	7×10^{-7}	9×10^{-7}	brightly yellow	orange	35.0

Results and Discussion

As is well known, the mean content of medical products and their metabolites in human hair is in the range of 10^{-3}% to 10^{-4}% (11). It is obvious that their quantitative determination is a prerogative of trace analysis and consequently requires the elaboration of hyphenated techniques, which include a preconcentration stage.

For the realization of the preconcentration stage of psychotropic drugs, the extraction version of a concentration process was chosen (12). In spite of the evident advantages that are responsible for the prominent place of liquid extraction in a number of concentration methods, the conventional version of their realization has some limitations.

Traditionally, the components to be determined are concentrated from the sufficiently large volume of the aqueous phase by several solvent portions. Its total volume is in the range of 10 to 50 mL. In this case a concentration degree is rather small. This fact increasingly necessitates the use of more sensitive methods for subsequent determination. Furthermore, it should be mentioned that even though the concentration degree appears to be reasonable, as a rule a very small part of the final extract (0.001%–0.01%) is used on the determination stage. It is evident that this makes the metrological characteristics of determination worse. The use of microliquid extraction and the subsequent analysis of the whole final extract make it possible to realize all potential advantages of the extraction concentration stage.

The main practical characteristic of taking into account the possibility of the conversion from macro- to microliquid extraction is represented by the partition coefficient D. Its values for the extraction system allow for the prediction of the conditions in which the desired concentration degree can be obtained. Unfortunately, numerical values of partition constants or D values for our model compounds were not available from literature. In this connection an extraction behavior of model compounds in the hexane–water system was investigated. All of these compounds were tertiary amines, and consequently the extraction process to a large extent depended on the pH value of the aqueous phase. The D values can be calculated theoretically on the basis of the acidity constants of these compounds. Unfortunately, in our case these data were lacking. Therefore, the D and optimal pH values for the extraction process have been experimentally determined. The results are presented in Figure 2. The validity of extraction apart from the pH value essentially depends on the time of phase contact. An analysis of the kinetic curves obtained for the investigated systems

revealed that the extraction equilibrium was reached already after 90 s (Figure 3).

It should be pointed out that D values are essentially unchanged as the relation volumes of organic and aqueous phases changes up to 1:100, respectively. This fact demonstrates the potential of microliquid extraction for the preconcentration of psychotropic drugs in a hexane–water system.

The basic criteria that have been used for the selection of a mobile phase in TLC separation are: (*a*) providing a good separation of substances; (*b*) a high speed of elution; (*c*) reproducibility of the relative-to-front (R_f) values; (*d*) maximum simplicity of the composition of mobile phase; and (*e*) a minimum time of evaporation after the chromatographic process.

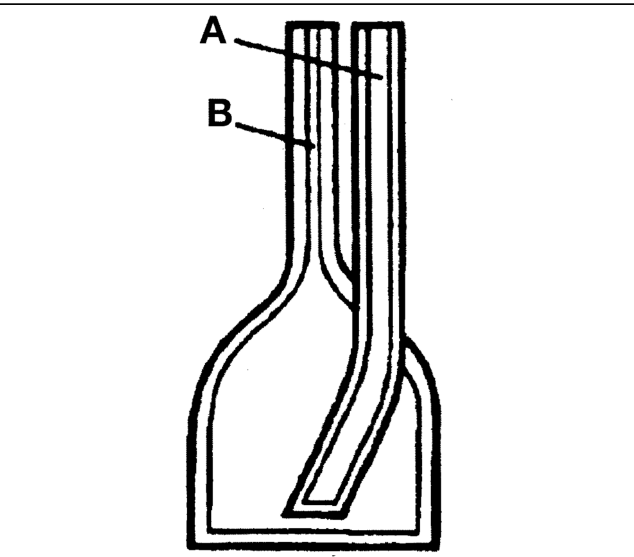

Figure 1. Vessel for microliquid extraction: (A) tube for liquid entering and (B) grade capillary for extract collection.

The choice and optimization of mobile phase composition have been performed in accordance with the PRISMA model (13). As our research has demonstrated, the most optimal mobile phase is acetone–chloroform–methanol (20:70:10, v/v/v) with 0.2 mL of tributylamine as the modificator. When employing this mobile phase, it is possible to gain the satisfactory separation of all of the model compounds (Table II).

Densitometric calibration curves for all of the model drugs were obtained. Although all of the calibration curves were nonlinear, there was an area of linearity corresponding with the real contents of drugs in the hair samples.

The developed screening technique was assumed to use both densitometric and visual detection, thus our interest was in the comparison of the limits of detection (LODs) for both cases. The results of this comparison are presented in Table II. As can be seen there was no sharp difference between densitometric and visual (Marqui's reagent) detection for all of the model drugs. The use of Dragendorff's reagent lead to higher LODs; this reagent was also nonselective for the investigated model compounds.

It should be recognized that in numerous investigations devoted to the analysis of hair and the determination of narcotics and psychotropics in hair, the problems concerning the sorption behavior of drugs on a hair matrix are virtually not discussed. However, these problems are of great importance when choosing a version of sampling, excluding the analyte loss on the different stages. The problem of determining the possible entering paths (endogenous or exogenous) of analyte into hair is also closely connected with the sorption problem.

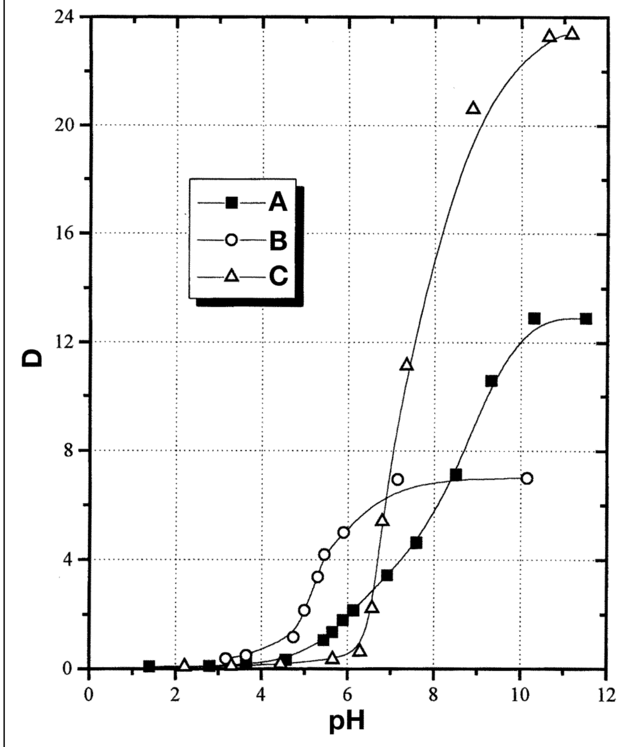

Figure 2. Partition coefficient versus the pH of aqueous phase: (A) AMI, (B) LEV, and (C) DIF.

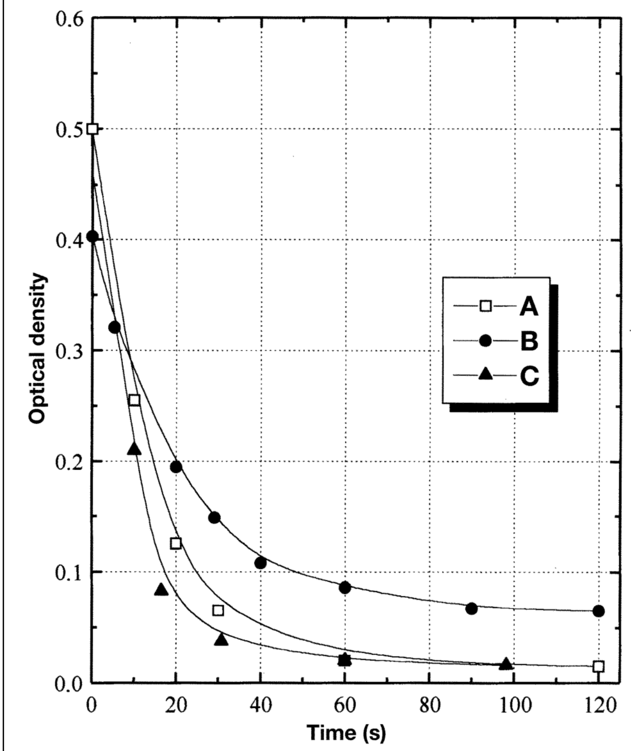

Figure 3. Optical density of aqueous phase versus time of extraction: (A) AMI, (B) LEV, and (C) DIF.

The sorption behavior of all of the model compounds on the model human hair samples was investigated. For the preparation of the model system, the hair from nonaddicted subjects was washed and soaked for 72 h in a basic aqueous solution containing the model compounds. The quantity of the model compounds in solution was controlled by spectrophotometry. The results of the sorption measurements for AMI as an example are presented on Figure 4. It is apparent that the sorbate quantity became practically invariable after 48 h. Similar behavior was also observed both for DIF and LEV. Although it is necessary to suppose that the nature of sorbate is exogenous, the penetration degree of sorbate into the matrix is a priori unknown. Even a rough estimation of the diffusion front penetration depth demonstrates that some part of the compound should be located in the inner structure of hair. Thus, sample modeling the real hair with endogenous pollution can be performed by means of a maximum elimination of the remaining (i.e., adsorbed) part of the model compound. We chose sufficiently rigorous conditions of cleaning, namely the continuous treatment of washing the solution by ultrasound and subsequent multiple washing by organic solvents. The analysis of kinetic recovery curves for model samples demonstrated that the recovery value did not virtually change and remained at a level of 90% already after 30 min of foregoing treatment. This fact can be explained in the framework of our assumptions that it was a sufficiently adsorbed model compound that was removed in the sampling process. The unrecovered part of the model compound (approximately 10%) that penetrated relatively deep into the interior structure of the hair can model the drug of endogenous origin. Model samples of hair with drugs prepared in such a manner were used for the creation and optimization of the developed screening technique. The determination of model drugs was performed according to the following scheme.

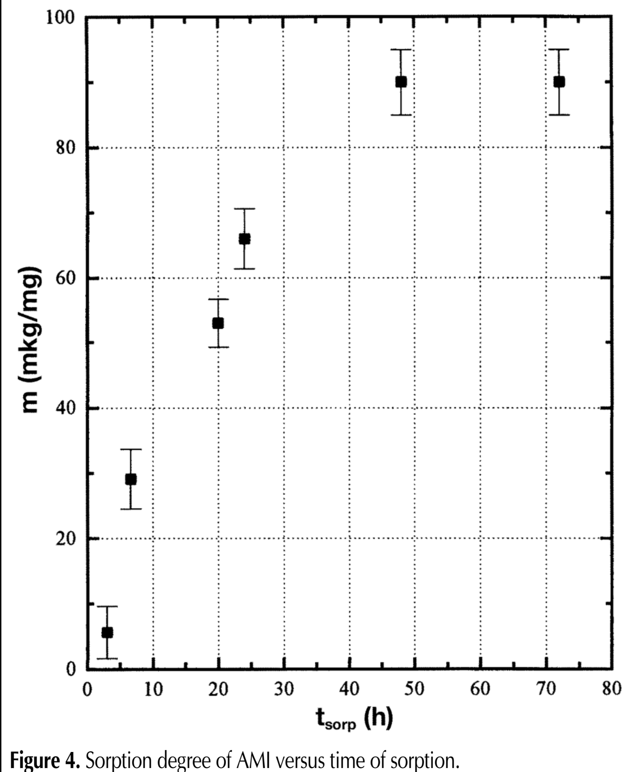

Figure 4. Sorption degree of AMI versus time of sorption.

The samples were washed by methanol with ultrasound treatment for 15 min, rinsed by methanol and acetone, cut into pieces with lengths of 2 to 4 mm, then 0.5M NaOH was added and samples were placed into an ultrasonic bath for 30 min. Afterwards, sampling aliquots were inserted in a microextractor and 100 µL hexane added. The organic phase was collected after extraction by a microsyringe and spotted onto a TLC plate with standard spots. After development and drying the chromatograms were scanned on a densitometer or sprayed with Marqui's reagent.

The developed approach was tested by the hair analysis of patients after a course of intensive treatment of drugs containing the investigated compounds. Patients' hair were analyzed within two weeks after the completion of the dose of psychotropics. The average content of the psychotropic drugs was found to be in the range of 3 to 7 ng/mg. Visual detection by the Marqui's reagent also gave positive results in all cases. These results suggest that the developed procedure based on the coupling of HPTLC and microliquid extraction can be used for screening psychotropic drugs in human hair.

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