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Integration of GC/EI-MS and GC/NCI-MS for simultaneous quantitative determination of opiates, amphetamines, MDMA, ketamine, and metabolites in human hair

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ABSTRACT

In this paper, the possibility of using a multiple ionization mode approach of GC/MS was developed for the simultaneous hair testing of common drugs of abuse in Asia, including amphetamines (amphetamine, AP; methamphetamine, MA; methylenedioxy amphetamine, MDA; methylenedioxy methamphetamine, MDMA; methylenedioxy ethylamphetamine, MDEA), ketamine (ketamine, K; norketamine, NK), and opiates (morphine, MOR; codeine, COD; 6-acetylmorphine, 6-AM). This strategy integrated the characteristics of gas chromatography–mass spectrometry (GC–MS) using electron impact ionization (EI) and negative chemical ionization (NCI). Hair samples (25 mg) were washed, cut, and incubated overnight at 25 °C in methanol–trifluoroacetic acid (methanol–TFA). The samples were extracted by solid phase extraction (SPE) procedure, derivatized using heptafluorobutyric acid anhydride (HFBA) at 70 °C for 30 min, and the derivatives analyzed by GC–MS with EI and NCI. The limit of detection (LOD) with GC/EI-MS analysis obtained were 0.03 ng/mg for AP, MA, MDA, MDMA, and MDEA; 0.05 ng/mg for K, NK, MOR, and COD; and 0.08 ng/mg for 6-AM. The LOD of GC/NCI-MS analysis was much lower than GC/EI-MS analysis. The LOD obtained were 30 pg/mg for AP and MDA in GC/EI-MS and 2 pg/mg in GC/NCI-MS. Therefore, the sensitivity of AP and MDA in GC/NCI-MS was improved from 15-fold compared with EI. The sensitivity of AP, MA, MDA, MDMA, MDEA, MOR, and COD was improved from 15- to 60-fold compared with EI. In addition, the sensitivity of 6-AM increased 8-fold through selection of *m/z* 197 for the quantitative ion. Moreover, K and NK could dramatically improve their sensitivity at 200- and 2000-fold. The integration of GC/EI-MS and GC/NCI-MS can obtain the high sensitivity and complementary results of drugs of abuse in hair. Six hair samples from known drug abusers were examined by this new strategy. These results show that integrating the characteristics of GC/EI-MS and GC/NCI-MS were not only enhancement of the sensitivity but also avoid wrong results and wrong interpretations of correct results.

1. Introduction

The analysis of abused drugs in hair sample has rapidly emerged as a useful tool for detecting and monitoring drugs [1–3]. Hair is unique in that drug intake information is stored for a much longer time period compared to other biological specimens such as blood and urine, enabling retrospective investigation of past consumption. Particularly, hair contains a relatively high parent drug to metabolite ratio, which means that it is easy to identify specific biomarkers [4]. Using segmental hair analysis may help determine the time period of drug exposure [5–7]. Furthermore, hair gives the additional advantages that it can be easily obtained, it is not easily adulterated, and it can be stored and transported without specific precautions due to its stability. Therefore, hair testing has found applications in evaluating environmental exposure to toxicants even from the intrauterine period of life, in doping control, and in drug abuse studies in the fields of forensic toxicology, clinical toxicology, and clinical chemistry. Although, there are some pitfalls of hair testing [8], involving possible external hair contamination [9–11], hair cosmetic treatments (dyeing, bleaching, and permanent waving) [12], racial bias [13,14], irregular speed of growth of hair from various anatomical parts of the body can complicate the interpretation of hair testing results. Knowledge of such pitfalls is useful since it can be used to avoid wrong results and wrong interpretations of correct results.

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Drugs concentration and sample sizes in hair sample is much lower than in urine, so the hair sample calls for more sensitive analytical methods. In order to verify the actual drug abuse, numerous methods have been developed for the analysis of drugs in hair. Gas chromatography coupled to mass spectrometry (GC–MS) using electron impact (EI) ionization mode is the most widely used technique in drug of abuse analysis in urine as well as in hair. The EI mode leads to a number of fragment ions providing more structural information. In addition, it allows identification of unknown compounds by comparison of their mass spectrum with reference mass spectra in commercially available libraries. The limit of detection (LOD) obtained were about 0.03–0.08 ng/mg with derivatives and 0.1–0.8 ng/mg without derivatives for common drugs of abuse in hair by GC/EI-MS [15–21].

Due to the extensive fragmentation in the EI mode, the chemical ionization mode (CI) could provide more selectivity as this technique often gives molecular mass information. In addition, the sensitivity was improved through the use of negative chemical ionization (NCI) analytical methods. The GC/NCI-MS offers highly sensitive analysis of the compound at low concentration about pg levels in hair [22–24]. In recent years, many forensic and toxicology labs have been switching to liquid chromatography/tandem mass spectrometry (LC–MS–MS) methods, which do not require derivatization or extensive sample clean-up procedures necessary in GC–MS analyses [25,26]. Although LC–MS–MS technique is a new trend and has great potential for hair testing, it is more expensive than GC–MS that often used in testing labs for drugs of abuse.

Recently, the abused club drugs and multiple-drug cocktails had become the worldwide trend. In order to increase the ability and extent of drug testing, development of simultaneous testing methods for drugs of abuse is in great demand. Previously, we had developed a simultaneous method to determine amphetamines, ketamine, opiates, and metabolites in hair using GC–MS using EI mode [27]. As far as we know, this is the first paper can simultaneously measure opiates, amphetamines, and their metabolites in human hair, due to the different preparation and derivatization procedures required. However, the sensitivity is needed to be improved for high-performance hair testing in further. The purpose of this paper was to evaluate the simultaneous hair testing using GC/NCI-MS for common drugs of abuse in Asia, including opiates, amphetamines, MDMA, ketamine, and metabolites. It was found that GC/NCI-MS is suitable for broad-spectrum drug testing in a single hair specimen. The integration of GC/EI-MS and GC/NCI-MS not only gained sensitivity enhancement but also avoid wrong results and wrong interpretations of correct results.

2. Experimental

2.1. Chemicals and reagents

All solvents and chemicals were analytical grade. Methanol, dichloromethane, isopropanol, ammonium hydroxide, acetonitrile, acetic acid, hydrochloric acid, ethyl acetate, potassium dihydrogen phosphate were purchased from MERCK (KGaA, Darmstadt, Germany). Drug standards of AP, MA, MDA, MDMA, MDEA, K, NK, MOR, COD, 6-AM, and internal standards of AP-d5, MA-d5, MDA-d5, MDMA-d5, MDEA-d5, K-d4, NK-d4 MOR-d3, COD-d3, 6-AM-d3, were purchased from Cerilliant (Austin, TX, USA). Heptafluorobutyric acid anhydride (HFBA) and trifluoroacetic acid (TFA) were purchased from Sigma (St. Louis, MO, USA). The Bond Elut Certify column was purchased from Varian (WalnutCreek, CA, USA).

The chemical structures of AP, MA, MDA, MDMA, MDEA, K, NK, MOR, COD, and 6-AM are shown in Fig. 1. Each compound was dissolved in methanol to make a stock solution with a concentration of 1 mg/mL, and this was used after dilution in methanol to the required concentration. Drug standards and internal standards solutions were stored at 4 °C.

2.2. Hair sample preparation

Authentic hair samples were collected from regional prevention centers for drug abuse. The procedure for the collection of the hair samples of this study was approved by the institutional review board of Chung Shan Medical University Hospital (approval number CS05138). These hair samples were generally cut as close as possible to the skin from the posterior vertex and folded in aluminum foil prior to placement in a paper envelope. The total length and weight of each hair were measured and special treatments such as dyeing, bleaching, or others were noted. Hair samples were stored under dry, dark conditions at room temperature. The samples were decontaminated with 2 mL dichloromethane for 5 min at room temperature. The hairs were then dried in an oven at 45 °C. After being dried, the hair samples were cut with scissors into very small lengths of less than 1 mm.

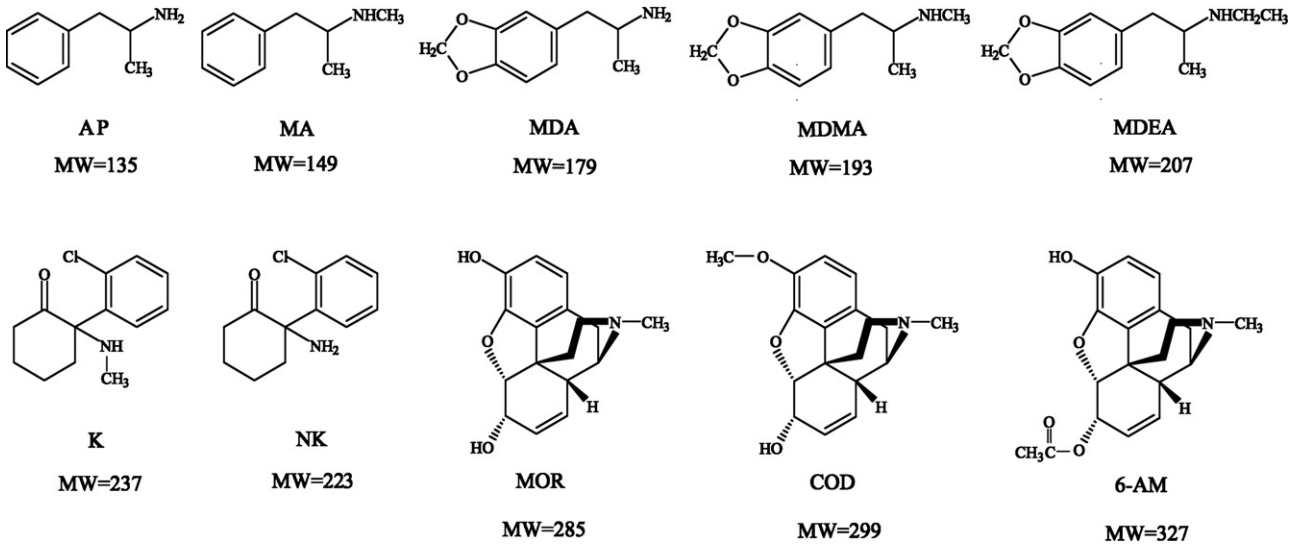

Fig. 1. The chemical structures of: AP, amphetamine; MA, methamphetamine; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine; MDEA, methylenedioxyethylamphetamine; K, ketamine; NK, norketamine; MOR, morphine; COD, codeine; 6-AM, 6-acetylmorphine.