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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.

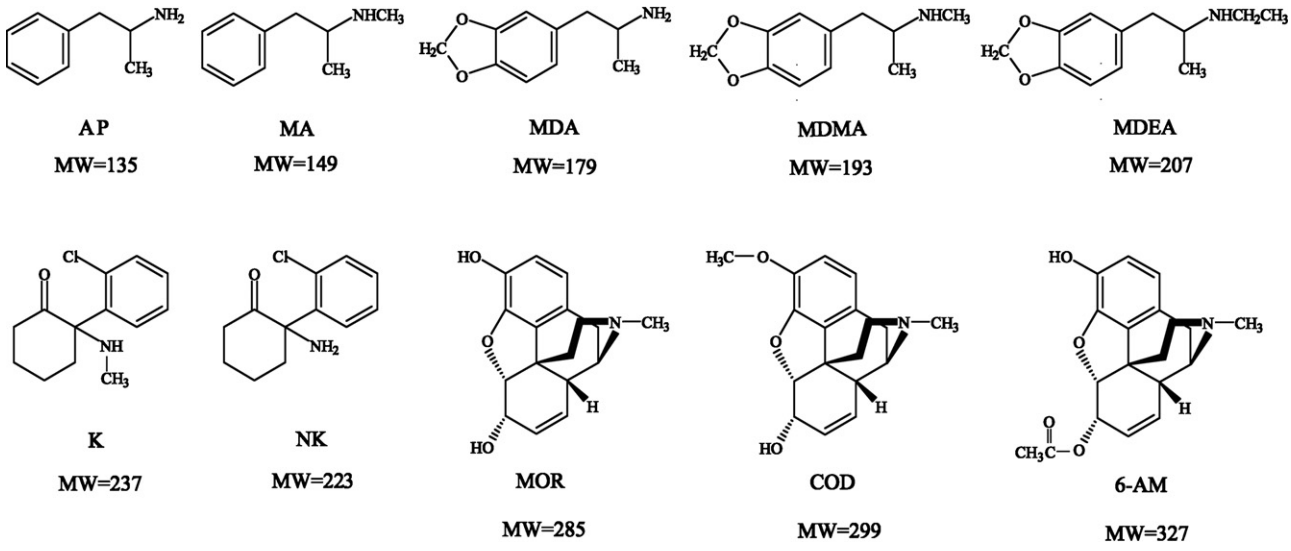

Fig. 1. The chemical structures of: AP, amphetamine; MA, methamphetamine; MDA, methylenedioxyamphetamine; MDMA, methylenedioxymethamphetamine; MDEA, methylenedioxyethylamphetamine; K, ketamine; NK, norketamine; MOR, morphine; COD, codeine; 6-AM, 6-acetylmorphine.

Table 1
Selected ions and retention times (RT) of the investigated drugs

Compound	EI			NCI		
	Ions	Ions of IS	RT (min)	Ions	Ions of IS	RT (min)
AP–HFBA	91, 118, 240	123, 244	2.69	311	316	2.81
MA–HFBA	118, 210, 254	213, 258	3.22	325	329	3.35
MDA–HFBA	162, 240, 375	167, 244	4.35	355	360	4.48
MDMA–HFBA	162, 210, 254	213, 258	4.98	369	373	5.12
MDEA–HFBA	162, 240, 268	165, 274	5.17	363, 403	409	5.32
NK–HFBA	340, 356, 384	360, 388	5.33	383	387	5.48
K–HFBA	236, 362, 370	366, 374	6.15	226, 397	401	6.32
MOR–2HFBA	411, 464, 670	467, 677	8.44	441, 637	640	8.66
COD–HFBA	282, 495	285, 498	8.59	475	478	8.81
6-AM HFBA	411, 464, 523	467, 526	9.06	197, 463	640	9.27

2.3. Analysis of real samples

Hair samples (25 mg) were incubated overnight with 2 mL of methanol–TFA (8.5:1.5) at 25 °C in the presence of 10 ng of each deuterated internal standard. The extracted solutions were collected in glass tubes. The remaining hairs were rinsed with 0.5 mL methanol to avoid drug remaining in the hair and the methanolic solutions were collected in the same glass tubes. The mixtures were then evaporated to dryness at 55 °C under a stream of nitrogen. After the extracted solutions had evaporated, the dry residue of the hair extract was added to 2 mL of 0.1 M phosphate buffer at pH 6.0. The hair extract was then added to a Bond Elut™ Certify cartridge, which was conditioned with 1 mL of methanol and 1 mL of a 0.1 M phosphate buffer at pH 6.0. The hair extract was passed very slowly through the cartridges. Then 1 mL of deionized water, 0.5 mL of 0.1 M acetic acid, and 1 mL of methanol were added in sequence. The cartridges were vacuum-dried for 5 min and eluted with a 1 mL (2×) mixture of dichloromethane–isopropanol–ammonium hydroxide (80:20:2, v/v/v). The eluates were collected in glass tubes, and then a 50 μL mixture of methanol–HCl (99:1, v/v) was added to ensure non-volatility of the drugs and the mixture was evaporated to dryness at 55 °C under a stream of nitrogen. Derivatization was performed with 100 μL of ethyl acetate and 100 μL of HFBA at 70 °C for 30 min. After derivatization, the mixture was dried under nitrogen and reconstituted in 25 μL of ethyl acetate. An aliquot of 2 μL of the derivatized extract was then directly injected into the GC/EI-MS and GC/NCI-MS instrument, respectively.

2.4. GC–MS analysis

2.4.1. Instrumentation

GC/EI-MS analyses were carried out with an Agilent 6890 gas chromatograph interfaced to an Agilent 5973 mass spectrometer, and an Agilent 6890 series automatic injector (GenTech, Arcade, NY, USA). Data handling and system operations were controlled by the Chemstation software (Rev. B.01.00) for GC/EI-MS. GC/NCI-MS analyses were carried out with an Agilent 6890N gas chromatograph interfaced to an Agilent inert 5973 mass spectrometer, and an Agilent 7683B series automatic injector (Palo Alto, CA, USA). Data handling and system operations were controlled by the Chemstation software (Rev. D.01.02) for GC/NCI-MS.

2.4.2. Chromatographic conditions

Separation was achieved with a capillary column (HP-5MS, 5% phenyl methyl siloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). A deactivated glass inlet liner (#5062-3587) was used (Agilent, Palo Alto, CA, USA). The GC injection port temperature was set at 230 °C in splitless mode and helium (99.999%) was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was held at 150 °C for 1 min, then programmed to 210 °C at 20 °C/min and held for 0.1 min, to 240 °C at 20 °C/min and held for 0.1 min, to 250 °C at 10 °C/min and held for 1 min, and finally to 280 °C at 20 °C/min and held for 2 min. The transfer line temperature was 280 °C.

2.4.3. MS detection

The mass detector was operated at 70 eV in EI mode. The quadrupole and ion source temperature were 150 °C and 230 °C in EI mode, respectively. The NCI mode was used with methane (99.99%) as a reagent gas in all MS measurement. The flow controller of methane was set to 40% of the maximal Agilent default value in NCI mode. The temperatures of quadrupole and ion source were set at 150 °C, respectively. All quantitative analyses were performed in the selected ion monitoring (SIM) mode, with a dwell time of 20 ms for each analyte. The diagnostic ions monitored for each substance and their relative retention times after derivatization with HFBA are listed in Table 1.

2.5. Analytical method validation

2.5.1. Linearity

The method linearity for each compound was investigated in the range of 0.05–5 ng/mg. Calibration curves were obtained with five calibration levels by adding 1.25 ng (0.05 ng/mg), 12.5 ng (0.5 ng/mg), 25 ng (1 ng/mg), 62.5 ng (2.5 ng/mg), and 125 ng (5 ng/mg) of pure standards, prepared as a mixture in methanol, to 25 mg of drug-free hair strands. Deuterated internal standards (10 ng each) were added and samples were analyzed following the complete procedure. Calibration curves were established with three replicates at each concentration. Linear regression analysis was performed on the peak area ratios of analyte to internal standard versus analyte concentrations.

2.5.2. Recovery

On the basis of the obtained results, the recovery was investigated by submitting samples to incubate with methanol–TFA (8.5:1.5) and solid phase extraction (SPE). The different concentrations of spiked hair (0.05, 0.5, and 2.0 ng/mg) were analyzed ($n = 3$), which were incubated overnight at 25 °C with 2 mL of methanol–TFA, then extracted and derivatized as we as described above. The deuterated internal standards (10 ng each) were added just before the eluates evaporated to dryness. The results were compared with data obtained from each standard compound of interest and each deuterated internal standard, derivatized and analyzed without SPE. The mean peak height ratios (analyte vs. IS) of extraction samples and extraction controls (the latter indicating full recovery) were compared and the percentage extraction efficiencies for each analyte were calculated (mean ± SD).

2.5.3. The limit of detection and the limit of quantitation

Sensitivity was evaluated by determination of the LOD and the limit of quantitation (LOQ). A series of decreasing concentrations of drug-fortified hair strands was analyzed to determine LOD and LOQ. The LOD was determined as the concentration with a signal-to-noise (S/N) ratio of at least 3, while the LOQ was the lowest concentration with a S/N ratio of at least 10.

2.5.4. Accuracy and precision

Different concentrations of spiked hair (0.05, 0.5, and 2.0 ng/mg) were analyzed ($n = 3$) in 1 day for the intra-day precision study. For the inter-day precision study, each concentration was analyzed on 5 different days. To determine the precision, the coefficients of variations (% CV) were calculated for replicate measurements. Accuracy (%) was expressed as the relative error from the expected value. It was calculated by the degree of agreement between the measured and nominal concentrations of the fortified samples.

3. Results and discussion

Simultaneous, specific and sensitive detection of drugs of abuse is great requirement, especially in hair sample. The simultaneous quantitative determination of several compound types is advantageous because forensic toxicologists are frequently confronted with limited sample size. In addition, the worldwide trend in drug abuse has become the use of multiple-drug cocktails. In this study, a new strategy was developed for the simultaneous determination of common drugs of abuse in Asia, including opiates, amphetamines, MDMA, ketamine, and their metabolites. By integrating the characteristics of GC/EI-MS and GC/NCI-MS, the later of which can be confirmed the results of GC/EI-MS analysis, enhanced sensitivity to correct interpretations of results. The major analytical procedures of hair analysis using GC–MS involve sample collection, decontamination; digestion/extraction, clean-up, derivatization, and GC–MS detected using EI and NCI.

3.1. Sample pretreatment

The preferred site of choice for hair collection has been the vertex of the head because less variability in the hair growth rate. After hair collection, hair samples were washed briefly with dichloromethane to remove external contaminants. Hair digestion/extraction procedure for drugs is the most sensible step of hair analytical procedures. The digestion/extraction procedure from hair must be performed with high recovery and without significant degradation of the investigated drugs. The presented methanol–TFA (8.5:1.5) extraction technique enables the simultaneous extraction of different drug classes from only one hair sample [27]. After methanol–TFA incubation overnight at 25 °C, the resulting organic phase was evaporated, and the residue was dissolved in a phosphate buffer solution. The aqueous solution was then processed by SPE, which provided substantial purification and reduction of background interferences.

Derivatization is usually performed after pre-concentration and clean-up of the extracts. In order to decrease the polarity and increase the volatility of the analytes, derivatization of these drugs and their metabolites prior to GC–MS analysis is required. Various derivatization reagents of drugs of abuse have been described [28]. As far as we know, there is no comprehensive derivatization reagent for simultaneously determining different drug classes and metabolites. Previously, we had developed a simultaneous method to determine amphetamines, ketamine, opiates, and metabolites in hair using GC–MS using EI ionization mode, BSTFA and HFBA were evaluated [27]. Although all the investigated compounds were observed in the GC–MS SIM chromatograms after derivatization with BSTFA or HFBA, the sensitivity of amphetamines after BSTFA derivatization is ten times lower than after HFBA derivatization. Thus HFBA was much better than BSTFA for the simultaneous derivatization of all the investigated drugs and their metabolites. Then the SPE mixture was evaporated and derivatized with HFBA, samples were analyzed by GC–MS operating in the SIM mode. The reliable GC–MS method includes HFBA derivatization of analytes after acidic extraction procedure was able to simultaneously quantify amphetamines, ketamine, and opiates in human hair.

3.2. Mass spectral characteristics of derivatives

It is well known that NCI gives strong signals to the investigated compounds owing to the electronegative atom(s) or functional groups in their molecular structure. Therefore, the analytes after HFBA derivatization were suitable analysis by GC/NCI-MS because it implies the addition of 7 or 14 fluorine atoms. In our experiment,

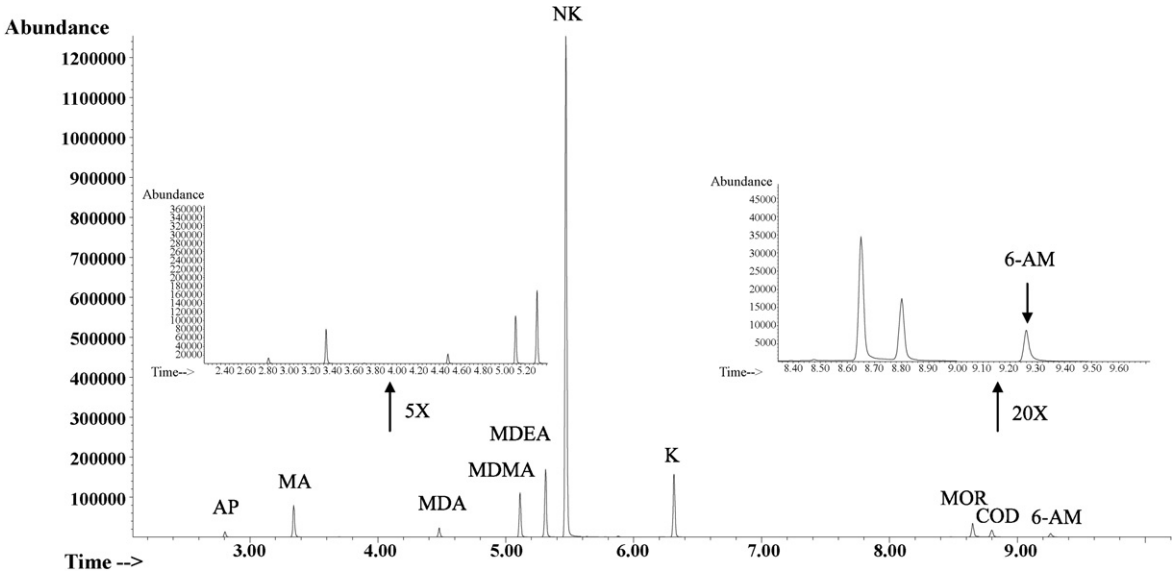

Fig. 2. GC/NCI-MS SIM chromatogram of the investigated drugs after derivatization with HFBA (the selection of the m/z 463 for 6-AM quantitative ion).

it was found all the investigated compounds were transformed to halogenate derivatives by HFBA, which provides both good GC properties and introduction of an electronegative moiety. Fig. 2 shows the GC/NCI-MS SIM chromatogram result for the analytes after derivatization with HFBA. Those investigated compounds show the enhanced intense signal in GC/NCI-MS except the 6-AM.

The chemical structures, molecular weights and NCI *full-scan* mass spectra of the heptafluorobutyrate derivatives are shown in Fig. 3. AP and MDA belong to primary amine structures; the molecular weights of AP and MDA derivatives after HFBA derivatization were 331 and 375 Da, respectively. The *full-scan* mass spectrums show the prominent signal at *m*/*z* 311 $(M\text{-}HF)^-$ and *m*/*z* 355 (M-

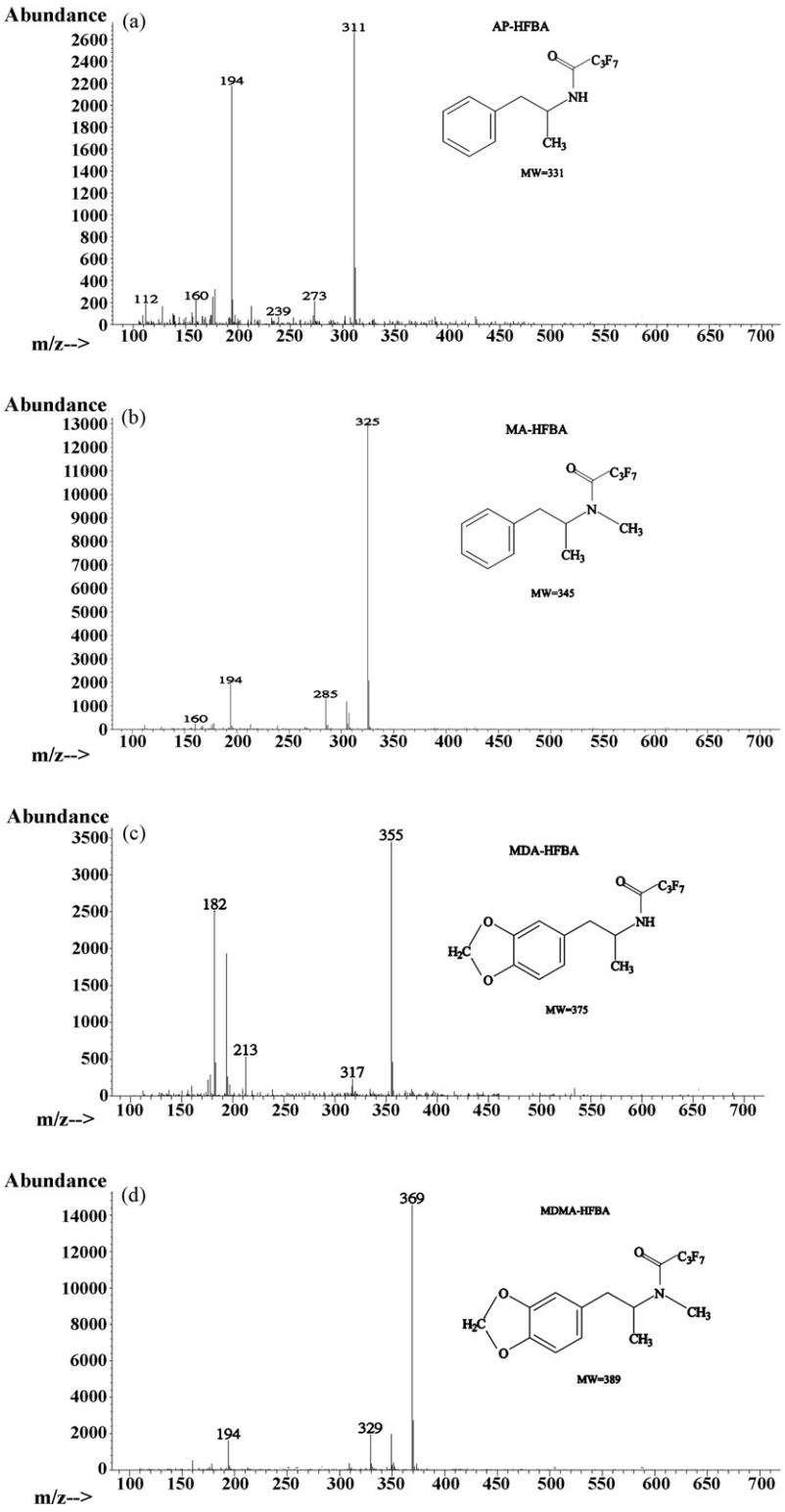

Fig. 3. GC/NCI mass spectrum of the investigated compounds after derivatization with HFBA.

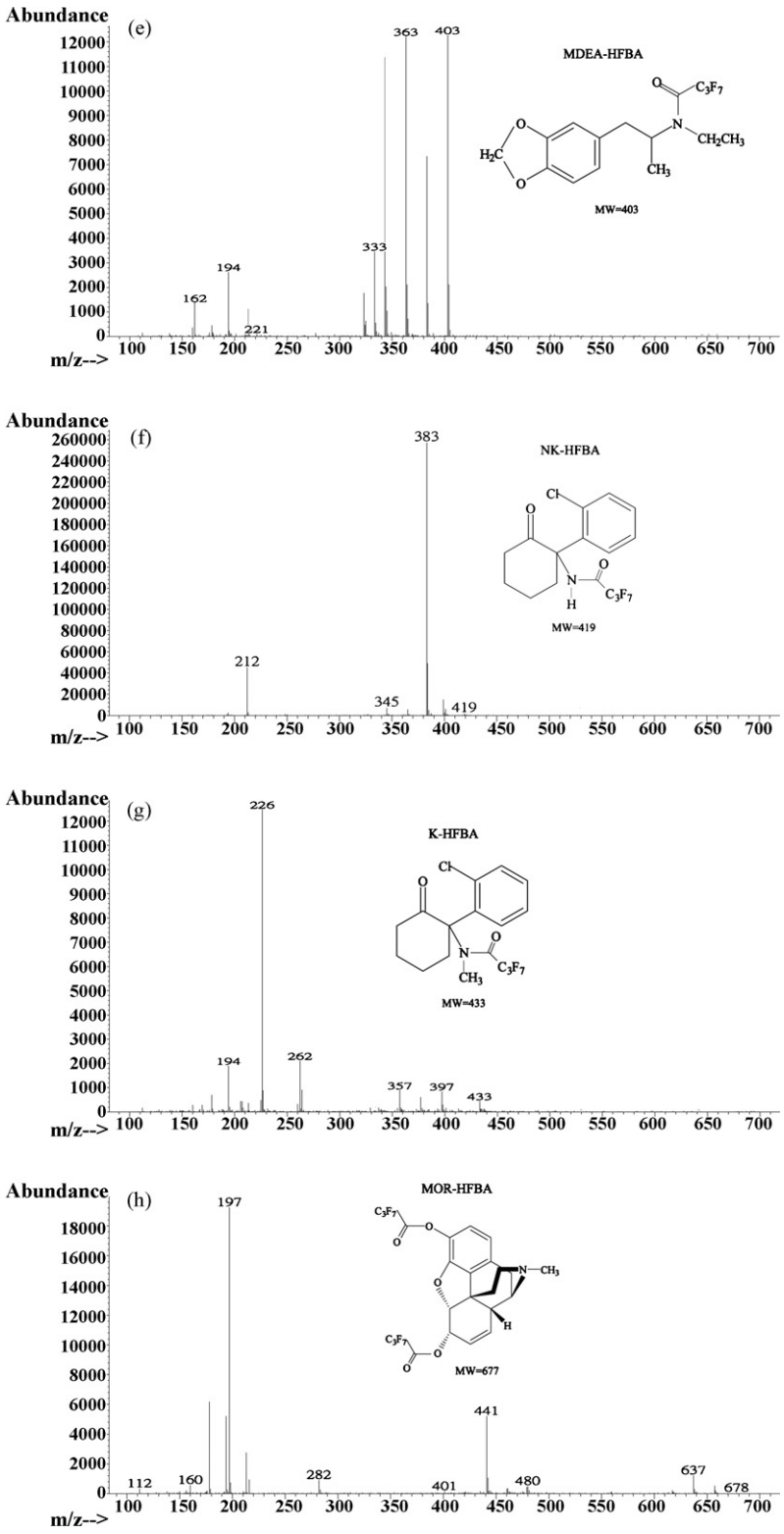

Fig. 3. (*Continued*)

$HF)^-$, respectively. MA and MDMA belong to secondary amine structures; the molecular weights of MA and MDMA derivatives after HFBA derivatization were 345 and 389 Da, respectively. The *full-scan* mass spectrums show the prominent signal at *m*/*z* 325 $(M\text{-}HF)^-$ and *m*/*z* 369 $(M\text{-}HF)^-$, respectively. The fragments of $(M\text{-}2\ HF)^-$, $(M\text{-}3\ HF)^-$, and $(M\text{-}4\ HF)^-$ were observed. The molecular weight of MDEA derivatives after HFBA derivatization was 403 Da. The *full-scan* mass spectrum shows the fragments of *m*/*z* 403 $(M^{\bullet})^-$, *m*/*z* 383 $(M\text{-}HF)^-$, *m*/*z* 363 $(M\text{-}2\ HF)^-$, *m*/*z* 343 $(M\text{-}3\ HF)^-$, and *m*/*z* 323 $(M\text{-}4\ HF)^-$ were observed. The molecular weight of K derivatives after HFBA derivatization was 433 Da. The *full-scan* mass spectrum shows the prominent signal at *m*/*z* 226

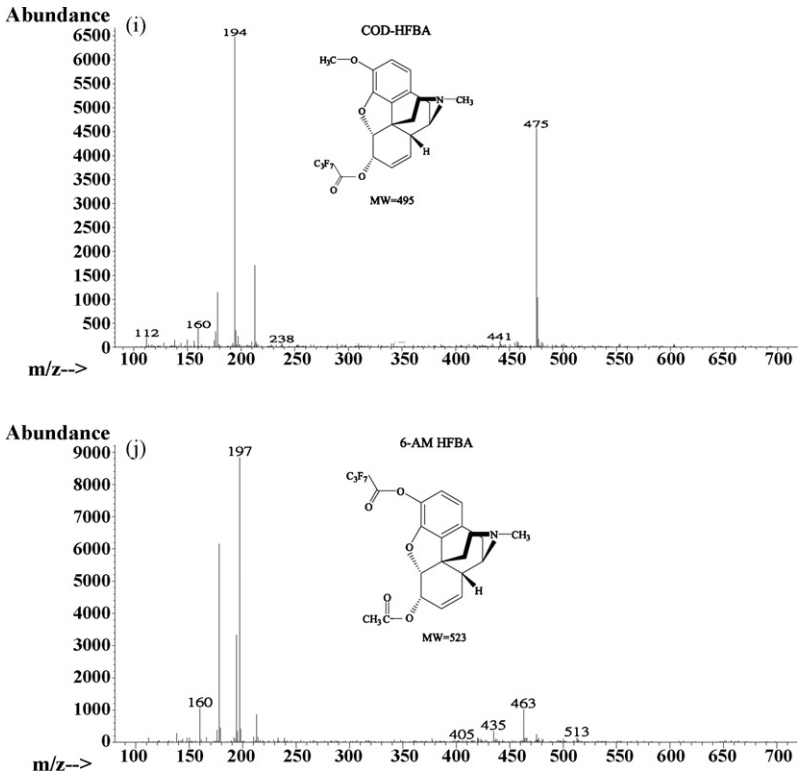

Fig. 3. (*Continued*).

$(M\text{-}C_{12}H_{12}ClO)^-$. The fragments of *m*/*z* 433 $(M^\bullet)^-$ and *m*/*z* 397 $(M\text{-}HCl)^-$ were observed. The molecular weight of NK derivatives after HFBA derivatization was 419 Da. The *full-scan* mass spectrum shows the prominent signal at *m*/*z* 383 $(M\text{-}HCl)^-$. The fragments of *m*/*z* 212 $(M\text{-}C_{12}H_{12}ClO)^-$ and *m*/*z* 399 $(M\text{-}HF)^-$ were observed. Morphine has two hydroxylic functions; hence the reaction with HFBA gives rise to a di-derivatized molecule, with a molecular weight of 677 Da. The *full-scan* mass spectrum shows two signals at high *m*/*z* values, 657 $(M\text{-}HF)^-$ and 637 (M-2 HF)$^-$. The fragment at *m*/*z* 441 derives from the opening and the rearrangement of the molecule ring structure, while the signal at *m*/*z* 197 corresponds to the fragment $[CF_3(CF_2)_2CO]^-$. The molecular weight of codeine derivatives after HFBA derivatization was 495 Da. The *full-scan* mass spectrum shows the prominent signal at *m*/*z* 475 $(M\text{-}HF)^-$. The molecular weight of 6-AM derivatives after HFBA derivatization was 523 Da. The *full-scan* mass spectrum shows low intensity signal at *m*/*z* 463 with loss of acetyl group $(M\text{-}CH_3COOH)^-$, almost all the total ion current is represented by the signal at *m*/*z* 197, it corresponds to a group introduced by the derivatization reaction.

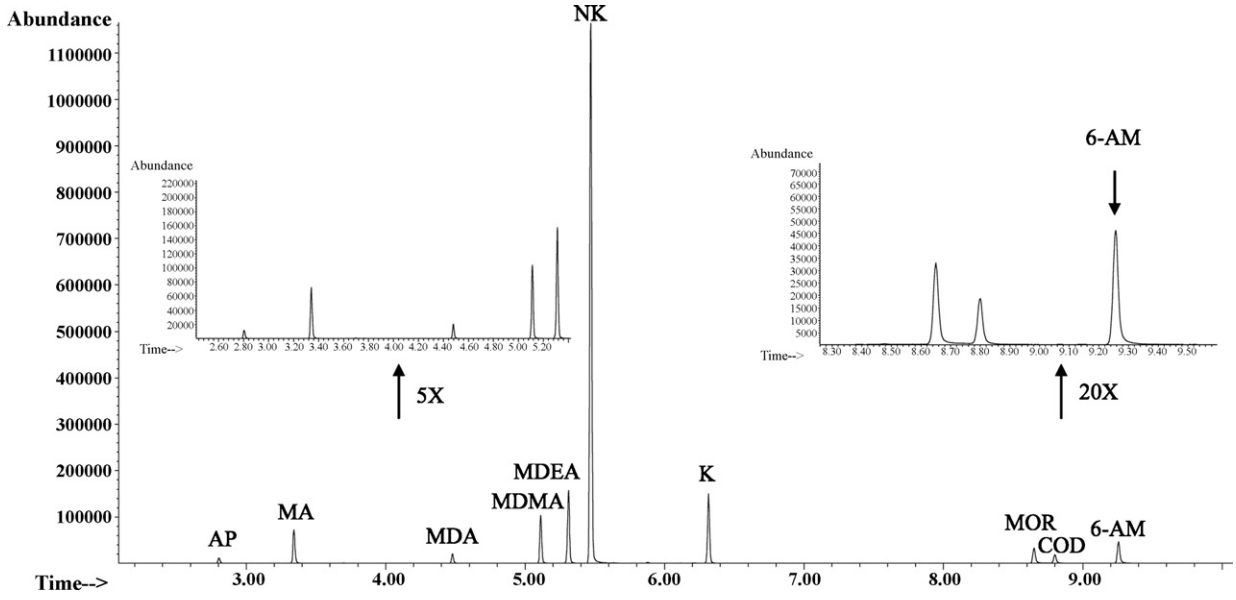

Fig. 4. GC/NCI-MS SIM chromatogram of the investigated drugs after derivatization with HFBA (the selection of the *m*/*z* 197 and *m*/*z* 463 for 6-AM quantitative ion).

Table 2
Validation data

Compound	Linearity[a] (r^2)	LOD[b] (pg/mg)	LOQ[c] (pg/mg)	Recovery (% mean ± SD[d], $n = 3$) (ng/mg)		
				0.05	0.5	2
AP	0.995	2	5	90.2 ± 2.4	93.8 ± 2.6	88.6 ± 5.8
MA	0.997	0.5	2	91.1 ± 1.2	96.2 ± 2.3	85.3 ± 4.7
MDA	0.999	2	5	85.5 ± 2.0	90.3 ± 2.8	87.2 ± 5.3
MDMA	0.997	0.5	2	80.2 ± 2.8	84.6 ± 3.5	85.9 ± 4.2
MDEA	0.996	0.8	3	80.4 ± 1.6	83.8 ± 2.5	86.6 ± 6.0
K	0.997	0.25	1	83.6 ± 2.8	90.3 ± 3.4	88.5 ± 4.1
NK	0.999	0.025	0.08	80.4 ± 1.3	92.7 ± 2.8	90.2 ± 5.7
MOR	0.996	2	5	80.5 ± 2.9	86.2 ± 3.5	84.6 ± 6.3
COD	0.999	2	5	88.7 ± 3.4	90.8 ± 2.0	86.5 ± 5.7
6-AM	0.995	10	40	85.8 ± 2.7	88.5 ± 2.8	89.2 ± 5.3

[a] Linearity is described by the correlation coefficient for the calibration curve.
[b] Limit of detection (LOD).
[c] Limit of quantification (LOQ).
[d] Standard deviation (SD).

The lower sensitivity of 6-AM was not observed in GC/NCI-MS. By comparison of the EI and NCI *full-scan* mass spectra, the intact structure of 6-AM derivatives were detected by GC/EI-MS. The EI *full-scan* mass spectrum of the 6-AM derivatives shows the prominent signal at *m*/*z* 523 and *m*/*z* 464, respectively. In contrast, the NCI *full-scan* mass spectrum shows the weak signal at *m*/*z* 463, the prominent signal at *m*/*z* 197 which is fragmentation of HFBA derivative reagent. Because 6-AM is a very important marker for differentiating heroin from other opiate use (i.e. codeine and morphine), it is essential to improve the signal of 6-AM in GC/NCI-MS. Therefore, we selected the *m*/*z* 197 for 6-AM quantitative ion, and MOR-d3 was chosen for internal standard of 6-AM because 6-AM-d3 after HFBA derivatization also showed the same signal of *m*/*z* 197.

Detailed and current values concerning chromatographic retention times and identification criteria, e.g. number of detected ions and peak area ratios (of at least three ions in GC–MS) along with other characteristics, are essentially comparable. NCI

Table 3
Intra- and inter-day precision and accuracy

Compound	Nominal concentration (ng/mg)	Intra-day ($n = 3$)		Inter-day ($n = 5$)	
		Precision (% CV)[a]	Accuracy (% bias)[b]	Precision (% CV)[a]	Accuracy (% bias)[b]
AP	0.05	4.4	5.2	6.1	8.4
	0.5	4.8	4.8	5.8	7.1
	2.0	5.9	6.7	8.7	6.8
MA	0.05	3.6	6.8	5.4	8.6
	0.5	3.2	4.5	6.5	6.9
	2.0	4.1	2.9	9.8	5.7
MDA	0.05	3.3	5.8	4.8	4.7
	0.5	2.9	6.3	5.3	5.9
	2.0	3.5	−4.7	6.6	3.4
MDMA	0.05	7.6	7.2	8.5	9.2
	0.5	6.2	8.3	6.4	12.5
	2.0	6.8	−5.9	8.2	3.2
MDEA	0.05	5.1	6.7	6.5	7.1
	0.5	4.7	9.1	5.0	12.3
	2.0	6.4	3.3	7.2	−3.6
K	0.05	7.9	4.6	10.3	8.5
	0.5	5.9	2.8	6.9	7.6
	2.0	8.2	−1.6	9.5	3.7
NK	0.05	2.2	3.7	1.3	6.3
	0.5	1.2	4.9	3.2	7.1
	2.0	2.5	−3.7	4.8	2.8
MOR	0.05	4.9	7.5	4.6	6.4
	0.5	3.6	8.5	6.8	10.2
	2.0	5.3	3.8	7.1	7.9
COD	0.05	3.3	8.2	6.6	10.2
	0.5	4.9	6.3	5.9	8.5
	2.0	5.3	3.9	7.2	4.2
6-AM	0.05	8.1	10.3	12.3	13.2
	0.5	5.7	8.5	9.6	7.9
	2.0	9.1	7.8	10.9	8.5

[a] The coefficient of variance (% CV): SD/mean × 100%.
[b] Calculated as [(mean calculated concentration − nominal concentration)/nominal concentration] × 100.

produced only few fragment ions and to reduce specificity in qualitative analysis, we selected more fragment ions to improve the specificity of the investigated compounds. The diagnostic ions monitored for each substance and relative retention times after HFBA derivatization are listed in Table 1. Fig. 4 shows GC/NCI-MS SIM chromatogram result for the investigated compounds after HFBA derivatization. Good spectrum of analytes was achieved on a DB-5 fused silica capillary column in 10 min. The capillary column and temperature programs used enable separation of the investigated compounds during the chromatographic process with no interferences.

3.3. Evaluation of validation data

Previously, we have evaluated the validation of the GC/EI-MS analysis method [27]. In this study, GC/NCI-MS method was validated by verifying extraction recovery, method linearity, LOD, LOQ, intra- and inter-day precision, and accuracy. Table 2 shows the parameters for quantitative validation of the methods, including linearity covering the concentration range of 0.05–5 ng/mg. The correlation coefficient of calibration curves were >0.995 for all analytes, indicating significant linear regression. The sensitivity of the method was evaluated by determining the LOD and the LOQ for each analyte. The LOD and LOQ values obtained were 2 and 5 pg/mg for AP, MDA, MOR, and COD; 0.8 and 3 pg/mg for MDMA; 0.5 and 2 pg/mg for MA and MDMA; 10 and 40 pg/mg for 6-AM; 0.25 and 1 pg/mg for K; 0.025 and 0.08 pg/mg for NK. Analytical recoveries were calculated by comparing the peak areas obtained by adding the investigated compounds and the internal standards in the extract from drug-free hair sample prior to and after the extraction procedure. The overall recoveries were more than 80% of all the investigated compounds.

Analytical precision and accuracy experiments were evaluated at three concentrations (low, middle, high), covering he linear dynamic range of each analyte. The results of intra- and inter-day precision and accuracy experiments are given in Table 3. The intra-day precision and accuracy of detecting all the three concentrations ranged from 1.2% to 9.1% and −5.9% to 10.3%, respectively, and the inter-day precision and accuracy values ranged from 1.3% to 12.3% and −3.6% to 13.2%, respectively. In conclusion, an acceptable good linearity was obtained of the method for the concentration ranges 0.05–5 ng/mg. The recoveries reported of the method have compromise of a range of analytes. The detection limits and the inter-day, intra-day reproducibility were acceptable.

3.4. Integrated GC/EI-MS and GC/NCI-MS

CI is a powerful tool in MS which lends selectivity and specificity to the ionization process. As an alternative approach to EI ionization, NCI can provide enhanced analyte detection in complicated matrices and assistance in confirmation testing. We have previously evaluated the LOD values of GC/EI-MS analysis, the LOD obtained were 0.03 ng/mg for AP, MA, MDA, MDMA, and MDEA; 0.05 ng/mg for K, NK, MOR, and COD; 0.08 ng/mg for 6-AM [27]. Because the HFBA derivative reagent has natural halogenated electronegative moieties, it can be analyzed not only GC/EI-MS but also GC/NCI-MS. The use of NCI generally involves a decrement in background noise and, as a consequence, an increment in the signal-to-noise ratio. It was found that the detection limits were improved more than one order compared with those observations in EI. Amphetamines (AP, MA, MDA, MDMA, and MDEA) and opiates (MOR and COD) can be transformed to halogenated derivatives by HFBA; the sensitivity was enhanced 15- to 60-fold after GC/NCI-MS analysis. Although the innate chemical structures of K and NK have chlorine atoms owing to the electronegative moieties, the chlorine atoms were lost in the ionization process due to the observation of m/z 397 $(M\text{-}HCl)^-$ for K and m/z 383 $(M\text{-}HCl)^-$ for NK in GC/NCI-MS. Therefore, the sensitivity of K and NK after GC/NCI-MS analysis can be enhanced at 200- and 2000-fold compared with GC/EI-MS analysis in virtue of the halogenated derivatives after HFBA derivatization. Although 6-AM also can be transformed to halogenated derivatives by HFBA; the GC/NCI-MS SIM chromatogram of the 6-AM derivatives shows low intensity signal. After the selection of m/z 197 for the quantitative ion, the sensitivity of 6-AM increased 8-fold, Figs. 2 and 4 show the difference.

Cut-off values are often established by the minimum detection levels. A suitable cut-off value related to the LOD will allow correct identification of drugs or metabolites in hair. Many hair testing labs for drug abuse use the cut-off levels established by the Substance Abuse and Mental Health Services Administration (SAMHSA) in the USA [29]. According to the SAMHSA, the detection criteria of hair by GC/EI-MS confirmation were as follows: the cut-off val-

Table 4
Concentration (pg/mg) of analytes in hair samples after GC/EI-MS and GC/NCI-MS analysis

	MA	AP	MDMA	MDA	MDEA	K	NK	MOR	COD	6-AM
1										
EI	327.8	36.7	ND	ND	ND	2366.2	243.7	ND	ND	ND
NCI	368.5	45.4	4.1	3.8	ND	2426.7	278.5	ND	ND	ND
2										
EI	ND	ND	324.6	ND	ND	684.91	104.5	ND	ND	ND
NCI	52.6	7.3	349.7	15.8	ND	660.32	101.3	14.0	8.1	22.0
3										
EI	3308.3	276.4	ND	ND	ND	206.5	ND	ND	ND	ND
NCI	3359.5	283.0	18.1	6.5	ND	216.6	15.8	ND	ND	ND
4										
EI	603.9	97.2	ND	ND	ND	ND	ND	ND	ND	ND
NCI	604.5	109.8	5.27	2.2	ND	ND	ND	16.2	8.1	22.7
5										
EI	12094.3	3001.3	ND	ND	ND	ND	ND	609.7	310.4	761.3
NCI	12231.3	2956.4	6.9	2.7	ND	ND	ND	638.5	322.9	745.8
6										
EI	ND	ND	5068.6	284.8	ND	1728.9	655.4	ND	ND	ND
NCI	ND	ND	5138.2	291.3	ND	1802.5	671.8	ND	ND	ND

ND: not detected.

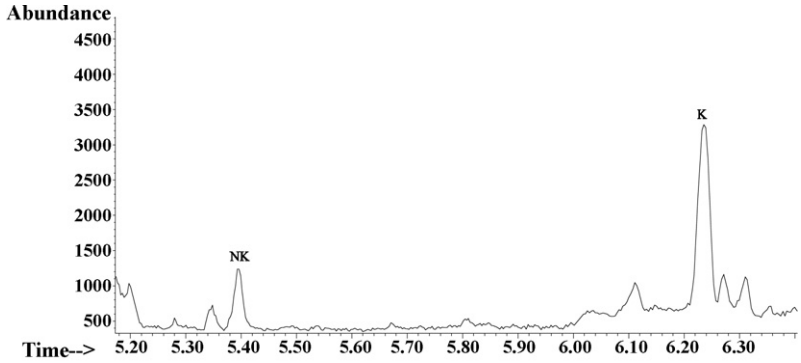

Fig. 5. GC/EI-MS SIM chromatogram of a real hair sample.

ues were 0.3 ng/mg for amphetamines (MA, AP, MDMA, and MDA); 0.2 ng/mg for opiates (MOR, COD, and 6-AM). No cut-off value has been established for ketamine. Not surprisingly, the LOD values of the investigated drugs by GC/NCI-MS analysis can be improved as compared with GC/EI-MS analysis. Obviously, owing to the high sensitivity of GC/NCI-MS, the new cut-off values for hair testing may be needed to reconsider in the future.

There are occasions where another ionization mode is desired without changing sources when we explored the integration of EI and NCI. The Agilent 5973x GC/MSD demonstrates the capability of acquiring high-quality EI spectra with the CI source. The ability to acquire both types of data without changing source results in increased productivity. However, we found the sensitivity of EI with the CI source decrease to about 1/8 comparison with sources optimized for EI. Therefore, a high-performance multiple ionization GC/MS source is in great demand in further.

3.5. Application to hair of suspected drug abusers

The comprehensive GC/NCI-MS assay has its advantages to enable simultaneous detection of opiates, amphetamines, MDMA, ketamine, and metabolites in a hair sample. The determination of drugs of abuse in hair samples were aimed at verifying the actual applicability of the described technique to real samples. GC/EI-MS and GC/NCI-MS were used to analyze six hair samples from known drug abusers. Table 4 shows the results of the simultaneous analysis using GC/EI-MS and GC/NCI-MS from drug abusers. In the GC/EI-MS analysis results of six subjects, Subject #4 tested positive for amphetamines, which means the subject had the experience of abuse with only one drug. The remaining five subjects had the experience of abuse with more than one drug. Subject #1 and Subject #3 tested positive for both amphetamines and ketamine, Subject #5 tested positive for both opiates and amphetamines, Subjects #2 and 6 tested positive for MDMA and ketamine.

The remaining samples of GC/EI-MS can be directly injected into GC/NCI-MS, which does not require any additional sample preparation. It was found that trace amounts of drugs can be detected by GC/NCI-MS because it has high sensitivity. For instance, Subject #1, Subject #3, and Subject #5 tested positive for MDMA and MDA, Subject #2 tested positive for amphetamines (MA and AP) and opiates (6-AM, MOR, and COD), Subject #4 tested positive for MDMA (MDMA and MDA) and opiates (6-AM, MOR, and COD). Those subjects seemed to take illicit drugs just one or two times because only trace amounts of drugs and their metabolites were detected.

It is critically important to demonstrate parent drugs and their metabolites were detected in hair testing. In general, the parent drugs can be detected in hair easily, its present higher concentrations than their metabolites which are scarcely incorporated in the hair matrix. However, the parent drug in hair might come from external environmental exposure, the detection of metabolites was really important because it can be used as evidence of active intake, prevent false positives and correct interpretations of results. The trace amounts of metabolites cannot be detected with GC/EI-MS, but the highly sensitive instrument of GC/NCI-MS can detect. The sensitivity of K and NK can be dramatically enhanced after GC/NCI-MS analysis, especially for NK (the metabolites of K). Therefore, the integration of GC/EI-MS and GC/NCI-MS method would be beneficial in the future to aid analysis of K smoke. As far as we know, K is widely used and smoking is the potential route of drug administration, however, contamination of hair from external K smoke has been minimally investigated. In our experiences of K analysis in hair samples by GC/EI-MS, only K has been detected in many cases. Fig. 5 shows the GC/EI-MS SIM chromatogram result of a real hair sample. K and trace of NK have been detected in the case. In

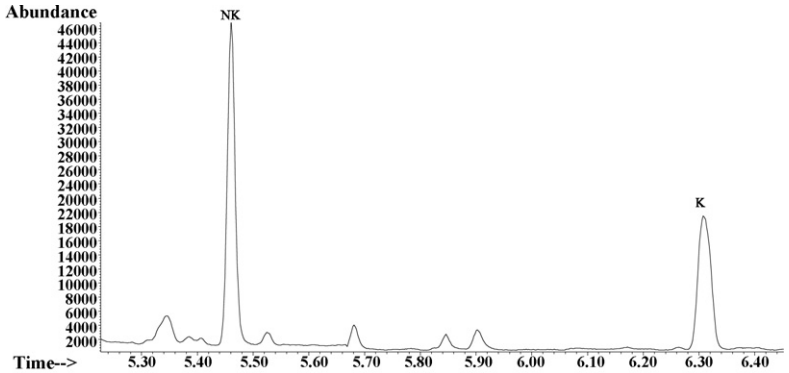

Fig. 6. GC/NCI-MS SIM chromatogram of a real hair sample.

order to improve the sensitivity and accurate detect metabolite-to-parent drug ratio in hair, the remaining samples of GC/EI-MS directly injected into GC/NCI-MS. Fig. 6 shows the GC/NCI-MS SIM chromatogram result of the sample, the concentrations of K and NK measured in this sample were 183.19 and 6.77 pg/mg, respectively. The case seems to be passive exposure to K in the K smoke environment. In conclusion, the accurate at metabolite-to-parent drug ratio in hair seem to be a viable approach to differentiating active users from false positives due to external contamination.

4. Conclusion

As far as we know, the presented study was the first time to integrate two GC–MS ionization methods for the simultaneous determination several types of drugs. It is commonly known that GC/EI-MS is the most widely used of all ionization methods for the analysis of drugs in hair, and GC/NCI-MS could dramatically improve the sensitivity when the investigated compounds owing to the electronegative moieties. The new strategy offers several advantages, including the enhancement of the sensitivity; avoiding wrong results and wrong interpretations of correct results and reducing the consumption of time, labor and hair sample. Although the ability to acquire both types of data without changing source results in increased productivity, a high sensitivity of multiple ionization GC/MS source is in great demand in further.

The reliable simultaneous sample preparation method with HFBA derivatization of the analytes after acidic extraction was able to simultaneously quantify opiates, amphetamines, MDMA, ketamine, and their metabolites in human hair. The HFBA derivative can be simultaneously analyzed not only GC/EI-MS but also GC/NCI-MS. The two different ionization techniques are complementary as more structural information can be obtained from the EI mode while more molecular mass information from the NCI mode. Both assays used the same sample, thus it is efficient and convenient without time-consuming sample preparation. Importantly, this study clearly demonstrated that the use GC/NCI-MS could confirm the results of GC/EI-MS. In addition, a consequence of the use GC/NCI-MS could detect the trace amounts of drugs and their metabolites in a hair sample, it will benefit further below the currently cut-off value. Moreover, the trace amounts of metabolites can be only detected with GC/NCI-MS in some authentic hair samples. There is no doubt that integrating the characteristics of GC/EI-MS and GC/NCI-MS for the simultaneous hair testing can provide valuable and valid information in the fields of forensic science and clinical toxicology.

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