— Minireview —

High-Performance Liquid Chromatographic Analysis of Drugs of Abuse in Biologic Samples

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Recently, drug abuse has become a serious social problem world wide. In Japan, methamphetamine (MP) is the most popular drug of abuse. In addition to MP, the use of 4,5-methylenedioxymethamphetamine (MDMA), called ecstacy, is rapidly increasing, especially among young people. The development of simple and convenient analytical methods for the analysis of these drugs of abuse is necessary for the prediction of and protection from human health risks. Many useful methods have been developed for qualification and quantification of drugs of abuse. Among these, gas chromatography with mass spectrometry (MS) and high-performance liquid chromatography with MS (HPLC-MS or LC-MS) or fluorescence (HPLC-FL) detection are widely used. As highly sensitive methods, precolumn or postcolumn derivatization methods are commonly utilized in HPLC. This review focuses on HPLC methods used for the practical analysis of drugs of abuse, mainly for amphetamine derivatives and MDMAs in biologic samples such as urine, blood, and hair.

Key words —— analysis, drug abuse, high-performance liquid chromatography

INTRODUCTION

Analyses of drugs of abuse are important for the prediction of and protection from the risk to human health, especially for young people. The use of drugs of abuse is increasing world wide and causing serious social problems. In Japan, abuse of methamphetamine (MP) is the most common and arrests for stimulant drug-related offences totaled 14624 in 2003.[1)] Recently, the use of 4,5-methylenedioxymethamphetamine (MDMA), which is called ecstacy, and 4,5-methylenedioxyamphetamine (MDA) is increasing among the young. Although the arrests for MDMA-related offences numbered 256 in 2003,[1)] which is very small compared with those for stimulants, the increase in its use among teenagers is serious. MDMA and MDA are used in tablet form, and thus can be easily taken orally, while MP that is mainly taken by injection. For the prediction of and protection from abuse of drugs, simple and sensitive methods for qualitative and quantitative analyses are required. Many chromatographic methods such as thin-layer chromatography, gaschromatography (GC), and high-performance liquid chromatography (HPLC) as well as immunoassays have been developed. Among these, HPLC has been a secondary choice. However, HPLC essentially can be applied to water-soluble compounds, and thus its use in the forensic and toxicologic fields is increasing rapidly.

In this review, HPLC methods developed in the past 5 years and their use in the analyses of stimulant- and MDMA-related compounds in biologic samples are described.

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DRUGS OF ABUSE

Many types of drugs have been abused and caused serious human health problems. Arrests for the possession and use of representative drugs of

abuse in Japan in 2003 are shown in Fig. 1. MP is the most popular and the arrests of those less than 20 years old for stimulant-related offences totaled 524 in 2003.[2] On the other hand, although the illegal use of MDMA is less than that of MP, the number of confiscated tablets containing MDMA is rapidly increasing. The total number of MDMA tablets confiscated in Japan was 393062 in 2003.[1,2]

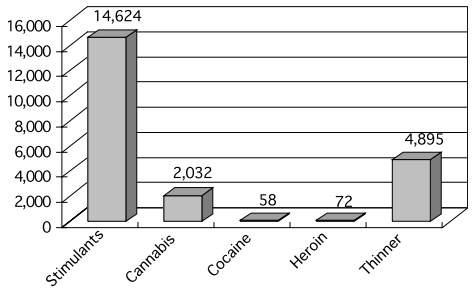

Fig. 1. Number of Arrestees for Representative Drugs of Abuse in Japan in 2003

HPLC DETECTION METHODS

HPLC is a very versatile method. The most common detection methods are ultraviolet (UV), electrochemical (EC), fluorescence (FL), and MS. For HPLC-UV and -FL methods, a derivatization procedure is generally required to increase sensitivity. LC-MS is a more versatile method for sensitive determination of many types of drugs including amphetamines. As a conventional method, GC-MS is well known and has generally been used in the forensic and toxicologic fields. However, GC also requires derivatization to increase the volatility of target compounds, and thus its use for water-soluble compounds has been limited. On the other hand, LC-MS generally requires no derivatization of target compounds. This is an important advantage of analytical procedure in terms of time reduction. As a result, LC-MS is becoming more commonly used than GC-MS.

Recently, the rapid emergency drug identification high-sensitivity (REMEDi-HS) system utilizing HPLC has been used in emergency hospitals for the detection of drugs of abuse as well as poison compounds.

ANALYSIS OF BIOLOGIC SAMPLES

Urine Sample

For the determination of illegal compounds such as amphetamines and opiates, urine is the most commonly used sample. Many practical methods utilizing HPLC have been developed, as described below.

Enantiomers of MP and its metabolite amphetamine (AP) were sensitively determined using FL detection after derivatization with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl). *S*(+)- and *R*(–)-enantiomers and *p*-hydroxymethamphetamine were detected in 19 Japanese abusers' urine samples. The degree of *N*-demethylation of *S*(+)-MP into the corresponding metabolite AP was significantly higher than that of the *R*(–)-enantiomer.[3] A semi-microcolumn separation was applied to achiral and chiral quantification of MP and AP using DIB-Cl as a label.[4] The conventional RP-column was also used for the detection of methamphetaimines in human urine.[5] AP and its metabolite, *p*-hydroxyamphetamine, in rat urine were sensitively determined after fluorescent labeling with dabsyl chloride. The recoveries of AP and *p*-hydroxyamphetamine were 97 and 94%, respectively. The detection limits of the method were 10 ng for both compounds.[6] Screening and quantification of MP were also achieved after dansyl chloride derivatization and solid-phase extraction with HPLC-FL detection and GC-MS. A good correlation (r = 0.95) between HPLC and GC-MS was obtained for urinary MP.[7]

Automated HPLC-FL or -UV using a column-switching and on-column derivatization method for AP was developed with *o*-phtaldialdehyde and *N*-acetyl-L-cysteine as labeling reagents. The proposed method is simple and rapid, with a total analytic time of approximately 8 min. The limits of detection were 25 and 10 ng/ml with UV and FL detection, respectively.[8] Direct determination of MP enantiomers using HPLC-UV was performed with a strong cation-exchange precolumn (SCX) and phenyl-β-cyclodextrin-bonded semi-microcolumn using the column-switching method. The detection limit for both enantiomers was 0.1 μg/ml.[9] *N*-Methylephedrine, a tertiary AP, was determined using precolumn derivatization with 9-fluorenylmethyl chloroformate. The limit of detection and quantification were 0.1 and 0.5 μg/ml, respectively.[10] Simple and rapid determination of APs and ephedrines with diode array detection was performed using solid-phase extraction. The recovery rates were at least 85%. Detection limits of APs and ephedrines were 0.2 and 0.5 mg/ml for human urine.[11]

Screening and identification of AP, MP and their derivatives were examined in the immunochemical [Triage and fluorescence polarization immunoassay (FPIA)] and chromatographic (REMEDi) techniques. REMEDi gave information on a single drug and main metabolites in the samples with minimal false-positive or false-negative results.[12)]

LC-electrospray ionization (ESI)-MS in the selected ion monitoring (SIM) mode was used for the simultaneous determination of dimethylamphetamine, its main metabolite dimethylamphetamine-*N*-oxide, and other metabolites MP and AP. A semimicro SCX column was used with the detection limits of 5–50 ng/ml.[13)] Selegiline-*N*-oxide, a specific metabolite of selegiline, was examined as a new indicator of selegiline administration in Perkinson's disease treatment. For discrimination of selegiline use from MP use, analysis of the urinary metabolites of selegiline is important. Selegiline-*N*-oxide was first detected in urine, in addition to AP and desmethylselegiline as metabolites.[14)]

Blood Samples

Blood samples are useful indetermining the short-term use of drugs of abuse. However, there are some difficulties from the legal point of view in obtaining abusers' blood samples.

MP and AP in human plasma samples were determined with FL detection using DIB-Cl as a labeling reagent. The detection limit was less than 0.87 ng/ml plasma. The method was used in two cases of illegally ingested MP.[15)] Simultaneous determination of free-form AM in rat blood and brain was performed using *in vivo* microdialysis with dansyl chloride as a FL-label. Pharmacokinetic parameters of AP in rat blood and brain were reported.[16)] The obesity drugs fenfluramine and phentermine in rat brain and blood microdialyzates were determined using HPLC-FL with DIB-Cl as a label.[17)] The method is very sensitive and detected < 23 fmol (S/N = 3) on the column for both compounds.

Pholedrine (4′-hydroxymethamphetamine), a cardiovascular agent, was determined in amperometric detection using ion-pair extraction with bis(2-ethylhexyl)phosphoric acid as an ion-pair reagent.[18)]

Pholedrine in a case of fatal intoxication was also studied using LC-MS/MS. The method developed is very sensitive with a limit of detection of 0.8 ng/ml (S/N = 3) and lower limit of quantitation of 3 ng/ml (S/N = 10).[19)] Analysis of underivatized APs and phenethylamines was performed with LC-atmospheric pressure chemical ionization (APCI)-MS using solid-phase extraction. Compounds examined were AP, MP, illicit designer phenethylamines such as MDA, 4,5-methylenedioxyethylamphetamine (MDEA), MDMA, *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butamanine (MBDB), and 4-bromo-2,5-dimethoxyphenethylamine (BDMPEA), and other phenetylamines such as benzyl-1-phenylethylamine, cathinone, ephedrine, fenfluramine, norfenfluramine, phentermine, 1-phenylethylamine, phenylpropanolamine, and propylhexedrine. The detection limits ranged from 1 to 5 μg/l serum, and recovery rates ranged from 58 to 96%.[20)] The possibility of creating a drug library with HPLC-AP-ESI was examined for identification of toxicologically relevant drugs in plasma. Forty different drugs were extracted from spiked blank plasma and patient samples. A search for significant peaks in the chromatogram in the MS library was shown to result in more than 95% positive identifications.[21)] Paramethoxyamphetamine and other amphetamine-related designer drugs such as MDMA, AP, and MDA were determined using LC with sonic spray ionization MS. Weighted (1/x) quadratic calibration curves were generated ranging from 10 to 1000 ng/ml (blood and urine) or 20 to 2000 ng/g (tissue) with correlation coefficients > 0.995.[22)] Selegiline and its three metabolites were sensitively assayed using a LC-APCI-MS/MS method. Lower limits of quantitation were 0.1 ng/ml for selegiline and *N*-desmethylselegiline, and 0.2 ng/ml for MP and AP. Extracted plasma samples retained quantitative accuracy after storage for at least 7 days at –20°C or up to 70 hr at room temperature.[23)]

Hair Samples

Hair yields useful samples in forensic and toxicologic sciences, because it is very stable and easy to handle compared with other biologic samples and can indicate long-term intake of drugs of abuse. A history of abuse can also be clarified by segmental analysis of hair.

A very sensitive HPLC-FL detection method for the determination of AP-related compounds such as MDMA, MDA, AP, and MP was developed using DIB-Cl as a label. Tentative chromatograms are shown in Fig. 2. The limits of detection for these compounds range from 11 to 200 pg/mg hair. The method was successfully used to determine MDMA and MDA in hair samples obtained from an MDMA abuser and clarify the history of use, as shown in Fig. 3.[24)] The DIB-Cl derivatization method was also

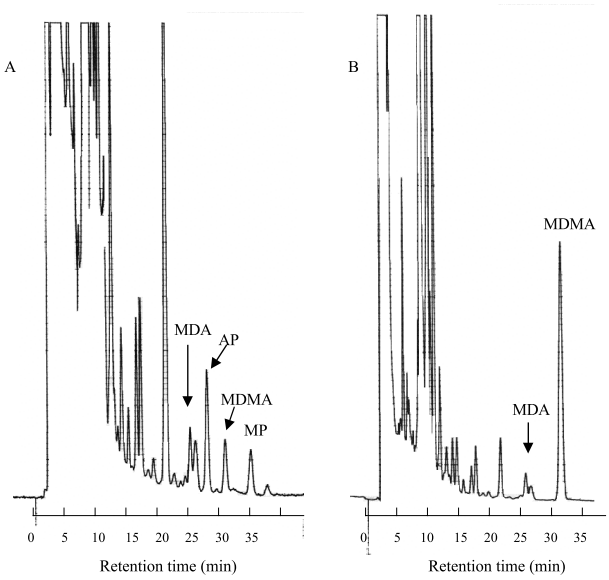

Fig. 2. Chromatograms

Chromatograms obtained from (A) spiked control human hair with MDA and AP 1 ng/mg, MDMA 2 ng/mg and MP 1.1 ng/mg, (B) segment 5 of abuser's hair containing MDA and MDMA 1.67 and 42.2 ng/mg, respectively. Detector sensitivity in (B) is four-fold lower than in A. Reprinted from Ref. 24 with permission of Wiley & Sons Ltd.

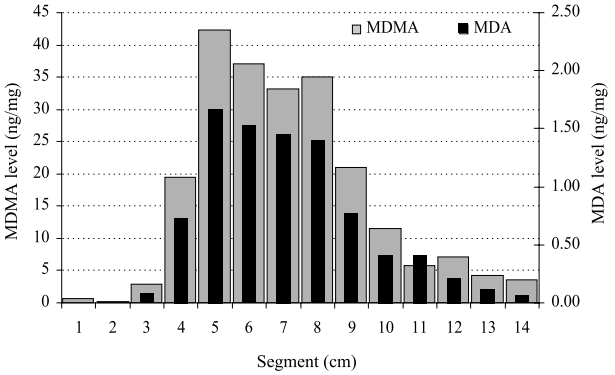

Fig. 3. Concentrations of MDA and MDMA in MDMA Abusers' Hair Strands Sectioned into 1-cm Segments

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applied to determine MP and AP in an abuser's hair samples, and very low amounts of both compounds were found.[15] Enantiomer-specific FL detection was also developed for the determination of MPs in abusers' hair using DIB-Cl as a label.

Segment analysis of MP and AP was developed using FL detection with DIB-Cl as a label.[25] Hair samples were segmentally analyzed based on 1-cm long segments. In four samples, only the *S*(+)-enantiomers of MP and AP were detected.[3] Furthermore, chiral and achiral semi-microcolumn HPLC methods with FL detection using DIB-Cl were applied to abusers' single-hair analyses. *S*(+)-Enantiomers were found in eight Japanese abusers' hair samples. The achiral method was used to study the concentrations of these compounds in single black and white hair strands of abusers.[26]

Fenfluramine and norfenfluramine in human hair as biomarker metabolites of *N*-nitrosofenfluramine (N-Fen) were determined using HPLC-FL with DIB-Cl as a label. The results obtained showed that the patients might have ingested N-Fen for a period of not less than five months.[27]

The chemiluminescence detection method was used to study the effects of permanent wave, dye, and decolorant treatments on MP and AP in abusers' hair. The concentrations of both compounds decreased in all cases. The results suggest that such hair treatments might interfere with the detection of MP and AP in hair.[28]

Simultaneous determinations of eight underivatized APs, *i.e.*, AP, ephedrine, methcathinone, *p*-methoxyamphetamine, MP, MDA, MDMA, and MDEA, in hair was performed using LC-APCI-MS. The analytes were digested in NaOH 1 M and extracted with 1-chlorobutane. The limits of detection ranged from 0.05 to 0.2 ng/g hair. The method was applied to 93 authentic hair samples obtained from detoxification and methadone treatment patients.[29] Polypyrrole-coated capillary in-tube solid-phase microextraction was used for the simultaneous assay of stimulants in spiked human hair samples with ES/MS detection.[30]

Other Samples

AP in rat brain was sensitively determined using *in vivo* microdialysis and ion-pairing LC with ES-MS/MS. Detection was performed with no postcolumn addition of an organic modifier. The detection limit was 0.001 μg/ml (5 nM) at S/N = 3. AP reached a maximum concentration of 0.086 ± 0.017 μg/ml over 20–40 min after a single 3.0-mg/kg i.p. administration.[31] AP, MP, and MDA derivatives in meconium were determined with LC-API-MS using 3,4-methylenedioxypropylamphetamine as an internal standard. Separation was achieved with a reverse-phase column using a linear gradient of ammonium bicarbonate 10 nM (pH 9) and methanol as mobile phases. The quantification limits were 0.005 μg/g meconium for AP, MP, and 4-hydroxy-3-methoxymethamphetamine and 0.004 μg/g meconium for MDA, MDMA, MDEA, and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine. The method was used to analyze meconium in newborns

to assess fetal exposure to AP derivatives.[32] Detection of MP and AP in abusers' clothing was performed with FL and UV detection. The limits of detection were less than or equal to 37.3 pg for UV and 0.4 pg for FL on column. MP and AP excreted *via* sweat from the human body was also successfully determined.[33]

CONCLUSION

Recently developed HPLC methods for the determination of stimulant-related compounds in biologic samples are versatile and convenient, and thus applicable to the analysis of many other drugs of abuse. The author expects further development in HPLC methods, which will contribute to predicting and protecting human health from the risk of drug abuse.

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