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# Development of a two-step injector for GC–MS with on-column derivatization, and its application to the determination of amphetamine-type stimulants (ATS) in biological specimens

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**Abstract**

A two-step auto-injector has been developed for the automated on-column derivatization and subsequent GC–MS of amine-type drugs and metabolites. To effectively derivatize such analytes, this injector has been designed to inject the derivatization reagent several seconds after the sample has been injected. Eleven kinds of amphetamine-type stimulants (ATS) and their typical metabolites were examined, using the trifluoroacetylation reagent *N*-methyl bis(trifluoroacetamide) (MBTFA). Although the quantitative derivatization of the hydroxyl groups was difficult, this technique was successfully applied to the determination of ATS in urine, blood, and hair specimens. The detection limits of methamphetamine and amphetamine in hair were 0.2 and 0.1 ng/mg hair, respectively, in the full-scan mode, when a 10 mg hair sample is analyzed.


*Keywords:* On-column derivatization; MBTFA; Trifluoroacetylation; ATS; Methamphetamine; MDMA; GC–MS; Sample injector; Automation

## 1. Introduction

Amphetamine-type stimulants (ATS), such as methamphetamine (MA), have been the most prevalent illicit drugs in many countries and regions, and are becoming more serious problems throughout the world [1,2]. In the analysis of such amine-type drugs and metabolites, the confirmation is usually carried out by GC–MS after perfluoroacylation to enhance sensitivity and specificity. However, manual sample preparation, which is often time-consuming, is necessary in conventional derivatization, for instance, with trifluoroacetyl (TFA) anhydride (TFAA) [3–5].

As an alternative for TFAA, *N*-methyl bis(trifluoroacetamide) (MBTFA) is available for trifluoroacylating primary and secondary amines, and the hydroxyl group under mild non-acidic conditions [6]. To cope with a large number of samples, we have used MBTFA as an on-column derivatization reagent, which allows the injection of an easy-to-prepare free-base ATS extract, and its instant trifluoroacetylation, without any tedious manual handling [7,8]. This derivatization method, especially in combination with the headspace technique, provides significant time savings in the GC–MS determination of ATS in urine [7,9]. Although no paper has described the use of MBTFA for the on-column derivatization of drugs in hair, Hidvegi et al. [10] recently reported its use for the on-column derivatization of ATS in serum.

For quantitatively derivatizing ATS, however, MBTFA should be injected several seconds after the sample has been injected, using a separate syringe, especially for quantitatively derivatizing less reactive *N*-alkylated ATS [4,5,7,8]. This is because the derivatization occurs most effectively on the separation column when the flow of MBTFA overtakes those of free-form ATS analytes, near the inlet of the column [7,11]. Thus, this technique is convenient, but its automation has remained a major challenge.

In order to achieve the automation of this technique, the authors have invented and developed a two-step auto-injector that first suctions MBTFA, and then the sample, followed by separately injecting the sample, and finally MBTFA, after an interval of several seconds [11]. Eleven kinds of ATS and their

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metabolites were examined as model analytes. This technique was successfully applied to the determination of ATS in urine, blood, as well as to ATS in hair, which is among the most difficult of analytical subjects [12].

## 2. Experimental

### 2.1. Chemicals and reagents

D-MA hydrochloride and DL-amphetamine (AP) sulfate were purchased from Dainippon Pharmaceutical (Osaka, Japan) and Takeda Pharmaceutical Industries (Osaka, Japan), respectively. Hydrochloride salts of DL-*p*-hydroxyamphetamine (*p*-OH-AP) and DL-*p*-hydroxymethamphetamine (*p*-OH-MA) were synthesized according to the method of Buzas and Dufour [13]. D-Dimethylamphetamine hydrochloride was synthesized according to the method of Inoue and Suzuki [14]. Hydrochloride salts of 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) were provided by Japan's ministry of health, welfare, and labor. Hydrochloride salts of *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) and (3,4-methylenedioxyphenyl)-2-butanamine (BDB) were synthesized according to the method of Shulgin and Shulgin [15]. Hydrochloride salts of D-ephedrine (Ep), DL-methylephedrine (MEp), and DL-norephedrine (NEp) were obtained from Alps Yakuhin (Gifu, Japan), Kansai Yakuhin (Osaka, Japan), and Tokyo Kasei (Tokyo, Japan), respectively. Stock standard solutions containing these compounds at 1.0 mg/mL each were prepared in water, and was diluted and/or spiked into drug-free biological samples or hair extracts at known concentrations.

MBTFA (derivatization reagent grade) and diphenylmethane (DPM) (analytical grade), used as an internal standard (I.S.), were purchased from Wako Pure Chemical Industries (Osaka, Japan). All organic and inorganic reagents used were of analytical grade or better quality. Deionized, distilled water was used throughout the experiments.

### 2.2. Sample preparation

#### 2.2.1. Urine

To a 0.75 mL urine sample was added 0.4 mL of concentrated carbonate buffer (1.0 M, pH 10) and 1.0 mL of ethyl acetate containing diphenylmethane (I.S.) at 3.0 μg/mL. The mixture was vigorously stirred for 2 min. After layer separation, the organic layer was separated into a vial containing 0.25 g of anhydrous sodium sulfate. An automated sample pretreatment system ATLAS mini-arm (Shimadzu, Kyoto, Japan) was utilized to perform the sample processing. The extract obtained was analyzed by GC–MS with on-column trifluoroacetylation, as described below.

#### 2.2.2. Blood

A 200 μL whole blood sample was diluted with 1.0 mL of 0.05 M phosphate buffer (pH 6.8), and this was applied onto a Bond Elut SCX cartridge (3 mL capacity; Varian, Harbor City, CA) that had been preconditioned with methanol–HCl (240:1; 5 mL), methanol (3 mL), and water (10 mL). After washing the cartridge with ethanol–water (1:1, 10 mL), the cartridge was dried in vacuum for 5 min, and the analytes were eluted with 28% ammonia–methanol (1:20, 3 mL). The eluate was then evaporated to approximately 150 μL under a gentle stream of nitrogen at room temperature. After adding 100 μL of ethyl acetate containing diphenylmethane (I.S.) at 1.0 μg/mL, the sample was dehydrated with 0.2 g of anhydrous sodium sulfate, and was separated into a microvial for GC–MS.

#### 2.2.3. Hair

A hair specimen (10 mg, cut into lengths of 2–3 mm) was washed, and ATS incorporated in hair were extracted into 1 mL of methanol–5 M HCl (20:1, v/v) solution, according to our previously reported method [16]. The extract was reconstituted in 1.0 mL of 0.05 M phosphate buffer (pH 6.8), and was purified in a similar manner as in the case of blood (a 1-mL capacity cartridge was used and the volumes of solvents were halved). After adding 100 μL of ethyl acetate containing diphenylmethane (I.S.) at 1.0 μg/mL, the sample was dehydrated with 0.15 g of anhydrous sodium sulfate, and was separated into a microvial for GC–MS. Spiked hair samples used for the validation of the present method were prepared in the same manner from 10-mg drug-free hair samples, by adding known amounts of MA and AP during the soaking extraction in the methanol–5 M HCl solution.

### 2.3. GC–MS

GC–MS was performed on a Shimadzu GCMS-QP-2010 gas chromatograph equipped with a quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Confirmation and quantitation were conducted in the full-scan and selected ion monitoring (SIM) modes, respectively with electron-impact ionization (70 eV). A Shimadzu AOC-20i auto-injector, which was adapted by replacing its ROM with a specially developed one based on the authors' invention, was used for the two-step injection of the sample and the derivatization reagent, as well as for the single injection of the sample (This ROM is currently available upon request to Shimadzu.). A DB-5MS or a DB-1MS capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; J&W Scientific, Folsom, CA) was used with helium as the carrier gas at a flow-rate of 3.0 mL/min and a split ratio of 3 (for hair and blood) or 5 (for urine and diluted standard samples). Typical operation conditions were as follows: injection port temperature, 230 °C; initial column temperature, 120 °C (140 °C for MDMA analogs), ramped to 170 °C at 15 °C/min, and ramped at 30 °C/min to 250 °C; interface temperature, 250 °C. The calibration curves for MA and AM were constructed by calculating the peak area ratios of target ions at *m/z* 154 for MA, *m/z* 140 for AM against *m/z* 168 for I.S. (diphenylmethane). The limit of detection was determined based on the detection limits of the above-mentioned target ion and at least two other qualifier ions on each mass chromatogram at S/N = 3, with the ratios of qualifier ions to the target ion, lying within 20% of the standard.

For additional analyses of the TFA derivative of MEp, tandem GC–MS (GC–MS/MS) were performed on a Varian Saturn

2100T iontrap GC–MS/MS system (collision gas, He; excitation amplitude, 59 V), in addition to GC–MS with chemical ionization (CI), using the Shimadzu GCMS-QP-2010 system (reagent gas, isobutane, 0.2 kg/cm$^2$).

### 2.4. On-column derivatization

The on-column trifluoroacetylation was performed by automatically injecting 1 μL of MBTFA 3 s after the sample injection. The mechanisms of the two-step injector and on-column trifluoroacetylation are described in the subsequent section. The operation parameter settings were as follows: the amount of MBTFA, typically 1 μL; that of the sample solution, 1 μL for urine and blood extracts, 2 μL for hair extracts; the air gap, 4 μL; the injection speed of MBTFA, 10 μL/s; and the interval between the injections of the sample and MBTFA, 3 s.

The yields of TFA derivatives were basically calculated by comparing their amounts with those prepared by the ordinary method using TFAA. However, the yield of mono-*N*-TFA derivative of ATS possessing a hydroxyl group, which cannot be prepared by using TFAA, was estimated by comparing its peak area with that of bis-*N*,*O*-TFA derivative obtained by the ordinary method using TFAA, on the total ion chromatogram.

## 3. Results and discussion

### 3.1. Mechanism of the two-step injector and on-column trifluoroacetylation

Fig. 1 illustrates the mechanism of on-column trifluoroacetylation using the two-step injector. The injector is equipped with a micro-syringe that is to inject both the sample solution and the derivatization reagent. First, the derivatization reagent is suctioned into the syringe, followed by air (creating an air gap to separate the sample from the derivatization reagent), next the sample, and finally 1 μL of air, so that the sample is positioned closer to the needle than the derivatization reagent. The use of a gas-tight syringe was effective to minimize cross-contamination of the sample and the derivatization reagent in their injections. After the sample has been injected into the instrument, the derivatization reagent MBTFA is injected when a predetermined period of time has elapsed. To optimize the performance of the injector, settings may be made according to the following operation parameters: the amount of derivatization reagent suctioned, that of the sample solution, the air gap, the injection speed of the derivatization reagent, and the interval between the injections of the sample and the reagent.

When the vaporized sample enters the column, the target analyte is retained on the inner wall of the column over an area close to the injection port, while the solvent is carried away faster. After this stage has been reached, the vaporized derivatization reagent flows into the column and comes in contact with the bands of analytes adsorbed over a certain area, thereby efficiently derivatizing the analytes at a certain column temperature (typically 120 °C).

### 3.2. Identification of derivatives with on-column trifluoroacetylation

The products from the on-column trifluoroacetylation with MBTFA of 11 kinds of ATS were investigated by GC–MS at an initial column temperature of 120 °C. Primary and secondary amine-type ATS without a hydroxyl group gave the same TFA derivatives as with the ordinary reagent TFAA. Among ATS possessing the hydroxyl group, NEp, Ep, *p*-OH-AP, and *p*-OH-MA, all of which have two reactive sites for trifluoroacetylation, predominantly gave an atypical mono-*N*-TFA derivative, though TFAA gives a common bis-*N*,*O*-TFA derivative. The mono-*O*-TFA derivative was predominantly detected for MEp as detailed below. The electron-impact mass spectra of such atypical derivatives and that of MEp are shown in Fig. 2.

Free-form ATS, especially those possessing a hydroxyl group, provide a less abundant peak with gross tailing, due to their adsorption onto the flow line, which makes their determi-

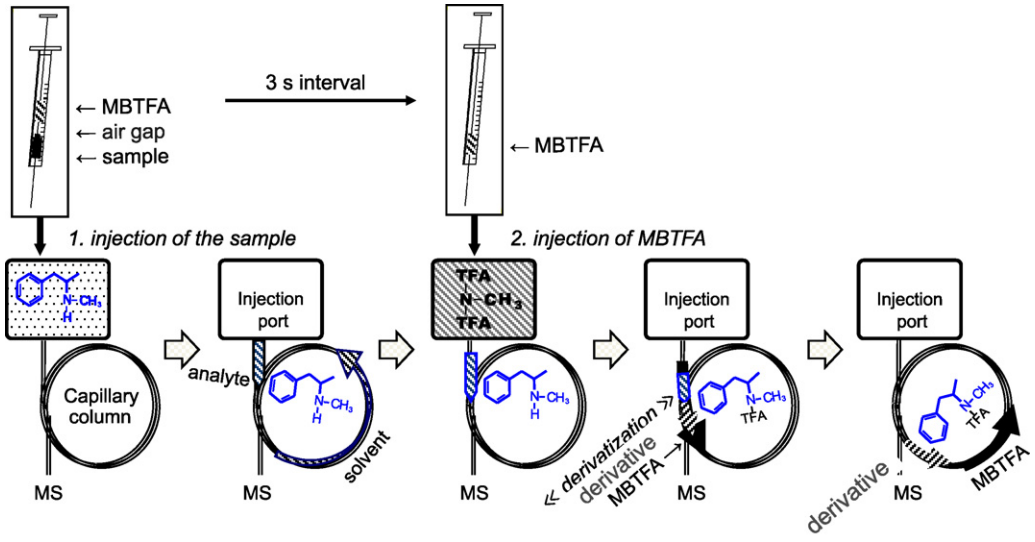


Fig. 1. Mechanism of on-column trifluoroacetylation using the two-step injector and MBTFA.

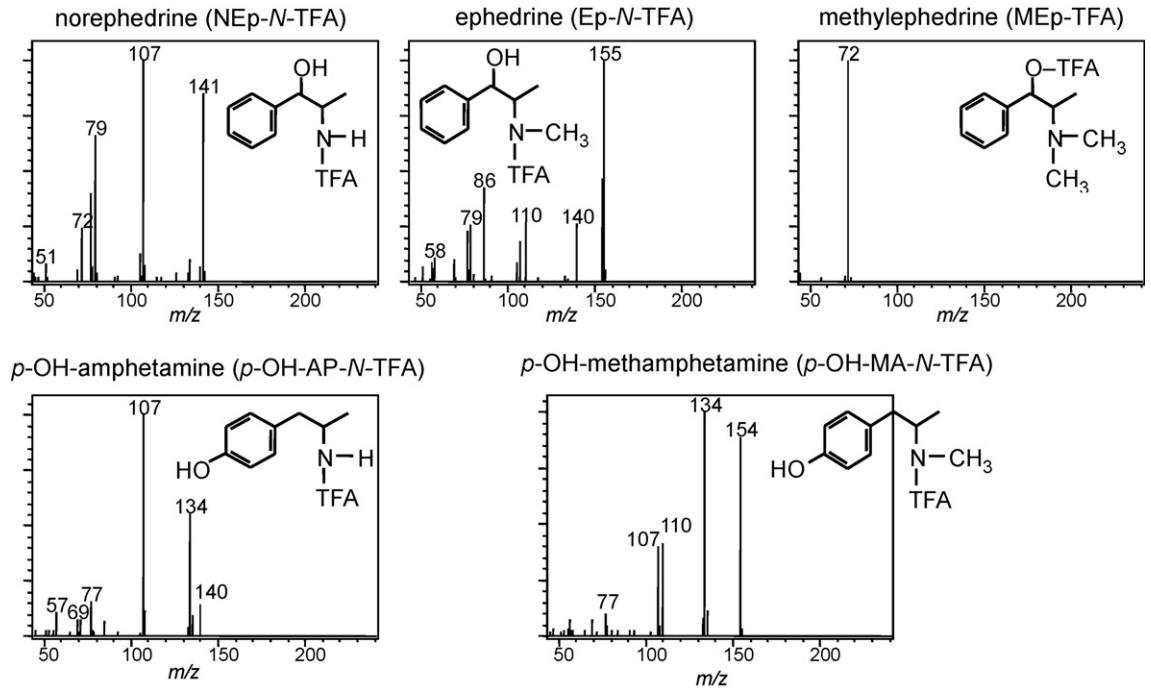


Fig. 2. Electron-impact mass spectra of atypical trifluoroacetyl derivatives of ATS possessing the hydroxyl group, detected in the present method.

nation impractical without derivatization. Although the present on-column method does not provide satisfactory derivatization yields of the hydroxyl group, the peaks of corresponding mono-*N*-TFA derivatives are as sharp as those of their typical bis-*N*,*O*-TFA derivatives, which allow their determination with much better precision and specificity than without derivatization.

### 3.3. Effects of column temperature

Because the on-column derivatization reactions occur in the column under the present experimental conditions, the effects of initial column temperature (reaction temperature) on the trifluoroacetylation of various types of ATS were investigated. Fig. 3 shows the changes in the derivatization yields, measured at a concentration of 10 μg/mL each. The yields were calculated by comparing their amounts with those prepared by the ordinary method using TFAA which is known to quantitatively derivatize both the amino and hydroxyl moieties of ATS, as detailed in Section 2. As shown in Fig. 3, the derivatization yields generally increased as the initial column temperature was raised. The highly reactive, primary amine-type ATS (i.e., AP and MDA) were semiquantitatively trifluoroacetylated (with yields of 90% or better), even at 80–100 °C. MA and MDMA, which are lesser reactive secondary amine-type ATS, were semiquantitatively derivatized at 120–140 °C. The derivatization yields of *p*-OH-AP remained lower than 40% by the present method. For Ep, although the mono-*N*-TFA derivative was predominant (73%), the bis-TFA derivative (6%) and free EP (18%) were also detected at an initial column temperature of 120 °C. For MEp, while a trace amount of free-form MEp was detected, the *O*-TFA derivative was predominantly detected at 140 °C or higher.

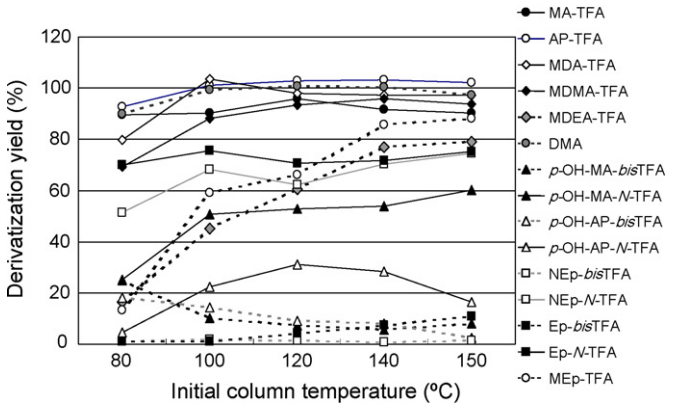


Fig. 3. Effects of initial column temperature on the derivatization yields of various ATS. The yields plotted were the averages for triplicate measurements of a chloroform solution containing all ot the analytes at 10 μg/mL each, as their free base.

A relatively high initial column temperature of 140 °C provided satisfactory derivatization yields of various types of ATS tested here by the on-column method. However, owing to the cold-on-trap effect, a lower initial column temperature, provides better peak shapes and separations, especially for volatile ATS such as AP and MA. In fact, slight peak deteriorations were observed for MA and AP when the initial temperature was set at 140 °C. Based on the above-mentioned results, it was concluded that the initial column temperature should be set at 120 °C for simple ATS, including AP, MA, and DMA (not derivatized), as well as for screening purposes; while 140 °C was optimal when the main targets are MDMA analogs and ephedrines. Fig. 4 shows the mass chromatograms of 11 ATS with on-column derivatization using MBTFA, where the initial column temperature was set at 120 °C.

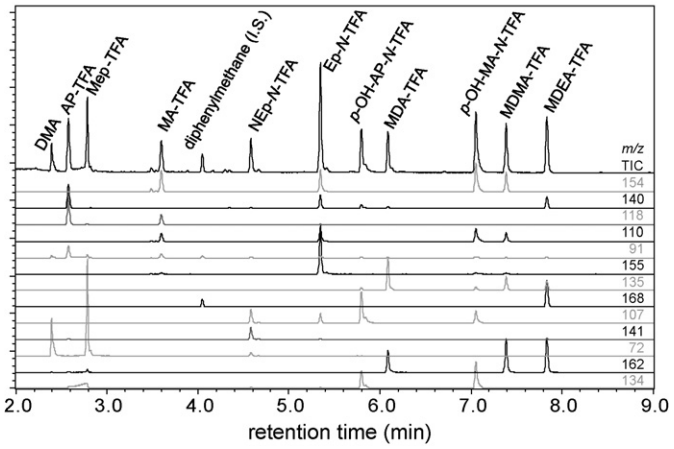


Fig. 4. Mass chromatograms of eleven ATS at 10 μg/mL each with on-column trifluoroacetylation using MBTFA. GC conditions: DB-5MS (0.32 mm × 30 m, i.d., 0.25 μm), 120 °C (3 min) to 200 °C (+10 °C/min).

### 3.4. Isomerization of the TFA derivative of MEp

In the ordinary GC–MS of the TFA derivative of MEp prepared with TFAA, a pair of characteristic peaks (having *m/z* 134 and 72, respectively, as their base fragment ions) with a broad "tailing" between the peaks, having *m/z* 134 as its base fragment ion, usually appear, as shown in Fig. 5. The former peak (having *m/z* 134) was most probably produced from the thermal isomerization of MEp–TFA, mainly at the injection port and slightly in the column, which makes the GC–MS determination of MEp problematic. However, the present on-column method was found to considerably reduce such isomerization, and only above-mentioned slight peak "leading" (due to the unavoidable thermal isomerization in its passage through the column and interface), was observed. Thus, the present on-column method was found to provide more a precise yet convenient GC–MS determination of MEp. This also demonstrates that derivatization by the present method occurs mainly in the column (120–140 °C) close to the injection port, instead of at the injection port (230 °C). The structure of the isomer was also investigated using GC–MS with chemical ionization and GC–MS/MS. As a result, its molecular weight was confirmed to be 275 (the same as MEp–TFA), and the base peak observed at *m/z* 134 was presumably due to $C_6H_5$–$CH_2$–$CH{=}N^{\bullet+}$ $HCH_3$ or $C_6H_5$–$CH{=}N^{\bullet+}$ $(CH_3)_2$, though the isomer's structure and its fragmentation could not be clarified. No such isomerization was observed for the other ATS tested here.

### 3.5. Optimization of injector operation parameters

When less reactive ATS and MBTFA are simultaneously injected using an ordinary injector, a certain amount of underivatized analyte is often detected. Fig. 6 shows GC–MS with on-column derivatization of MDMA and MDA using an ordinary injector and the present two-step injector. When an ordinary injector was employed and MBTFA was simultaneously injected with the sample, a substantial amount of underivatized MDMA remained, as shown in Fig. 6A. In addition to its lower reactivity, this would be primarily due to its much longer retention time than that of MBTFA, and thus MBTFA is carried away much faster than MDMA. However, the use of the two-step injector has made its semiquantitative (≥90%) derivatization possible (Fig. 6B).

In the on-column derivatization with MBTFA of lesser reactive MDEA, considerable amount of free-form MDEA was often detected when the reagent was somewhat deteriorated during storage (for example, when the ampoule of the reagent was opened 2 weeks before . . .). This was probably because of the stereo effects of the more bulky ethyl group at the nitrogen atom, and the lower reactivity due to the ethyl group. To improve the derivatization yields of less reactive MDMA analogs, the following injector operation parameters were optimized: the amount of MBTFA, that of sample solution, that of the air gap that separates the reagent and the sample, the injection speed of the derivatization reagent, and the interval between the injections of the sample and the reagent. As a result of the experiments,

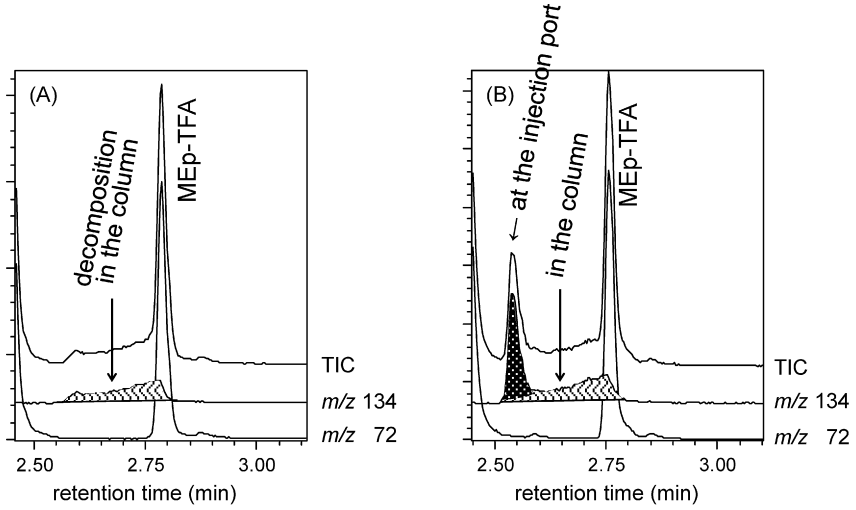


Fig. 5. Comparison of on-column trifluoroacetylation using MBTFA (A) with conventional one using trifluoroacetyl anhydride (B) isomerization of the TFA derivative of methylephedrine. GC conditions: DB-5MS (0.32 mm × 30 m, i.d., 0.25 μm), 120 °C (3 min) to 200 °C (+10 °C/min).

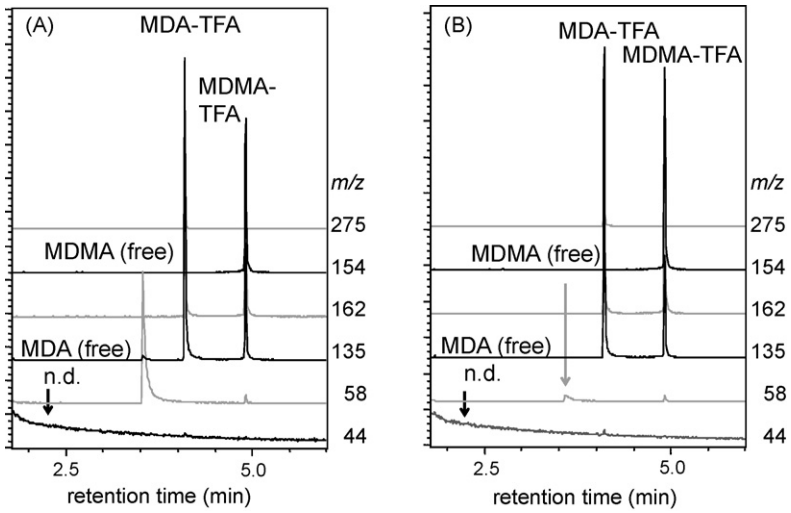


Fig. 6. Comparison of the on-column trifluoroacetylation of ATS using the two-step injector (A) with that using an ordinary injector (B). GC conditions: DB-5MS (0.32 mm × 30 m, i.d., 0.25 μm), 120–170 °C (+15 °C/min) to 250 °C (+30 °C/min).

the injection of 1 μL MBTFA was found to be enough for most ATS, except for MDEA which needs 2 μL MBTFA for its semi-quantitative derivatization. The optimal injection speed of the derivatization reagent and the interval between the injections of the sample and MBTFA were 10 μL/s and 3 s, respectively. Also, the optimal volume of the air gap was 4 μL. Fig. 7 shows the mass chromatograms of MDMA analogs, with on-column trifluoroacetylation by using the two-step injector and the optimized conditions settings.

### 3.6. Validation

The present GC–MS procedure with on-column trifluoroacetylation using the two-step injector was evaluated for spiked hair extract samples, containing MA and its metabolite AP at known concentrations. Table 1 summarizes the validation data. The detection limits of MA and AP spiked into drug-free hair extracts were 0.2 and 0.1 ng/mg hair, respectively, in the full-scan mode, when a 10 mg hair sample is used. In the forensic analysis of ATS in hair, a cutoff concentration of 0.5 ng/mg is generally accepted [17]. Also, an administrative cutoff of 5.0 ng/mg hair for the parent drug MA was proposed by Miki et al. [5,12], and is adopted in the Forensic Science Laboratories of Japan's police when the results are to be used as legal evidence for prosecuting illicit drug use. Thus, the satisfactory analytical performance of the present method was ensured even for the analysis of hair, which is among the most difficult of analytical subjects. Fig. 8 shows the GC–MS of MA and AP spiked into a drug-free hair extract, with automated on-column trifluoroacetylation.

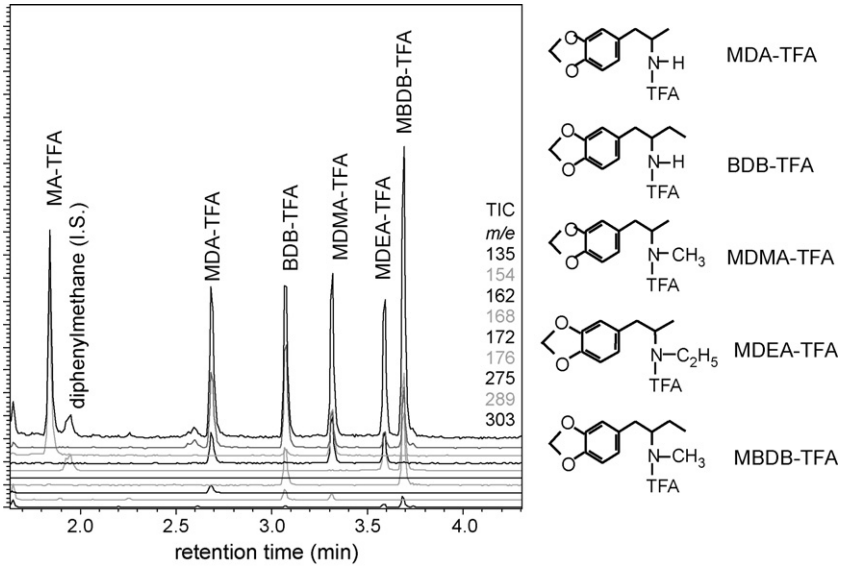


Fig. 7. Mass chromatograms of MDMA analogs with on-column trifluoroacetylation using the two-step injector. GC conditions: DB-5MS (0.32 mm × 30 m, i.d., 0.25 μm), 140 °C (1 min) to 200 °C (+10 °C/min).

Table 1
Validation data of the present GC–MS procedure for APs in hair with on-column trifluoroacetylation using the two-step injector[a]

| | Amphetamine (AP) | Methamphetamine (MA) |
|---|---|---|
| Recovery[b,c] | 95% | 93% |
| Derivatization yield[b,c,d] | 100% | 95% |
| Calibration graph | | |
| Linear range | 0.1–10 ng/mg hair | 0.2–30 ng/mg hair |
| Correlation of coefficiency | $r^2 = 0.995$ | $r^2 = 0.983$ |
| Limits of detection | | |
| SIM mode | 0.05 ng/mg hair | 0.1 ng/mg hair |
| Full-scan mode | 0.1 ng/mg hair | 0.2 ng/mg hair |
| Accuracy (%) ($n = 5$)[b] | 101.2 | 98.1 |
| Precision[b] | | |
| Within assay ($n = 8$) | 3.2% | 4.6% |
| Inter-assay ($n = 8$) | 4.5% | 5.7% |

[a] Evaluated by using 10-mg hair samples spiked with known amounts of AP and MA during the extraction process.
[b] Evaluated at 2 ng/mg hair for AP, and 10 ng/mg hair for MA.
[c] Average of five samples prepared separately.
[d] Derivatization yields by on-column trifluoroacetylation using MBTFA, compared with those by conventional trifluoroacetylation using trifluoroacetyl anhydride.

### 3.7. Application to ATS in urine and blood samples

The present method was also applicable for the determination of ATS in urine and blood extracts. The detection limits of MA, AP, MDMA, and MDA in the full-scan mode were 0.1, 0.05, 0.2, and 0.1 μg/mL, respectively, for urine, and 0.05, 0.01, 0.1, and 0.02 μg/mL, respectively, for blood, when the present GC–MS method was applied to the sample extracts prepared as described in Section 2. The recoveries (the ratio of the derivative detected to the analyte spiked into the sample) of MA, AP, MDMA, and MDA were 94%, 97%, 92%, and 95%, respectively, for urine at 5 μg/mL each, and 89%, 91%, 86%, and 89%, respectively, for whole blood at 1 μg/mL each.

Typical GC–MS results for urine specimens from two drug users (without the hydrolysis of conjugated metabolites) are shown in Fig. 9. Although its derivatization yield was estimated to remain at about 60%, HMMA, a specific metabolite of MDMA, was detectable, in addition to the parent drug MDMA, and its *N*-demethylated metabolite MDA. Fig. 10 shows GC–MS chromatograms obtained from a whole blood sample spiked with MA and AP at 1.0 and 0.1 μg/mL, respectively.

### 3.8. Advantages of the on-column trifluoroacetylation using the newly developed two-step injector

Under the present experiment conditions, the derivatization reactions take place within the column. This eliminates the tedious work of manually performing the derivatization process on the sample, thereby significantly improving the efficiency and throughput of the analysis. Also, the operator can avoid the health hazards of the derivatization reagent, which are usually harmful. In conventional trifluoroacetylation using TFAA, excess reagent and its by-product, which are detrimental to column lifetime due to their strong acidity, should be evaporated before analysis. However, no negative effects have been noticed in daily and frequent use of MBTFA (about 40 injections of 1-μL MBTFA per day at a split ratio of 3 or 5, for 16 years), owing to the neutral properties of MBTFA and its acylamide by-product. With respect to the hardware construction, the sample injector invented is identical to conventional ones, and it did not require any additional hardware components for the derivatization process. Therefore, it could be constructed as small as conventional ones with the corresponding minimum increase in production costs for replacing the ROM with a newly developed one.

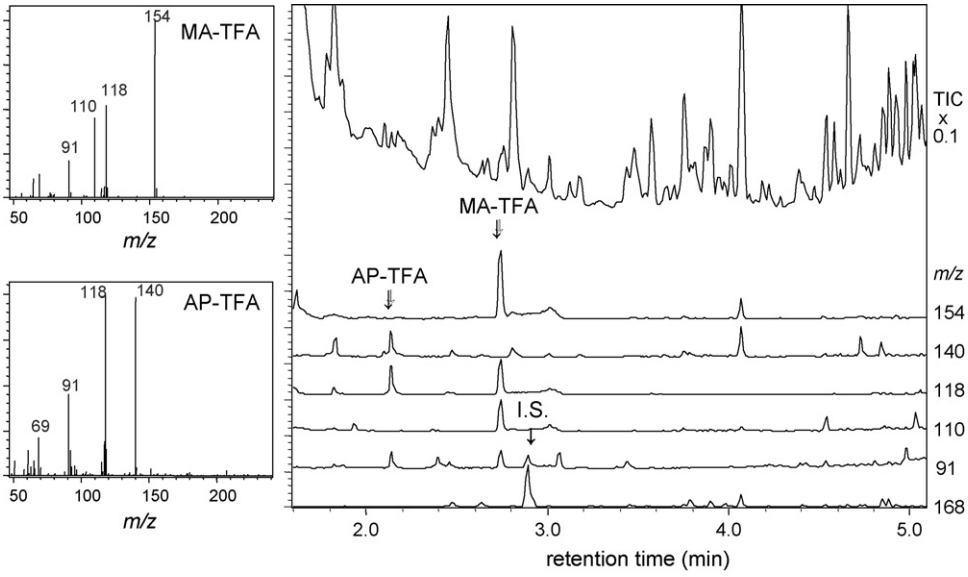


Fig. 8. GC–MS of MA and AP spiked into a hair extract, with automated on-column trifluoroacetylation. GC conditions: DB-1MS (0.32 mm × 30 m, i.d., 0.25 μm), 120–170 °C (+15 °C/min) to 280 °C (+30 °C/min).

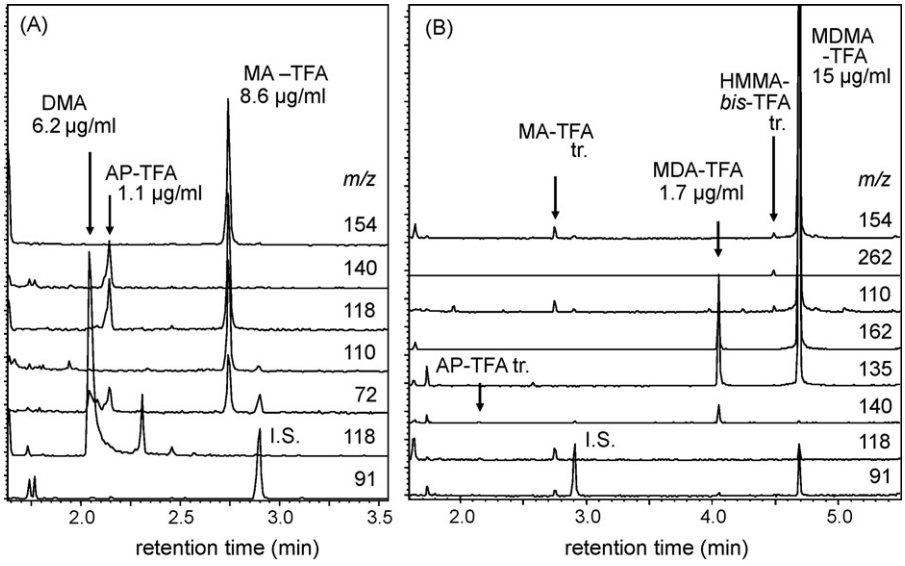


Fig. 9. GC–MS chromatograms obtained from urine specimens from two ATS users (without the hydrolysis of conjugated metabolites), with automated on-column trifluoroacetylation. GC conditions: DB-1MS (0.32 mm × 30 m, i.d., 0.25 μm), 120–170 °C (+15 °C/min) to 250 °C (+30 °C/min).

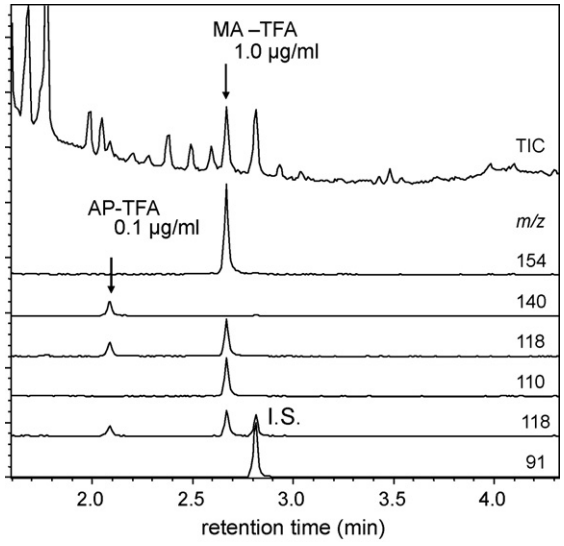


Fig. 10. GC–MS chromatograms obtained from whole blood spiked with methamphetamine and amphetamine at 1.0 and 0.1 μg/mL each. GC conditions: DB-5MS (0.32 mm × 30 m, i.d., 0.25 μm), 120–170 °C (+15 °C/min) to 250 °C (+30 °C/min).

## 4. Conclusion

By the use of the newly developed two-step injector, the fully automated GC–MS determination with on-column trifluoroacetylation of ATS extracted from various biological samples has become possible. The two-step injector will serve as a useful tool in laboratories which analyze a number of samples for amine-type drugs and metabolites, such as ATS, which have become the most prevalent illicit drugs in many countries and regions. The application to other drugs and derivatization reagents, such as *N*-methyl-bis(heptafluorobutyramide) (MBHFBA), is under investigation.

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