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Applications of Hadamard Transform to Gas Chromatography/Mass Spectrometry and Liquid Chromatography/Mass Spectrometry

Cheng-Huang Lin,*,† Takashi Kaneta,‡ Hung-Ming Chen,† Wen-Xiong Chen,† Hung-Wei Chang,† and Ju-Tsung Liu§

Department of Chemistry, National Taiwan Normal University, 88 Section 4, Tingchow Road, Taipei, Taiwan, Department of Applied Chemistry, Graduate School of Engineering, and Division of Translational Research, Center for Future Chemistry, Kyushu University, Motooka, Fukuoka 819-0395, Japan, and Forensic Science Center, Military Police Command, Department of Defense, Taipei, Taiwan

Successful application of the Hadamard transform (HT) technique to gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS) is described. Novel sample injection devices were developed to achieve multiple sample injections in both GC and LC instruments. Air pressure was controlled by an electromagnetic valve in GC, while a syringe pump and Tee connector were employed for the injection device in LC. Two well-known, abused drugs, 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) and *N*,*N*-dimethyltryptamine (DMT), were employed as model samples. Both of the injection devices permitted precise successive injections, resulting in clearly modulated chromatograms encoded by Hadamard matrices. After inverse Hadamard transformation of the encoded chromatogram, the signal-to-noise (S/N) ratios of the signals were substantially improved compared with those expected from theoretical values. The S/N ratios were enhanced ~10-fold in HT-GC/MS and 6.8 in HT-LC/MS, using the matrices of 1023 and 511, respectively. The HT-GC/MS was successfully applied to the determination of MDMA in the urine sample of a suspect.

In separation science, the limit of detection (LOD) for analytes is an important issue. Thus, much research has focused on the development of sensitive detectors and improvements in the sensitivity of detectors for separation techniques such as gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE). These studies have resulted in the development of sophisticated detectors. Therefore, in some cases, further improvement in LOD is physically limited by the detection principle. The use of mathematical methods is one alternative for the improvement of the LOD and resolution of analytical instrumentation. In chromatographic separations, correlation methods have been employed for improving the LOD. In 1970, Smit demonstrated correlation gas chromatography in which a sample was injected into a column according to a pseudorandom binary sequence (PRBS).[1] Correlation chromatography is a powerful method for reduction of the LOD. Therefore, correlation chromatography has been applied to not only GC[2–5] but also to LC[6–11] and to CE.[12,13] The Hadamard transform (HT) technique is a technique that is analogous to the correlation method. The HT technique has been applied in many fields, including time-of-flight mass spectrometry,[14–16] Raman,[17,18] fluorescence imaging,[19–21] ion mobility spectrometry,[22,23] and NMR.[24,25] In our previous studies,[26–29] Hadamard transformation has been successfully applied to capillary electrophoretic separations where the signal-

* Corresponding author. E-mail: chenglin@ntnu.edu.tw.
† National Taiwan Normal University.
‡ Kyushu University.
§ Forensic Science Center.

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to-noise (S/N) ratios were substantially improved by sample injections according to the PRBS. Nonconventional sampling techniques including correlation and HT methods have been reviewed by Kaljurand and Smit.[30]

With respect to chromatographic separations, some studies have reported on the use of HT for GC[31] and LC.[32] The advantages of the HT technique using pseudorandom injections in GC have also been suggested but only by means of computer simulation.[31] Recently, Trapp reported high-throughput multiplexing GC using the HT method.[33]

Conversely, in LC, the only report of HT relates to the application of HT to UV absorption detection, in which the Hadamard mask was placed in front of a photomultiplier tube to simplify the measurement of the spectra for the analytes.[32] To date, there has been no report on the use of HT based on multiple injections according to PRBS in LC, although substantial improvement would be expected.

A Hadamard matrix on the order of n, H_n, is an $n \times n$ of +1's and −1's with the property of the scalar product of any two distinct rows being 0. Thus, H_n must satisfy the following equation,

$$H_n H_n^{\mathrm{T}} = H_n^{\mathrm{T}} H_n = nI_n \quad (1)$$

where H_n^{T} is the transpose of H_n and I_n is the unit matrix on the order of n. A fundamental equation of the Hadamard transformation is given by

$$[\eta] = [S] \times [C] \quad (2)$$

where η is a series of data, i.e., the observed chromatogram, encoded by a cyclic S-matrix, S, which is the $(n - 1) \times (n - 1)$ matrix consisting of "zero" and "one" elements, and C is a series of data representing a chromatogram. A cyclic S-matrix on the order of $(n - 1)$ is obtained by omitting the first row and column of H_n and then changing +1's to 0's and −1's to +1's. To encode the chromatogram, C, a sample and eluent are introduced into a column according to the PRBS derived from the cyclic S-matrix. When the elements of the PRBS are "one" and "zero", sample and eluent plugs are introduced into the column, respectively. As a result, the encoded chromatogram, η, is obtained. The encoded chromatogram is decoded to the chromatogram, C, by multiplying an inverse matrix of S, S^{-1}, as follows.

$$[C] = [S]^{-1} \times [\eta] \quad (3)$$

Consequently, the decoded chromatogram shows improvement in the S/N ratio (Fellgett advantage).

In both correlation and HT methods, the key technology, based on multiple input techniques according to PRBS, is the injection device, which permits the continuous introduction of a sample. In CE, two kinds of injection methods, i.e., electrokinetic injection[29] and optically gated injection,[26,27] have been employed to achieve successive injections. Multiple injection devices for GC have also been developed for correlation GC, in which the solenoid valve,[1,2] cylindrical slide valve,[3] and fluidic logic gate[4] were used. Conversely, in correlation LC, the input signals modulated by PRBS were generated by valve systems[6–9,11] and by an electrochemical concentration modulator.[10] In this study, we developed novel injection devices for HT-GC/MS and HT-LC/MS, respectively. The injection devices, which are quite simple, permit precise sample introduction resulting in clearly modulated chromatograms at the command of PRBS. Two well-known, abused drugs, 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) and *N*,*N*-dimethyltryptamine (DMT), were selected as model samples. The design of injection devices, details of experimental conditions for HT-GC/MS and HT-LC/MS, and the determination of a drug in an actual sample are reported herein.

EXPERIMENTAL SECTION

Reagents. 3,4-Methylenedioxy-*N*-methylamphetamine (MDMA), *N*,*N*-dimethyltryptamine (DMT), and suspects' urine samples were generously donated by the Military Police Command, Forensic Science Center, Taiwan. All the other chemicals were of analytical grade and were obtained from commercial sources.

Extraction Procedure and Safety. A volume of 1 mL of the urine sample was made alkaline by the addition of excess K_2CO_3. The free bases were then extracted into 4 mL of a hexane/CH_2Cl_2 (3:1, v/v) solution by mixing for 1 min. After shaking for 5 min, 0.1 mL of acetic anhydride was added to derivatize the MDMA. After centrifugation, the upper layer (3 mL) was collected, and this organic phase was then evaporated to dryness. The residue was dissolved in 0.5 mL of methanol for subsequent GC/MS experiments. The proper and safe handling of urine samples followed the regulations of the Department of Health, Executive Yuan (Taiwan).

Apparatus. A gas chromatograph (GC 5890 Hewlett-Packard, Avondale, PA) equipped with a mass spectrometer (Hewlett-Packard 5972 mass selective detector) was used to detect the analytes. A capillary column (30 m × 0.25 μm i.d.) with an HP-5MS (cross-linked 5% PH ME siloxane) bonded stationary phase film, 0.25 μm in thickness (Agilent Technologies), was used. The inlet temperature was maintained at 250 °C and the column oven was also held at 250 °C (carrier gas: helium, flow rate 1.2 mL/min operating in either the splitless or split mode). The mass spectrometry conditions were as follows: ionization energy, 70 eV; and ion source temperature, 110 °C. The selected ion monitor (SIM) mode was used for MDMA and DMT by selecting ion peaks at m/z = 162 and 58, respectively. The dwell value was set at 80; 10 dots/s could be recorded. Data were collected using Hewlett-Packard Chem-Station software with transfer to an ASCII text file. The average was calculated for each 30 dots that were treated as one bin to fit the HT calculation. The LC/MS system (Finnigan LCQ Classic LC/MS/MS) consisted of a Constametric 4100 solvent delivery system (LDC Analytical, Gelnhausen,Germany), a manual injection valve from Shimadzu, a reversed phase column (Jasco C18 T-5, 5 μm, 15 cm × 4.6 mm i.d.; Nacalai Tesque, Kyoto, Japan), and an electrospray ionization (ESI) probe operated in the positive ion mode. The mass signal was recorded under the SIM mode, where the data recording speed was ~0.63 dot/s. The Xcalibur data system was used for collecting data, which were transferred to an ASCII text file. The scan mode used was SIM;

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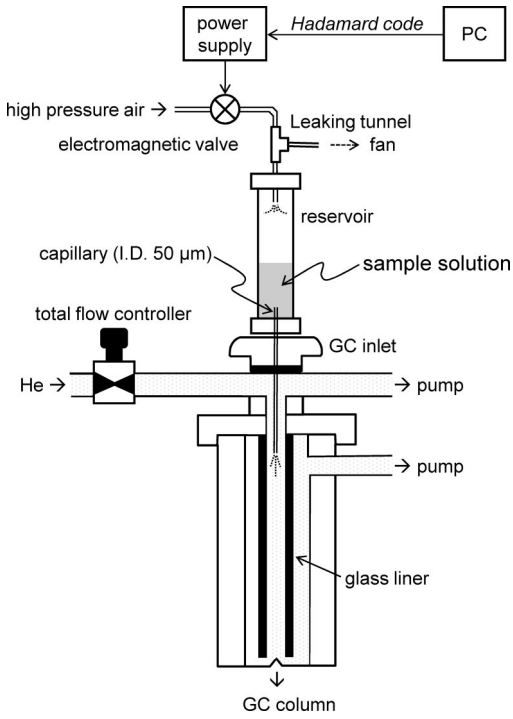

Figure 1. Schematic diagram of HT-GC/MS injection device.

the capillary temperature and spray voltage were set to 300 °C and 4.5 kV, respectively. The tube lens offset and capillary voltage were set at −10 and 6 V, respectively; sheath gas and auxiliary gas flow rates were 70 and 20 (arb), respectively. The HT-GC- and HT-LC-chromatograms were calculated using the LabView program, as described previously.[34]

RESULTS AND DISCUSSION

Injection Device. Figure 1 shows a schematic diagram of the injection device for HT-GC/MS. A sample solution was replaced in the reservoir of a stainless tube (o.d., $^1/_4$ in.; length, 5 in.). The sample solution was introduced into a glass liner through a capillary (i.d., 50 μm) by air injection pressure from a compressed air cylinder. A personal computer turned the electromagnetic valve on and off, according to a series of Hadamard codes. When the electromagnetic valve was opened, high-pressure air was immediately squeezed into the reservoir, leading to the introduction of the sample solution through the capillary into the glass liner where the capillary was immersed in the sample solution. The injection volume of the sample solution could be adjusted by changes in the air pressure, capillary i.d., capillary length, and injection time. The air pressure (ranged from 1.6 to 1.9 atm) was inversely proportional to the injection time (ranged from 6 to 3 s). The optimized condition was obtained when the air pressure was 1.67 atm and the injection time was set at 3 s, using a short capillary (i.d., 50 μm; 5 cm in length). The injection volume was estimated at ~100 nL for a single injection (stability: intraday, 92.7 ± 7.4 nL; interday, 95.7 ± 7.4 nL). The injected volume was recognized by comparing the relationship between a single peak obtained from a regular GC injector (0.1 μL of sample solution) and average of the peaks obtained from the Hadamard injection. The time gap between successive injections was less than 1 s, which depended on air pressure, inner pressure of the glass liner, and the size of leaking tunnel, respectively. The glass liner usually remained at high temperature (~250 °C), so that the ejective solution, containing the analytes and solvent, were evaporated immediately. When the electromagnetic valve was closed, the inner pressure of the glass liner (i.e., carrier gas, helium) pushed back the sample, resulting in interruption of the sample introduction by the carrier gas. The flow rate of helium could be set at 0.6−1.2 mL/min, leading to a 0−8 psi head pressure. Once the carrier gas pushed the sample solution back and entered into the reservoir, excess air escaped from a small leaking tunnel (i.d., 0.4 mm), and was then drained by a fan to keep the reservoir pressure equal to the atmospheric pressure (waiting for the next injection). Thus, the injection device permitted sample injections according to PRBS in GC.

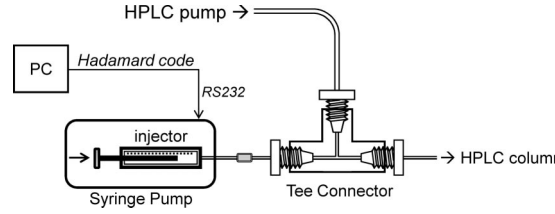

Figure 2. Schematic diagram of HT-LC/MS injection device.

Figure 2 shows a schematic diagram for the HT-LC/MS injection device. In this case, the sample solution was placed in a homemade reservoir (a brass injector; i.d., 6.0 mm), and $^1/_{16}$ in. (i.d., 0.13 mm) pipes were used in the system. A sample solution was pushed out by a commercial syringe pump (model 22 syringe pump; Harvard apparatus), which was controlled by a personal computer through an RS232-port. When the flow rate was set at 5.4 μL/min, the volume of sample solution was estimated to be ~0.3 μL/injection (stability: intraday, 0.34 ± 0.03 μL; interday, 0.30 ± 0.04 μL). Meanwhile, a commercial HPLC pump was used to provide eluent (mobile phase: acetonitrile/water) for separation. The sample solution and eluent interflowed in a Tee connector and alternately entered the separation column. Once the separation was finished, the effluent entered the mass spectrometer by means of the ESI process. The pressure of the syringe pump was adjusted to be greater than that of the HPLC pump. Thus, when the syringe pump was operated, only the sample solution could pass through the Tee connector. Conversely, when the syringe pump was stopped (the sample injection was interrupted), only the eluent could pass through the Tee connector. Thus, multiple sample injections were accomplished by controlling the operation of the syringe pump. The initial pressure of the HPLC pump was adjusted to less than 200 psi. When an ESI mode was used, the pressure increased to 500 psi. Hence, the optimal pressure of the syringe pump was set at 500−550 psi. The eluent used in this experiment was water and acetonitrile (50:50, v/v), pumped at a rate of 1.0 mL/min. The time gap between the sample solution and the eluent depended on the viscosity of solvent used and the pump pressure, respectively. At this point, the optimized time gap ranged from 6 to 11 s. The separation efficiency of DMT was influenced by the acidity of the mobile phase. When a basic mobile phase was used, the peak of DMT was broadened, whereas when 0.1% formic acid was added to the mobile phase, good separation efficiency was obtained. On the other hand, if the ratio of water

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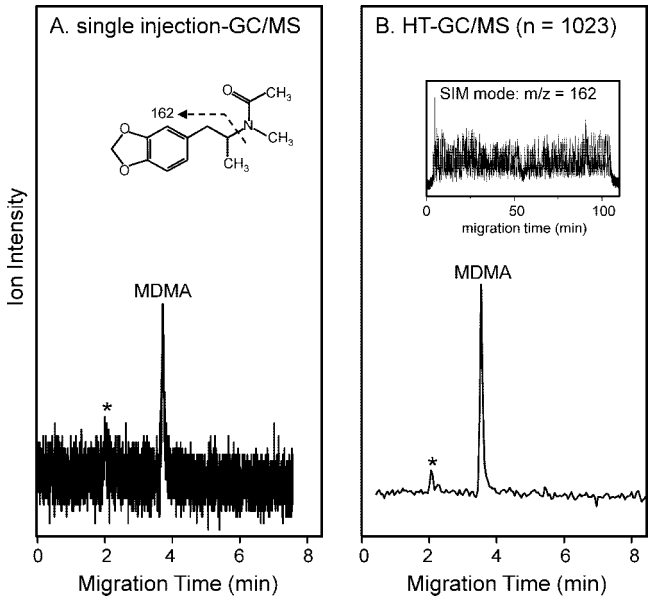

Figure 3. (A) A typical GC/MS chromatogram of MDMA derivative obtained by single injection based on the SIM mode (ion peak at m/z = 162 was selected for monitoring); the concentration of MDMA was 10 μg/mL before derivatization. (B) HT-GC/MS (order of matrix, 1023) chromatogram of MDMA derivative. Inset, the raw data of TIC (by selecting the ion at m/z = 162) chromatogram before inverse Hadamard transformation.

was higher than 50% in volume, or methanol was used instead of acetonitrile, the inner pressure was increased (>1000 psi), which made the sample injection and the ESI process difficult.

Hadamard Transformation. MDMA is a semisynthetic entactogen of the phenethylamine family and is most commonly known today by the street name "ecstasy." DMT is a naturally occurring tryptamine and human neurotransmitter. It is well-known that phenethylamines (such as MDMA) and tryptamines (such as DMT) provide a strong imine fragment (m/z = 58) under electron impact (EI; 70 eV) mode. Thus, optimum derivatization for MDMA is needed. The derivatization was performed via a reaction with acetic anhydride, leading to characteristic mass fragments of MDMA (m/z = 58, 100, and 162). Figure 3A shows a typical GC/MS chromatogram of the MDMA derivative obtained by single injection, based on the SIM mode (ion peak at m/z = 162 was selected for monitoring). The concentration of MDMA was 10 μg/mL before derivatization; yield was 60−70%. The period of sample injection was 3.010 s; sample injection volume was ~0.1 μL in a 10:1 split mode. The characteristic MDMA peak in methanol, as well as a tiny impurity (marked as "∗"), were found. The S/N ratio appeared to be poor in the chromatogram obtained by single injection. Under the same experimental conditions, when an HT injection was performed (n = 1023), the S/N ratio was dramatically improved to 9.13-fold, as shown in Figure 3B. The inset shows the raw data of the total ion current (TIC) (selecting the ion at m/z = 162) chromatogram before inverse HT was applied. The total operation time was more than 120 min, suggesting that the HT-GC/MS injection device works very well. If a faster electromagnetic valve and higher air pressure is used, the operation time can be shortened and the use of a splitless mode is also possible. Another test sample, DMT, was also

Table 1. Relationship between the Order of Matrix, Enhancement of the S/N Ratio, and Mass Conditions for Analysis[a]

matrix order	enhancement of S/N ratio	mass conditions
	Theoretical	
255	8.02	
511	11.32	
1023	16.01	
	Observed	
	HT-GC/MS	
sample	MDMA-derivative	SIM mode: m/z = 162
255	6.77	
511	8.44	
1023	9.13	
sample	DMT	SIM mode: m/z = 58
255	6.05	
511	7.10	
1023	10.87	
	HT-LC/MS	
sample	DMT	SIM mode: m/z = 189
255	5.29	
511	6.84	

[a] The enhancement of the S/N ratio was calculated as the ratio of the S/N values obtained in the chromatograms, measured by HT-GC/MS, HT-LC/MS, and a single injection method.

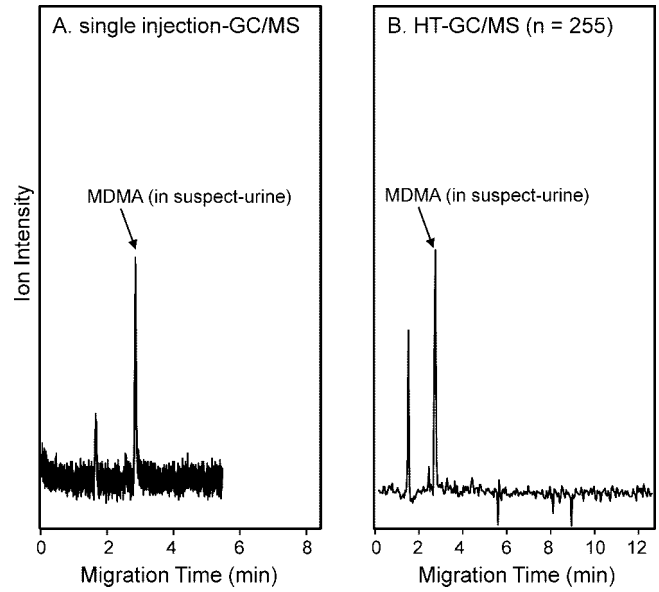

Figure 4. Typical GC/MS chromatograms of a urine derivative from a suspect based on the SIM mode (ion peak at m/z = 162 was selected for monitoring). (A) Single-injection; (B) HT-GC/MS (order of matrix, 255).

examined using this approach. Since DMT is a tertiary amine, its derivatization is difficult, so an imine fragment (m/z = 58) was selected for monitoring. Table 1 shows the relationship between the order of matrix, the enhancement of the S/N ratio, and the mass conditions for analysis of MDMA and DMA, respectively. As seen in Table 1, the enhancement of the S/N ratio increases with increasing order of the Hadamard matrix, as expected from theoretical observation, although the experimental values are slightly smaller than the theoretical ones. In order to evaluate the applicability of the present method to an actual sample, a profile of MDMA extracted from a urine sample of a suspect was

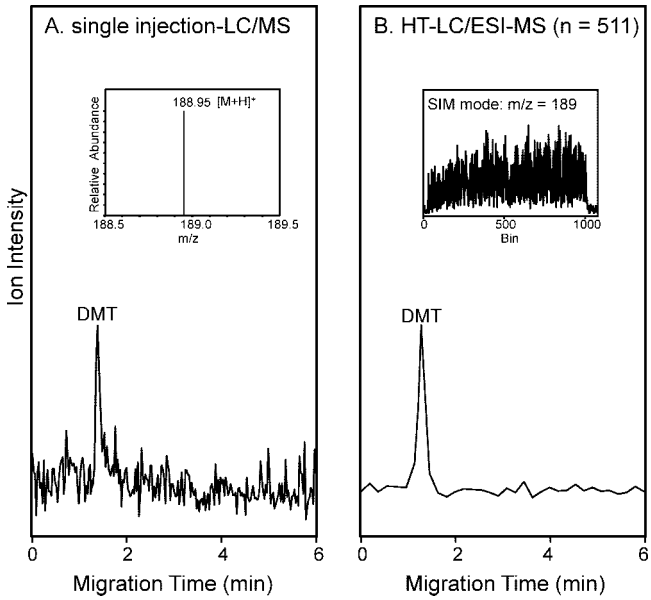

Figure 5. (A) A typical LC/MS chromatogram of DMT obtained by single injection based on the SIM mode (ion peak at $m/z = 189$ was selected for monitoring); the concentration of DMT was 1.0 μg/mL. (B) HT-LC/MS (order of matrix, 511) chromatogram of DMT. Inset, the raw data of TIC (by selecting the ion at $m/z = 189$) chromatogram before inverse Hadamard transformation.

also attempted and was successful, as shown in Figure 4 (frame A, single injection; B, HT injection; $n = 255$). The concentration level detected in the urine sample from a suspect was 5 μg/mL.

Figure 5A shows a typical LC/ESI-MS chromatogram of DMT obtained by single injection based on the SIM mode (the parent ion, $[M + H]^+$ ion at $m/z = 189$, was selected for monitoring). The concentration of DMT was 1.0 μg/mL. The S/N ratio was ~3, i.e., the LOD was estimated to be ~1.0 μg/mL. Under the same experimental conditions, when an HT injection was performed ($n = 511$), the S/N ratio was improved 6.84-fold, as shown in Figure 4B. The inset shows the raw data of the TIC (by selecting the ion at $m/z = 189$) chromatogram before inverse Hadamard transformation. In Table 1, the results obtained by HT-LC/MS are also summarized. As with HT-GC/MS, the enhancement of the S/N ratio increases with the order of the Hadamard matrix. The results indicate that the present injection device, with a simple design, permits precise multiple injections for the HT-LC/MS method.

CONCLUSION

In this study, we developed novel injection devices for HT-GC/MS and HT-LC/MS. The utility of the HT-GC/MS and HT-LC/MS injection devices was demonstrated using two well-known abused drugs (MDMA and DMT) as model compounds. In both cases, the devices permitted precise sample injections continuously, resulting in substantial improvement in the S/N ratio through the application of the Hadamard transformation. The enhancement factors of the S/N ratios were in good agreement with the theoretical values. On the other hand, the present HT-LC/MS method is based on a regular C-18 HPLC column. In the near future, if nanocolumns can be used, the sample injection volume can be decreased and a higher order of matrix can be applied. Thus, the present methods have a variety of applications and could potentially be used in practical trace analysis.

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