LC/MSを用いた健康食品中の18種類の違法添加物の一斉分析

誌名	食品衛生学雑誌
ISSN	00156426
著者名	田上,貴臣 青山,愛倫 武田,章弘 淺田,安紀子 土井,崇広 梶村,計志 沢辺,善之
発行元	[日本食品衛生学会]
巻/号	55巻1号
掲載ページ	p. 34-40
発行年月	2014年2月

Report

Simultaneous Identification of 18 Illegal Adulterants in Dietary Supplements by Using High-Performance Liquid Chromatography-Mass Spectrometry

Takaomi TAGAMI*, Airin AOYAMA, Akihiro TAKEDA, Akiko ASADA, Takahiro DOI, Keiji KAJIMURA and Yoshiyuki SAWABE

Osaka Prefectural Institute of Public Health: 1–3–69 Nakamichi, Higashinari-ku, Osaka 537–0025, Japan; * Corresponding author

We developed a method for the identification of 18 illegal adulterants in dietary supplements for erectile dysfunction by using high-performance liquid chromatography-mass spectrometry. The separation was achieved on a Cosmosil $3C_{18}$-EB column. The mobile phase consisted of 0.1% formic acid solution and 0.1% formic acid in acetonitrile, with gradient elution at a flow rate of 0.15 mL/min. The proposed method may be useful for the identification of illegal adulterants and for quality control of dietary supplements.

(Received 17 May, 2013)

Key words: dietary supplement; erectile dysfunction; medicinal ingredient; simultaneous identification

Introduction

In recent years, impairment of health owning to ingestion of dietary supplements that contain medicinal ingredients has become a social problem[1]. Since this may be due to the presence of illegal adulterants, it is necessary to analyze commercial dietary supplements to confirm their purity. Inhibitors of phosphodiesterase type 5 (PDE-5), such as sildenafil, tadalafil, and vardenafil are administered to treat erectile dysfunction and pulmonary arterial hypertension, and they have been recently detected in dietary supplements for erectile dysfunction[2), 3)]. The known side effects of PDE-5 inhibitors include headaches and visual abnormalities. The combined use of PDE-5 inhibitors and nitric monoxide donors could be fatal[1]. Various ingredients whose structures have been modified from those of medicinal ingredients, such as sildenafil, in order to avoid identification have also been detected in dietary supplements[4)–7)]. To efficiently analyze the many adulterants that may be present in commercial dietary supplements, a simultaneous identification method is needed. In this study, we developed a method to identify simultaneously 18 adulterants in dietary supplements by using LC/MS.

Materials and Methods

Chemicals and reagents

LC/MS grade acetonitrile, methanol, acetic acid, and formic acid were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). LC/MS grade ammonium formate and ammonium acetate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydroxythiohomosildenafil, dimethylsildenafil, acetilacid, thiohomosildenafil, thiodimethylsildenafil, and chloropretadalafil were purchased from TLC Pharma Chem (Ontario, Canada). Hydroxyhomosildenafil, thiosildenafil, pseudovardenafil, gendenafil, and norneosildenafil were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Aminotadalafil was purchased from Toronto Research Chemicals (Ontario, Canada). Sildenafil, tadalafil, vardenafil, xanthoanthrafil, and a positive control containing approximately 18% acetildenafil were kindly provided by the National Institute of Health Sciences in Japan. The positive control of acetildenafil was used as a standard. Standard stock solution of homosildenafil (approximately 75.5 ppm) was provided by the Department of Pharmaceutical Sciences, Tokyo Metropolitan Institute of Public Health. The chemical structures of compounds used in this study are shown in Fig. 1.

Instrumentation and chromatographic conditions

The LC-electrospray ionization-MS experiments were performed using a Prominence Ultra Fast Liquid Chromatograph (UFLC) system and an LCMS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). A Cosmosil $3C_{18}$-EB column (particle size, 3 μm; i.d., 2.0 mm; length, 250 mm, Nacalai Tesque, Kyoto, Japan) was used. The following gradient system was used. Mobile phase A consisted of 0.1% formic acid solution and mobile phase B 0.1% formic acid in acetonitrile. The gradient elution program, with a constant flow rate of 0.15 mL/min, was started with solution A at 70%, followed by

*tagami@iph.pref.osaka.jp

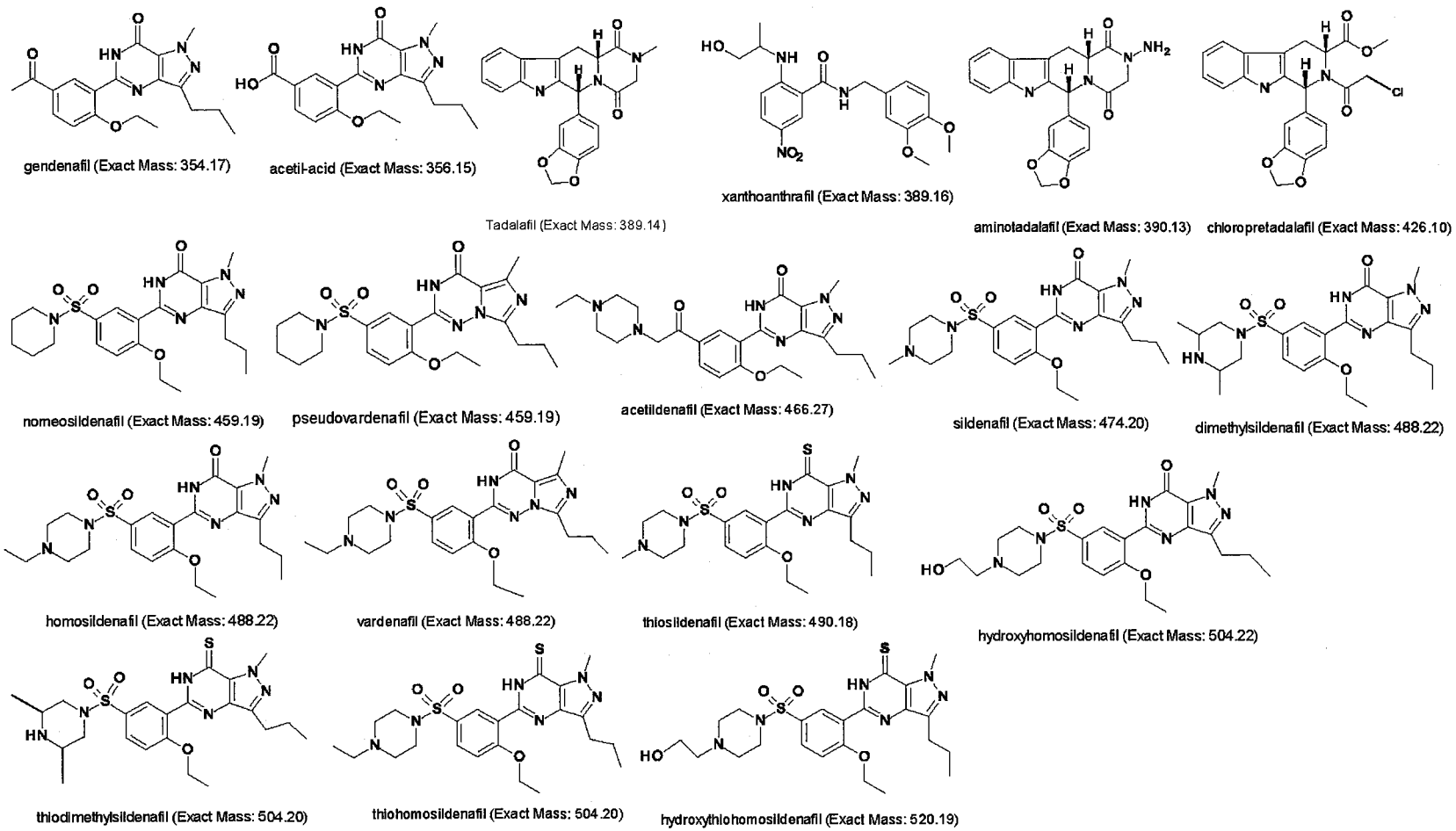

Fig. 1. Chemical structures of the 18 adulterants examined

Table 1. Monitoring ions to extract ion chromatograms

Compound	*m/z*
Gendenafil	355
Acetil-acid	357
Tadalafil	390
Xanthoanthrafil	390
Aminotadalafil	391
Chloropretadalafil	427
Norneosildenafil	460
Pseudovardenafil	460
Acetildenafil	467
Sildenafil	475
Dimethylsildenafil	489
Homosildenafil	489
Vardenafil	489
Thiosildenafil	491
Hydroxyhomosildenafil	505
Thiodimethylsildenafil	505
Thiohomosildenafil	505
Hydroxythiohomosildenafil	521

a linear decrease to 30% in 50 min. The injection volume was 1 μL. The column temperature was maintained at 40℃. The values of the instrument parameters were as follows: source temperature, 350℃; desolvation temperature, 250℃; and desolvation gas flow, 600 L/h. In the scan mode analysis, the mass range of the spectra was *m/z* 100–800. All LC-MS runs were performed in the positive mode.

Preparation of standard solutions

The standards were each dissolved in methanol to make standard stock solutions. The working standard solution was then prepared from the standard stock solutions.

Sample preparation

The dietary supplement (10 mg) to be investigated was either a powdered tablet or the contents of a capsule. The sample was extracted with 10 mL of methanol

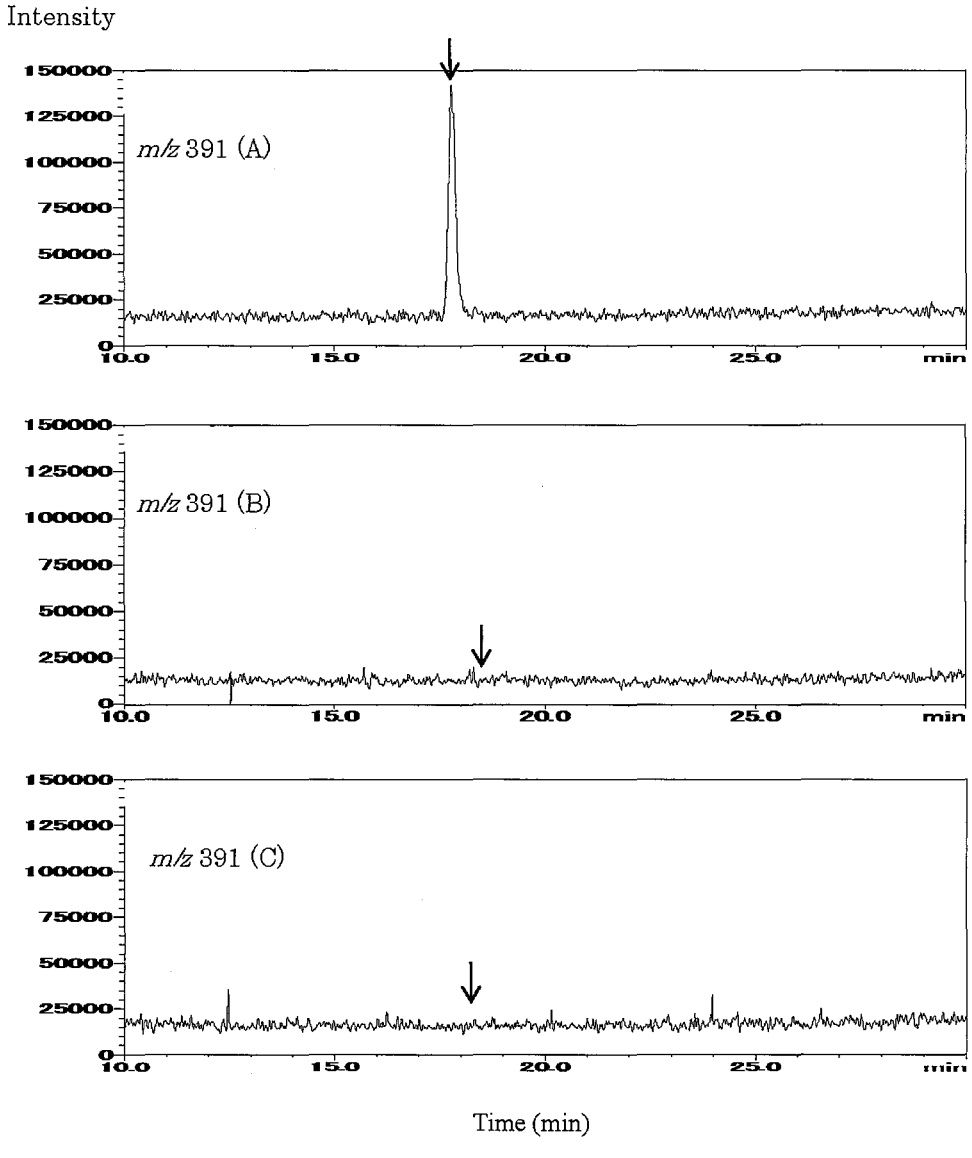

Fig. 2. Extracted ion chromatograms of standard solution of aminotadalafil (concentration: 1 μg/mL)
A: mobile phase 1, B: mobile phase 2, C: mobile phase 3

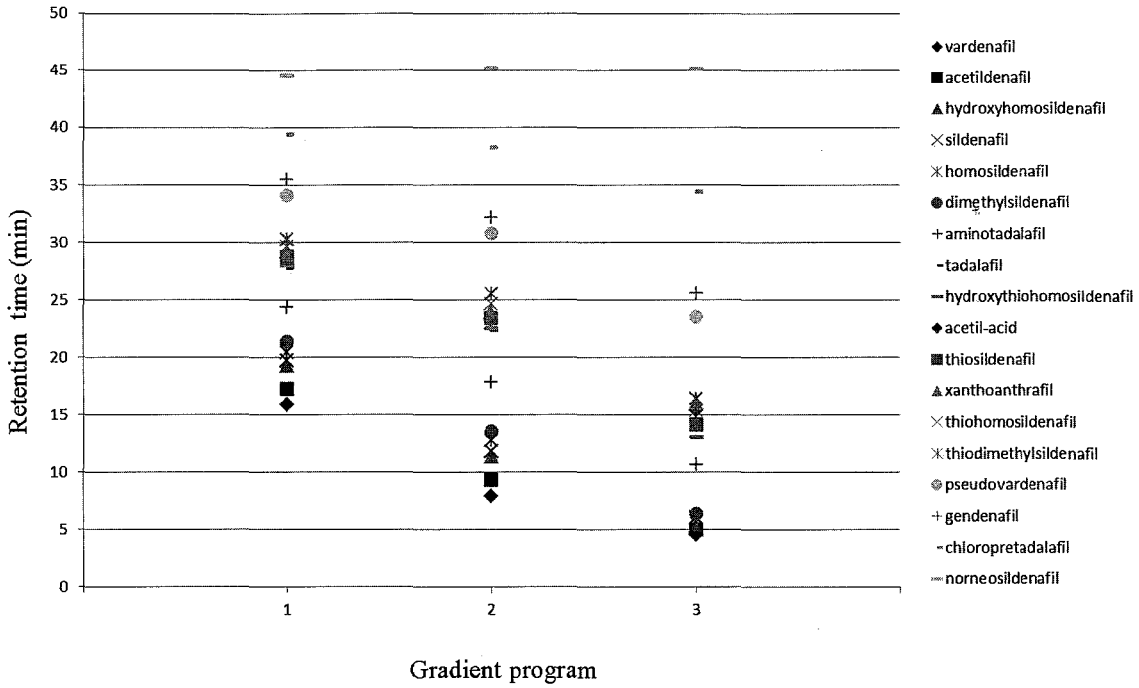

Fig. 3. Retention times of the 18 compounds using 3 different gradient programs

The concentration of each compound was 1 μg/mL

by sonication for 10 min using a 8510J-MT ultrasonicator (BRANSON, Danbury, CT, USA) according to the previous reported method[8]. The solution was then filtered using a 0.45-μm pore size membrane. The eluate was diluted 10-fold with methanol to form the sample solution.

Results and Discussion

Selection of chromatographic separation conditions

Initially, the composition of the mobile phase was investigated to enable separation of all 18 compounds. Three kinds of mobile phases (mobile phase 1: mobile phase A, 0.1% formic acid solution and mobile phase B, 0.1% formic acid in acetonitrile; mobile phase 2: mobile phase A, 0.1% formic acid in 5 mM ammonium formate and mobile phase B, 0.1% formic acid in acetonitrile; and mobile phase 3: mobile phase A, 0.1% acetic acid in 5 mM ammonium acetate and mobile phase B, 0.1% acetic acid in acetonitrile) were used to analyze the standard solutions of all 18 compounds (each at a concentration of 1 μg/mL). To detect the protonated molecules of the 18 compounds, the *m/z* values shown in Table 1 were selected to plot the extracted ion chromatograms. Our results showed that the sensitivity of aminotadalafil was low and the peak of aminotadalafil (1 μg/mL) was not detected in mobile phases 2 and 3 (Fig. 2). Thus, mobile phase 1 was selected as the mobile phase.

Secondly, the gradient condition was investigated. The gradient elution program, with a constant flow rate of 0.15 mL/min, was started with solution A at 80% (gradient program 1), 70% (gradient program 2), or 60% (gradient program 3), followed by a linear decrease to 20% (gradient program 1), 30% (gradient program 2), or 40% (gradient program 3) in 50 min. The gradient program 2 was selected because the retention times of the compounds were widely distributed (Fig. 3).

Extracted ion chromatograms of standard solution including all 18 compounds (each at a concentration of 0.5 μg/mL) are shown in Fig. 4.

Comparison of columns

To choose the best column, the standard solution including all 18 compounds (each at a concentration of 0.5 μg/mL) was analyzed by the proposed method using a Cosmosil $3C_{18}$-EB column and a conventional octadecylsilyl-silica gel (ODS) column (Cosmosil $3C_{18}$-MS-II column (particle size, 3 μm; i.d., 2.0 mm; length, 250 mm; Nacalai Tesque). It is very important to separate the peaks of different illegal adulterants, especially when a dietary supplement contains multiple compounds possessing the same molecular weight. Hydroxyhomosildenafil, thiodimethylsildenafil, and thiohomosildenafil have the same molecular weight, and the molecular ion (*m/z* 505) was the major peak for each compound in the positive mode. Hydroxyhomosildenafil, thiohomosildenafil, and thiodimethylsildenafil were clearly separated (the resolutions were 49.0 and 3.3), and sharp peaks were obtained on the $3C_{18}$-EB column (Fig. 5A). On the other hand, thiohomosildenafil and thiodimethylsildenafil were not clearly separated on the conventional ODS column (Fig. 5B). The proposed method could also separate vardenafil, homosildenafil, and dimethylsildenafil (the resolutions were 17.3 and 3.2 at *m/z*: 489, tadalafil, and xanthoanthrafil (the resolution was 4.2 at *m/z*: 390), and pseudovardenafil and norneosildenafil (the resolution was 40.4 at *m/z*: 460). Thus, it was consid-

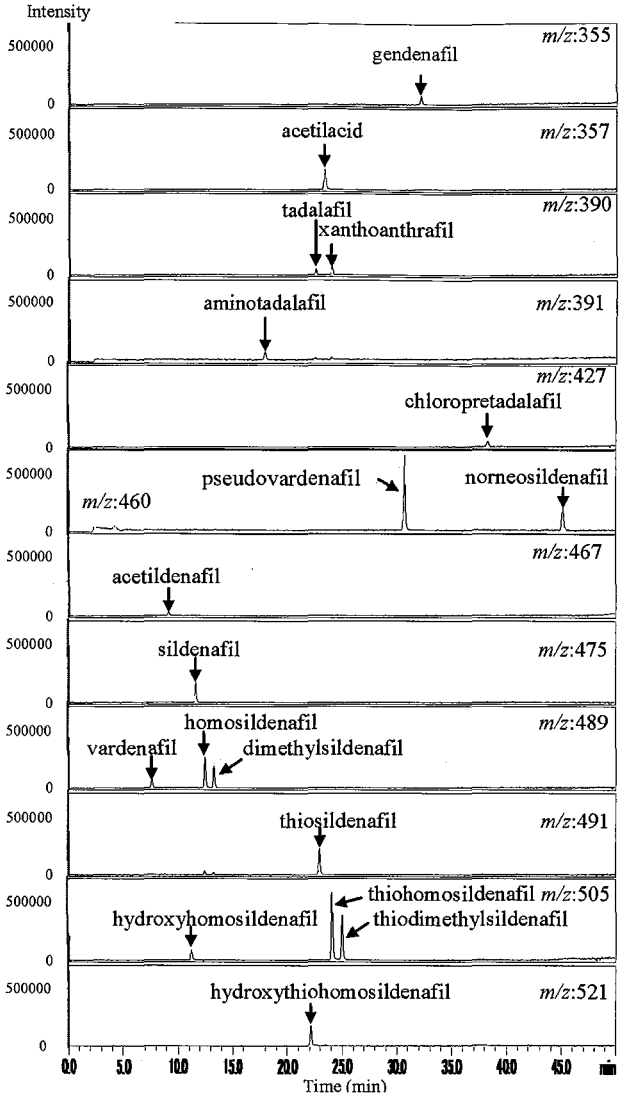

Fig. 4. Extracted ion chromatograms of standard solution including all 18 compounds (each at a concentration of 0.5 μg/mL)

ered that the proposed method is suitable for in distinguishing the peaks of compounds possessing the same molecular weight.

Detection limit

The detection limit of each compound was estimated based on a signal-to-noise ratio of 3. The detection limit of each compound was lower than 0.1 μg/mL. Depending on the sample preparation, the detection limits of the 18 compounds were below 1 mg/g. Thus, the proposed method has sufficient sensitivity for detecting illegal adulterants, since the prescribed dosage of sildenafil, tadalafil, and vardenafil is over 5 mg.

Application of the proposed method

We applied the proposed method to analyze 6 dietary supplements (A to F), which were examined in 2011 and were found to contain one or more illegal adulterants. We bought the dietary supplements *via* the Internet in 2011. Dietary supplements A and B contained sildenafil. Dietary supplement C contained pseudovardenafil. Dietary supplement D contained hydroxyhomosildenafil and aminotadalafil. Dietary supplement E contained thiohomosildenafil, thiosildenafil, and homosildenafil. Dietary supplement F contained hydroxythiohomosildenafil, aminotadalafil, thiosildenafil, dimethyl sildenafil, and thiodimethylsildenafil. The peak derived from each compound was clearly detected in all cases. Extracted ion chromatograms of dietary supplement F are shown in Fig. 6. Thus, the proposed method is adequate for the detection of illegal adulterants in commercial dietary supplements.

Conclusion

In this study, we developed a method to identify 18 illegal adulterants in dietary supplements. The proposed method can distinguish the peaks of multiple compounds possessing the same molecular weight, and is suitable to identify harmful illegal adulterants in dietary supplements.

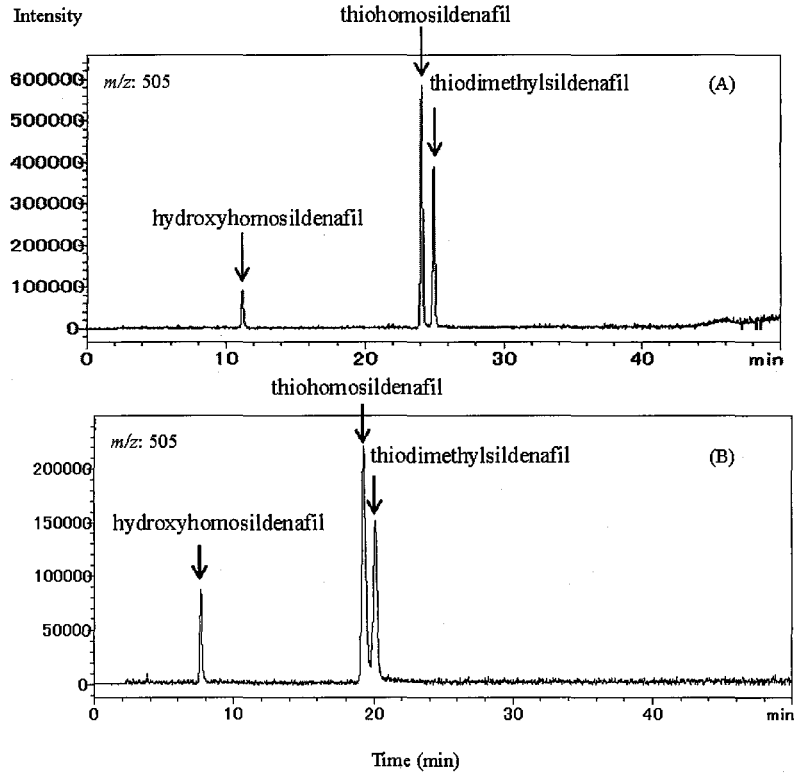

Fig. 5. Extracted ion chromatograms (m/z: 505) of standard solution including all 18 compounds (each at a concentration of 0.5 μg/mL) using the proposed method (A) and the conventional octadecylsilyl-silica gel column (B).

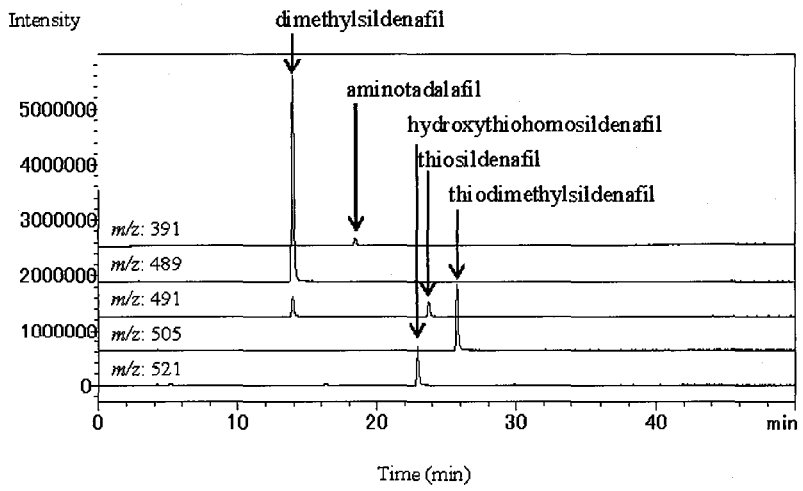

Fig. 6. Extracted ion chromatograms of dietary supplement F.

Acknowledgements

The authors are grateful to Dr. Goda (National Institute of Health Sciences, Japan) for providing helpful suggestions and reference materials. The authors are also grateful to the Department of Pharmaceutical Sciences, Tokyo Metropolitan Institute of Public Health for providing standards and helpful suggestions.

References

1) Yasuda, I. The medicinal supplies resemble ingredients in health foods. Shokuhin Eiseigaku Zasshi (Food Hyg. Saf. Sci.), **51**, 402–407 (2010).
2) Li,Y., Hu, J., Shi, Y., Sun, A., Sun, C. Simultaneous determination of seven adulterants in slimming functional foods by HPLC-ESI-MS/MS. Food Anal. Methods, **4**, 505–516 (2011).
3) Zhang, Y., Huang, Z., Ding, L., Yan, H., Wang, M., Zhu, S. Simultaneous determination of yohimbine, sildenafil, vardenafil and tadalafil in dietary supplements using high-performance liquid chromatography-tandem mass

spectrometry. Journal of Separation Science, **33**, 2109–2114 (2010).
4) Kumasaka, K., Kawahara, N., Doi, K., Kojima, T., Goda, Y. Determination of (*R*)-xanthoanthrafil, a phosphodiesterase-5 inhibitor, in a dietary supplement promoted for sexual enhancement. Chem. Pharm. Bull., **56**, 227–230 (2008).
5) Hasegawa, T., Saijo, M., Ishii, T., Nagata, T., Haishima, Y., Kawahara, N., Goda, Y. Structural elucidation of a tadalafil analogue found in a dietary supplement. Shokuhin Eiseigaku Zasshi (Food Hyg. Saf. Sci.), **49**, 311–315 (2008).
6) Demizu, Y., Wakana, D., Kamakura, H., Kurihara, M., Okuda, H., Goda, Y. Identification of mutaprodenafil in a dietary supplement and its subsequent synthesis. Chem. Pharm. Bull., **59**, 1314–1316 (2011).
7) Uchiyama, N., Saisho, K., Kikura-Hanajiri, R., Haishima, Y., Goda, Y. Determination of a new type of phosphodiesterase-5 inhibitor, thioquinapiperifil, in a dietary supplement promoted for sexual enhancement. Chem. Pharm. Bull., **56**, 1331–1334 (2008).
8) Tagami, T., Takeda, A., Asada, A., Aoyama, A., Doi, T., Kajimura, K., Sawabe, Y. Simultaneous identification of hydroxythiohomosildenafil, aminotadalafil, thiosildenafil, dimethylsildenafil, and thiodimethylsildenafil in dietary supplements using high-performance liquid chromatography-mass spectrometry. Shokuhin Eiseigaku Zasshi (Food Hyg. Saf. Sci.), **54**, 232–236 (2013).

LC/MS を用いた健康食品中の 18 種類の違法添加物の一斉分析（調査・資料，英文）

田上貴臣 * 青山愛倫 武田章弘 淺田安紀子
土井崇広 梶村計志 沢辺善之

食衛誌 **55**(1), 34～40 (2014)

強壮効果を標榜したいわゆる健康食品からは，医薬品成分が検出された事例がある．近年では摘発を逃れるために，強壮効果のある医薬品成分の構造の一部を変えた医薬品成分類似体が検出される事例がしばしば見受けられる．健康食品中の多数の成分を効率的に分析するためには，一斉分析法が必要である．そこで，LC/MS を用い，強壮効果を標榜する健康食品に添加される恐れのある 18 種類の化合物の一斉分析法について検討した．その結果，今回検討した分析法は，強壮効果を標榜する健康食品に添加される恐れのある 18 種類の化合物を分析することが可能であると考えられた．このことから，今回検討した分析法は，健康食品中の医薬品成分の効率的な検査法の 1 つとして有用であると考える．

* 大阪府立公衆衛生研究所