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Simultaneous Analysis of 12 Aphrodisiacs in Traditional Medicine using Liquid Chromatography-Tandem Mass Spectrometry

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ABSTRACT

Traditional medicines are often preferred over synthetic drugs due to their perceived safety and lower incidence of side effects. However, some unscrupulous industries illegally add chemical adulterants to these products to enhance their effects. A rapid and reliable analytical method is necessary to ensure the quality and safety of traditional medicines. This research aimed to validate the use of liquid chromatography-tandem mass spectrometry (LC-MS) to simultaneously identify 12 aphrodisiac compounds in traditional male sexual enhancers. The LC system employed 0.1% formic acid as an ion modifier, while the mass spectrometer used a triple quadrupole (TQD) as a mass analyzer in positive Electrospray Ionization (ESI) mode. The analysis was carried out using multiple reaction monitoring (MRM) transitions. Method validation was performed by assessing selectivity, precision, the matrix effect, and the limit of detection (LOD). Selectivity was assessed by comparing retention times between spiked and reference standards, with a difference of ±1 min shifts. Intra-day precision ranged from 2.0% to 8.6%. Extraction efficiency values showed no matrix effect, ranging from 86.2% to 110.9%. LOD values for the 12 compounds ranged from 1.6 ± 0.58 to 18.2 ± 6.14 µg/g. The method was applied to 49 samples of traditional male sexual enhancers in five different dosage forms. Our study demonstrates that LC-MS can confirm the content of aphrodisiac compounds in traditional medicine samples.

Keywords: aphrodisiac compounds, mass spectrometry, traditional medicine, male sexual enhancer

INTRODUCTION

The use of traditional medicines has increased significantly in recent years as people believe they are relatively safe and do not cause side effects compared to synthetic drugs. Public acceptance of traditional medicine in East Java, Indonesia, is relatively high, especially in the lower-middle-class group, with around 58% considering it an alternative treatment (Andriati & Wahjudi, 2016). However, the desired effect of traditional medicine takes longer than that of synthetic drugs, leading some manufacturers to add chemical adulterants to their products illegally to accelerate the desired effect (Kulkarni *et al*., 2014). One type of traditional medicine that is in high demand is products claiming to treat erectile dysfunction (Mulhall *et al*., 2008). Such products contain PDE-5 inhibitors, including sildenafil citrate and other aphrodisiac compounds. Although sildenafil citrate, tadalafil, and vardenafil hydrochloride are accepted for the treatment of sexual dysfunction, incorrect use can result in side effects such as headache, flushing, dyspepsia, nasal congestion, and rhinitis (Smith *et al*., 2013). Therefore, monitoring the distribution of PDE-5 inhibitor drugs should be intensified.

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Tadalafil, when used in combination with Chinese herbal medicine, provides a higher efficacy value, but regulations are needed to prevent unwanted side effects (Wang *et al*., 2020). Currently, chemical adulterants added to traditional medicines with claims to increase male stamina are not only sildenafil, tadalafil, and vardenafil hydrochloride but also other aphrodisiac compounds, including methyl testosterone and yohimbine hydrochloride. The consumption of these chemical adulterants can cause health problems and drug interactions when taken with other drugs (Kloner, 2005). To monitor the quality and safety of traditional medicines, the Indonesian FDA conducts sample testing in the market. However, irresponsible companies add sildenafil derivative compounds to avoid lawsuits and make it harder to detect their content using routine testing methods.

Several analytical methods based on chromatographic techniques have been developed to detect the content of aphrodisiac compounds in traditional medicines. These methods include thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) with different detection systems (Do *et al*., 2015; Nickum & Flurer, 2015). Both methods are effective in detecting chemical adulterants, but detecting sildenafil derivatives can be challenging due to their similar characteristics. Chromatographic methods rely on the differences in compound polarities to determine chemical adulterants. TLC separates compounds on a stationary phase and produces spots with different Rf values. Similarly, compounds can be identified using HPLC or GC, which produce peaks with different retention times after eluting through a column. However, the TLC method for sildenafil derivatives requires certain toxic reagents to increase compound separation. On the other hand, HPLC-UV can be used to analyze sildenafil-derived compounds, but in some cases, low-resolution values are observed (Nickum & Flurer, 2015). Ion detection with a certain m/z mass spectrometry can be used to detect sildenafil derivatives that are difficult to identify (Singh *et al*., 2009). The gas chromatography-mass spectrometry (GC-MS) method has also been used for the assay of sildenafil derivatives, but this requires a chromatographic system with a high column temperature setting due to the compounds' large molecular weight and high boiling point (Mokhtar *et al*., 2016; Jeong *et al*., 2016).

Liquid chromatography-tandem mass spectrometry (LC-MS) has been developed as a single compound identification and quantification method for low levels of sildenafil (Wang *et al*., 2005). Additionally, simultaneous analysis of 32 sildenafil-derived compounds can be performed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), which provides a good resolution profile and specific ionic fragment m/z values for each analyte in just 15min of running time (Azimi *et al*., 2012). A faster LC-MS/MS test method has been developed, which takes only 4.5 min of analysis time but can detect only a single compound of sildenafil in honey (Sharma *et al*., 2015). High-resolution mass spectrometry is useful for determining the types of compounds present in a sample, as it reflects mass accuracy, retention times, spectrum patterns based on the library, and an overview of the formulas of the compounds (Wang *et al*., 2018). However, there have been no reports on the identification of sildenafil derivatives and other aphrodisiacs in Indonesian traditional medicines. Thus, this study aims to develop a fast and reliable method for identifying sildenafil derivatives and other aphrodisiacs to improve the safety monitoring of traditional medicines.

METHODS AND MATERIAL

Reference Standards

The reference standards for sildenafil citrate, tadalafil, vardenafil hydrochloride, yohimbine hydrochloride, and methyl testosterone were obtained from the National Quality Control Laboratory of Drug and Food, Indonesian FDA. Thiodimethylsildenafil, nor-acetildenafil, hydroxythiohomosildenafil, propoxyphenyl hydroxyhomosildenafil, thiosildenafil, hydroxy-homosildenafil, and aminotadalafil were purchased from TLC Pharmaceutical Standards Ltd. (Aurora, Canada). HPLC-grade acetonitrile, HPLC-grade methanol, and p.a. formic acid were purchased from Merck. Distilled water (18.2MΩ) was obtained from the Milli-Q Advantage A10 ultrapure water purification system.

Traditional Medicine Samples

Surveillance sampling around Jakarta, Indonesia, yielded 49 male sexual enhancer traditional medicine samples in the dosage forms of powders, capsules, soft capsules, solutions, and caplets. Traditional medicine samples that did not contain the 12 tested aphrodisiac compounds were used as a blank matrix.

Instruments

An HPLC-tandem mass spectrometer (LC-MS/MS) system consisting of a Shimadzu LC-20 AD (Shimadzu, Japan) coupled with a Sciex Triple QuadTM 3500 mass spectrometer (Sciex, USA) was used. Instrument settings and data processing were supported by Analyst® software for setting test conditions, data acquisition, processing, and data analysis. Chromatographic separation was generated using a Cortecs C18 Waters column (4.6 mm x 50 mm, 2.7 µm) (Waters, USA). Sample extraction was done using a Thermo LP vortex mixer (Thermo Fisher Scientific, USA) and Hettich Rotofix 32 A centrifuge (Hettich, Germany).

Standard Solutions

Standard solutions were prepared by dissolving each reference standard in methanol to obtain a 1 mg/mL concentration and stored at -20°C. Intermediate standard solutions were prepared by dissolving each standard solution in a mixture of 0.1% formic acid-0.1% formic acid in acetonitrile (1:1, v/v) to obtain a concentration of 200 ng/mL. The blank matrix solution was prepared by dissolving a 250 mg sample in a mixture of methanol-acetonitrile (75:25, v/v) diluted with a mixture of 0.1% formic acid-0.1% formic acid in acetonitrile (1:1, v/v).

Extraction Procedure

The sample extraction was conducted following the application note from Waters (Azimi *et al*., 2012). Ten pack samples were mixed and homogenized to obtain a representative sample. Then, 250 mg of the sample was weighed and transferred to a 15-ml centrifugation tube. Next, it was dissolved in 10 ml of methanol-acetonitrile (75:25, v/v), vortexed for 1 min, sonicated for 15 mins, and centrifuged for 10 mins at 3500 rpm. The supernatant was separated and diluted 50 times with a mixture of 0.1% formic acid and 0.1% formic acid in acetonitrile (1:1, v/v). Finally, the solution was filtered through a 0.45 µm membrane filter.

LC-MS Instrumentation and Setting

Chromatographic separation was performed using a Cortecs C18 Waters column (4.6 mm x 50 mm, particle size 2.7 µm). The mobile phase was a binary system with a flow rate of 0.6 ml/min. Mobile phase A was 0.1% formic acid, and mobile phase B was 0.1% formic acid in acetonitrile. The gradient system was initiated with 10% B, increased to 30% B within 2 mins, rose to 90% B within 2 mins, returned to 10% B within 0.25 min, and was held for 1.25 min before the next injection of sample solution. The column temperature was set at 40°C during the analysis. The injection volume of the sample solution was 10 µL. Mass spectrometry analysis was performed using a Sciex Triple QuadTM 3500 mass spectrometer equipped with a Turbo VTM ion source with ESI in positive mode. Analysis was done using the MRM transition.

Method Validation

Analytical method validation is necessary to confirm that the method is suitable for its intended purpose by evaluating certain performance characteristics as per analytical requirements (Indrayanto, 2018). Qualitative analysis was conducted by using retention time (Rt) data of peaks at the m/z value of quantifier and qualifier ions for each compound. Method validation was set by determining the profiles of selectivity, precision, sample matrix effect, and limit of detection (LOD). Validation was performed following the guidelines for standard method performance requirements by evaluating some performance characteristics, including selectivity, precision, sample matrix effect, and LOD (Method *et al*., 2016; Steiner *et al*., 2020). Selectivity refers to the ability of the analytical method to differentiate the target compound from other components present in the sample matrix. Selectivity was determined by comparing the Rt of the peak and the value of the m/z quantifier and qualifier ions between the spiked sample solution and the standard solution. Precision was determined by injecting the standard mixture solution eight times, and the parameter observed was the RSD value of the chromatogram response of each compound. The sample matrix effect was observed by comparing the spiked sample's response with the standard solution's response with the same concentration, which is called extraction efficiency. The extraction efficiency was calculated using the formula EE=A/B x 100, where A is the response of the spiked sample, and B is the response of the standard solution. The extraction efficiency is expressed as a percentage (%). The LOD value was determined using the signal-to-noise (S/N) ratio by considering the profiles of quantifier and qualifier ions from each compound.

Data Collection and Analysis

The data collection and analysis were conducted using the mass spectrometer's Analyst® software. The chromatogram profile, Rt value,

Table I. Retention time, parent ion, and daughter ion data of the 12 aphrodisiac compounds during LC-MS/MS analysis with MRM transition.

Analyte	Rt of standard (min)	Rt of the *spiked sample* (min)	Quantifier ion (m/z)	Qualifier ion 1 (m/z)	Qualifier ion 2 (m/z)	Qualifier ion 3 (m/z)
Yohimbine hydrochloride	2.58	2.58	355.2	144.1	117.2	212.2
Vardenafil hydrochloride	2.73	2.73	489.3	151.0	312.2	299.2
Nor-acetildenafil	2.78	2.79	453.3	113.1	97.3	297.2
Hydroxyhomosildenafil	2.94	2.94	505.3	99.2	487.2	283.1
Sildenafil citrate	2.98	2.98	475.3	100.1	283.2	311.1
Propoxyphenyl hydroxyhomosildenafil	3.10	3.10	519.3	99.0	283.0	129.2
Aminotadalafil	3.33	3.33	391.1	269.1	262.1	233.3
Hydroxythiohomosildenafil	3.53	3.52	521.3	99.0	129.1	503.0
Thiosildenafil	3.58	3.58	491.2	100.2	299.3	99.3
Tadalafil	3.59	3.59	390.1	268.1	135.1	169.2
Thiodimethylsildenafil	3.68	3.68	505.3	299.0	113.1	327.2
Methyl testosteron	4.08	4.08	303.3	97.1	109.2	123.2

peak area, and m/z value of quantifier and qualifier ions in MRM transition mode were analyzed. The analysis was performed both qualitatively and quantitatively. The qualitative analysis involved comparing the RT of the spiked sample solution with the RT of the standard solution. Quantitative analysis was performed by statistically calculating the peak area of the spiked sample and standard solutions.

RESULTS AND DISCUSSION

In chromatographic-based analysis, the main criterion is the ability to elute peaks with sufficient resolution. To produce acceptable chromatograms, it is important to select a proper mobile phase with an appropriate gradient system that can provide good selectivity between the separated compounds and produce appropriate ionic fragments for all tested compounds (Shi *et al*., 2014). The optimization of the mobile phase was carried out by adding modifier ions to improve the chromatogram profile. Good separation was obtained using 0.1% formic acid modifier ions in both the aqueous phase (mobile phase A) and acetonitrile phase (mobile phase B) (Shi *et al*., 2014). In the gradient system, 12 aphrodisiac compounds were well eluted in 5.5 mins with good separation. Mass spectrometry analysis started with optimizing the appropriate ion fragments for each compound by adjusting the voltage at the ESI ion source. A triple quadrupole was operated in positive ion mode, and fragmentation occurred in the mass analyzer Q2, where the precursor ion was split into fragment ions (Table I).

Method Validation

A selectivity test was carried out by comparing the peak retention times between the spiked sample and reference standard solutions. Their retention times were found to be ±1min different. However, the chromatogram profile of the spiked sample showed no matrix effect in the sample (Figure 1). The precision value was evaluated as intra-day precision, giving results in the range of 2.0–8.6%. Tadalafil had the highest precision value among the other compounds, with an RSD value of 2.0%. Meanwhile, the lowest precision value was that of thiosildenafil, with a Relative Standard Deviation (RSD) value of 8.6%. The concentration of the reference standard solution used for the precision test of the 12 compounds was in the range of 187.1–216.4 ng/mL (Table II).

The extraction efficiency was determined by comparing the response areas of the spiked sample to those of the reference standard at the same concentration. The extraction efficiency of the compounds was found to be in the range of 86.2–110.9% (Table III). This value indicates that the solvent used in the extraction process, consisting of methanol-acetonitrile (75:25, v/v), is quite selective in dissolving the 12 target compounds without matrices influence. Thus, this method can be used to analyze 12 aphrodisiac compounds in traditional medicine samples that generally contain various natural compounds.

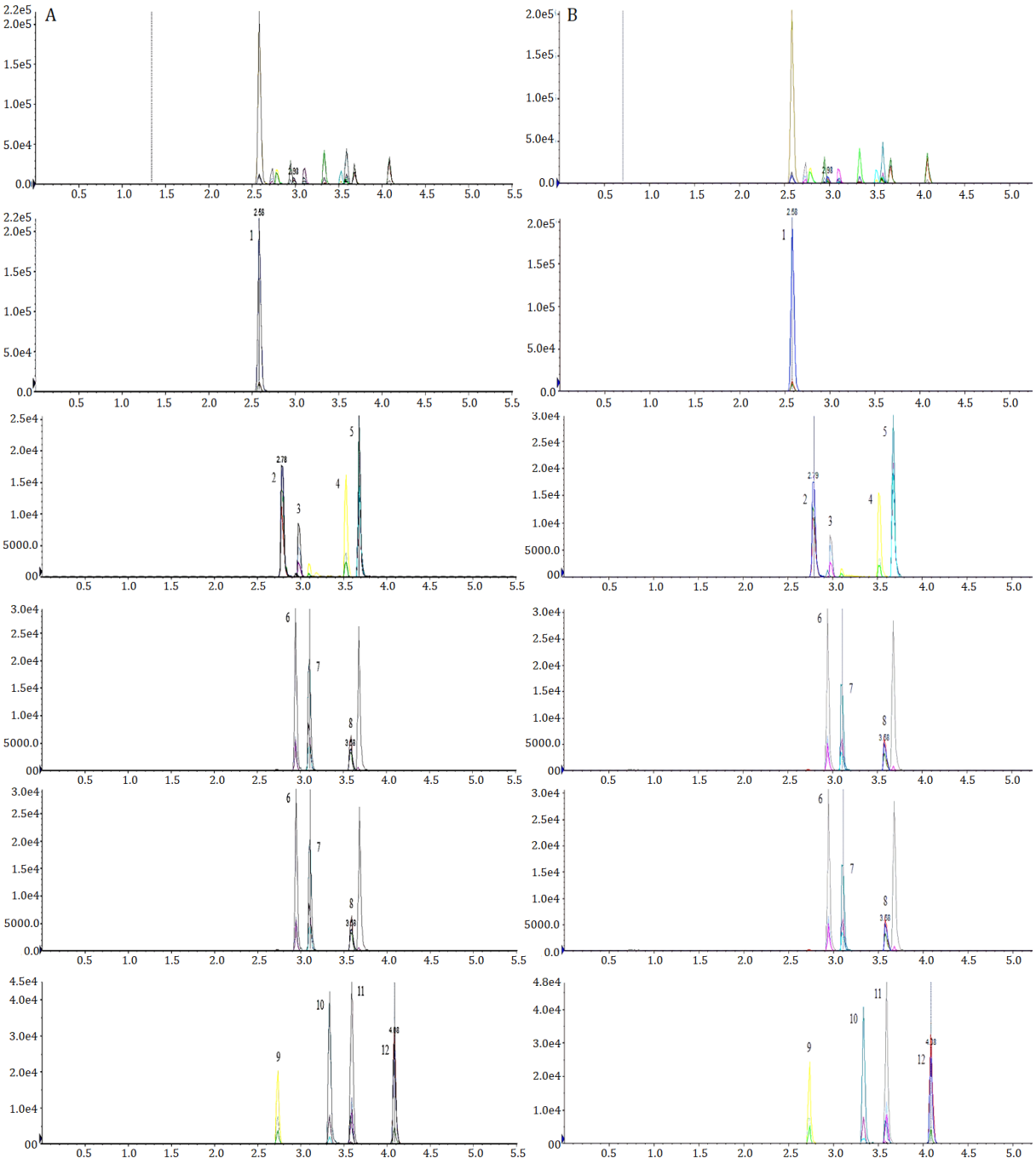

Figure 1. Chromatogram profiles of the reference standard solution (A) and spiked sample solution (B) consisting of a mixture of 12 aphrodisiac compounds, including yohimbine hydrochloride (1), nor-acetildenafil (2), sildenafil citrate (3), hydroxythiohomosildenafil (4), thiodimetilsildenafil (5), hydroxyhomosildenafil (6), propoxyphenyl hydroxyhomosildenafil (7), thiosildenafil (8), vardenafil hydrochloride (9), aminotadalafil (10), tadalafil (11), and methyl testosterone (12).

Table II. Precision data of the 12 aphrodisiac compounds.

Analyte	Concentration (ng/mL)	Average of the Area	Precision (RSD, %) (n=8)
Yohimbine hydrochloride	216.1	507648.8	5.3
Vardenafil hydrochloride	192.6	47122.9	6.3
Nor-acetildenafil	211.6	47331.8	5.9
Hydroxyhomosildenafil	187.1	55354.3	4.4
Sildenafil citrate	194.5	19817.8	5.3
Propoxyphenyl hydroxyhomosildenafil	192.2	47236.1	3.9
Aminotadalafil	202.1	88473.5	8.5
Hydroxythiohomosildenafil	188.2	38435.1	6.1
Thiosildenafil	216.4	11445.4	8.6
Tadalafil	211.0	96884.5	2.0
Thiodimethylsildenafil	209.1	55034.6	6.1
Methyl testosterone	200.5	63803.3	3.3

Table III. Extraction efficiency results and LOD values of the 12 aphrodisiac compounds.

Analyte	Concentration (ng/ml)	Extraction efficiency (%)	S/N Ratio	LOD (µg/g, n = 2)
Yohimbine hydrochloride	216.1	94.6	3.0	2.2 ± 0.01
Vardenafil hydrochloride	192.6	106.3	5.1	6.0 ± 1.68
Nor-acetildenafil	211.6	96.7	3.2	2.0 ± 0.06
Hydroxyhomosildenafil	187.1	98.0	4.5	6.2 ± 0.90
Sildenafil citrate	194.5	92.1	4.8	4.9 ± 0.41
Propoxyphenyl hydroxyhomosildenafil	192.2	86.2	5.5	5.3 ± 0.69
Aminotadalafil	202.1	86.7	5.0	5.1 ± 1.43
Hydroxythiohomosildenafil	188.2	98.5	2.6	18.2 ± 6.14
Thiosildenafil	216.4	100.7	4.6	11.3 ± 1.79
Tadalafil	211.0	95.8	2.6	5.2 ± 1.61
Thiodimethylsildenafil	209.1	110.9	3.7	3.5 ± 0.74
Methyl testosteron	200.5	91.8	4.0	1.6 ± 0.58

LOD is the ability of a method to detect the target compound at the lowest concentration while still giving an identical profile to the target compound. The detection limit of an analytical method indicates how low the analyte level can be detected (Proctor, 2008). LOD was obtained by measuring the S/N ratio of the quantifier ion with the rules that the qualifier ions 1, 2, and 3 meet the requirements (Table I). The obtained S/N ratio values ranged from 2.6–5.5, with LOD values between 1.6 ± 0.58 to 18.2 ± 6.14 µg/g. The acceptance criteria for LOD correlate with the No Observed Adverse Effect Level (NOAEL) value of the compounds, in which the LOD value should be lower than the NOAEL value. For example, the LOD value of sildenafil citrate was much lower than its NOAEL value: 4.9 µg/g and 3 mg/kg/day (17.1 mg/g), respectively.

The Use of Validated Methods for Analysis of Marketed Samples

The validated method was used to analyze the content of 12 aphrodisiac compounds in traditional male sexual enhancer medicine samples. A total of 49 samples were tested, which consisted of 5 different dosage forms: powders, capsules, solutions, soft capsules, and caplets, with 14, 31, 2, 1, and 1 item, respectively. These samples were collected as part of post-market surveillance sampling for supervision and prosecution due to the misuse of chemical adulterants.

From the 49 samples tested, eight types of aphrodisiac compounds were detected. Two samples in capsule dosage form were found to contain three chemical adulterants, namely hydroxythiohomosildenafil, thiosildenafil, and hydroxyhomosildenafil.

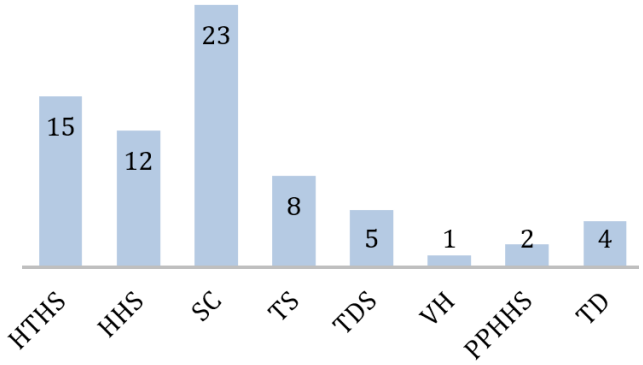

Figure 2. Profiles of the aphrodisiac compounds found in the male sexual enhancer traditional medicine samples. HTHS = hydroxythiohomosildenafil; HHS = hydroxyhomosildenafil; SC = sildenafil citrate; TS = thiosildenafil; TDS = thiodimetilsildenafil; VH = vardenafil hydrochloride; PPHHS = propoxyphenyl hydroxyhomosildenafil; TD = tadalafil.

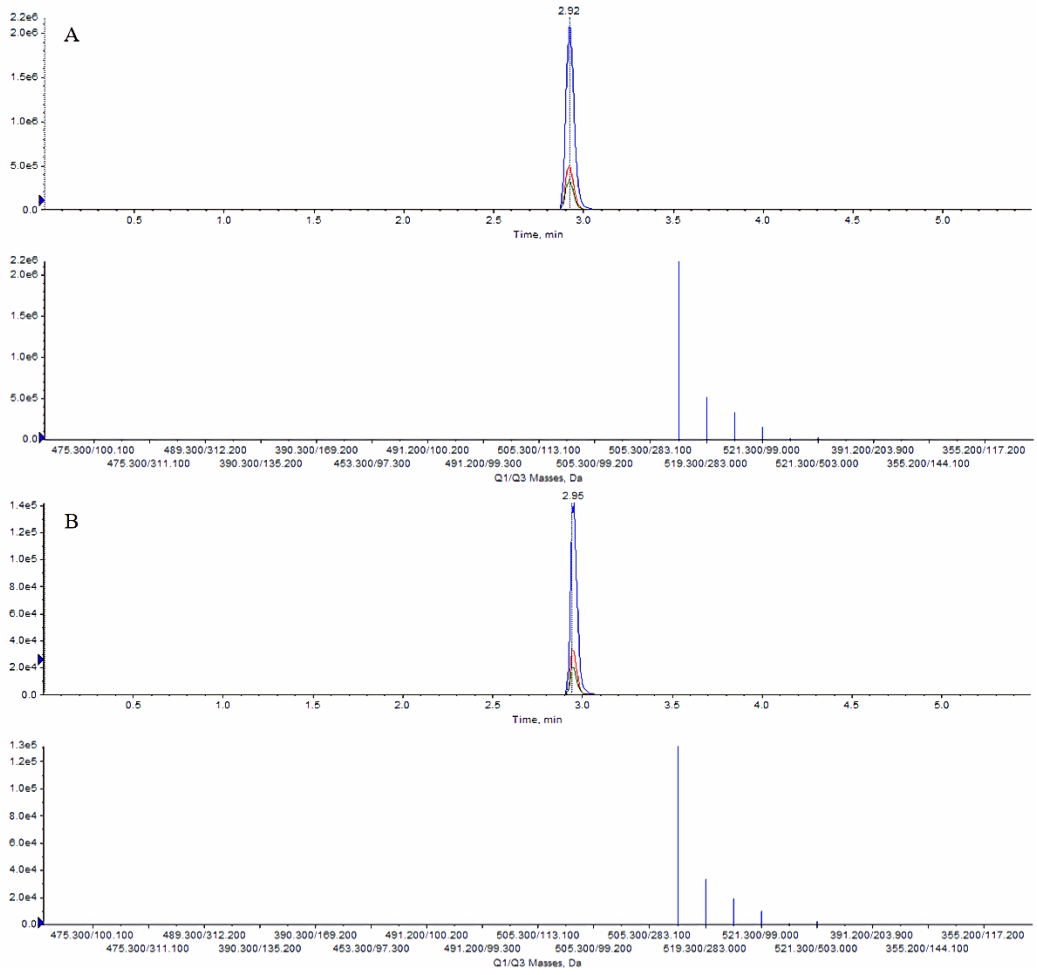

Figure 3. Chromatogram profiles of a tested capsule containing propoxyphenyl hydroxyhomosildenafil (A) compared to the reference standard of propoxyphenyl hydroxyhomosildenafil (B).

Additionally, 16 samples were found to contain two chemical adulterants, with hydroxy-thiohomosildenafil and hydroxy-homosildenafil being the most common (Figure 2).

Sildenafil citrate was the most common compound found in the samples; it was present in 23 samples. Hydroxythiohomosildenafil and hydroxyhomosildenafil were present in 15 and 12 samples, respectively. On the other hand, vardenafil hydrochloride, propoxyphenyl hydroxyhomosildenafil, and tadalafil were rarely found, with 1, 2, and 4 samples, respectively. The sample test results were categorized as positive for containing aphrodisiac compounds if the chromatogram profile and m/z value of the ion with the MRM transition were identical to the reference standard (Figure 3).

These results demonstrate that the developed method is applicable for determining the content of sildenafil citrate and its derivatives with high sensitivity and selectivity. Moreover, the method can be used to monitor the quality and safety of traditional medicines.

CONCLUSION

A simultaneous analytical method has been developed to determine 12 aphrodisiac compounds using HPLC-tandem mass spectrometry, which has been proven selective and sensitive. The selectivity of the method is based on m/z fragment ion values, which are specific to each compound, rather than retention times. This method can be used as a screening method to identify the content of chemical adulterants in traditional male sexual enhancers. The method is sensitive, with a low limit of detection (LOD) of 1.6 ± 0.58 to 18.2 ± 6.14 µg/g, which makes it useful as a confirmation method if there are doubts about test results using other methodologies. The developed method is also rapid, with an analysis time of only 5.5 mins, allowing for fast follow-up actions by authoritative bodies monitoring traditional medicines in the market.

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REFERENCES

Andriati, A., & Wahjudi, R. M. T. (2016). Tingkat penerimaan penggunaan jamu sebagai alternatif penggunaan obat modern pada masyarakat ekonomi rendah-menengah dan atas. *Masyarakat, Kebudayaan Dan Politik*, *29*(3), 133. https://doi.org/10.20473/mkp.v29i32016.133-145

Azimi, S., Mistry, N., Wood, M., Corporation, W., & Park, A. (2012). *UPLC-MS / MS for the Screening , Confirmation and Quantification of 32 Drugs illegally added to Herbal / Dietary Supplements for the Enhancement of Male Sexual Performance*. 298.

Do, T. T. K., Theocharis, G., & Reich, E. (2015). Simultaneous detection of three phosphodiesterase type 5 inhibitors and eight of their analogs in lifestyle products and screening for adulterants by high-performance thin-layer chromatography. *Journal of AOAC International*, *98*(5), 1226–1233. https://doi.org/10.5740/jaoacint.14-285

Indrayanto, G. (2018). Recent development of quality control methods for herbal derived drug preparations. *Natural Product Communications*, *13*(12), 1599–1606. https://doi.org/10.1177/1934578x1801301208

Jeong, Y. dong, Suh, S. I., Kim, J. Y., In, M. K., & Paeng, K. J. (2016). Simultaneous Determination of Sildenafil, Tadalafil, and Vardenafil in Pharmaceutical Preparations by High-Temperature Gas Chromatography/Mass Spectrometry. *Chromatographia*, *79*(23–24), 1671–1678. https://doi.org/10.1007/s10337-016-3185-y

Kloner, R. A. (2005). Pharmacology and drug interaction effects of the phosphodiesterase 5 inhibitors: Focus on α-blocker interactions. *American Journal of Cardiology*, *96*(12 SUPPL. 2), 42–46. https://doi.org/10.1016/j.amjcard.2005.07.011

Kulkarni, K. M., Patil, L. S., Khanvilkar, V. V, & Kadam, V. J. (2014). Fingerprinting Techniques in Herbal Standardization. *Indo American Journal of Pharmaceutical Research*, *4*(02), 1049–1062.

Method, S., Requirements, P., Method, S., Requirements, P., & Guide, E. (2016). *Appendix F : Guidelines for Standard Method Performance Requirements*.

Mokhtar, S. U., Chin, S. T., Kee, C. L., Low, M. Y., Drummer, O. H., & Marriott, P. J. (2016). Rapid determination of sildenafil and its analogues in dietary supplements using gas chromatography-triple quadrupole mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *121*, 188–196. https://doi.org/10.1016/j.jpba.2016.01.034

Mulhall, J., King, R., Glina, S., & Hvidsten, K. (2008). *Importance of and Satisfaction with Sex Among Men and Women Worldwide: Results of the Global Better Sex Survey*. 788–795. https://doi.org/10.1111/j.1743-6109.2007.00765.x

Nickum, E. A., & Flurer, C. L. (2015). Determination of phosphodiesterase-5 inhibitors and analogs using high-performance liquid chromatography with ultraviolet detection. *Journal of Chromatographic Science*, *53*(1), 38–46. https://doi.org/10.1093/chromsci/bmu010

Proctor, C. H. (2008). A simple definition of detection limit. *Journal of Agricultural, Biological, and Environmental Statistics*, *13*(1), 99–120. https://doi.org/10.1198/108571108X273476

Rebiai, A., Hemmami, H., Zeghoud, S., Ben Seghir, B., Kouadri, I., Eddine, L. S., Elboughdiri, N., Ghareba, S., Ghernaout, D., & Abbas, N. (2021). Current Application of Chemometrics Analysis in Authentication of Natural Products: A Review. *Combinatorial Chemistry & High Throughput Screening*, *25*(6), 945–972. https://doi.org/10.2174/1386207324666210309102239

Sharma, P. K., Bhasin, N., Joseph, P., Pillai, M. G., Schreiber, A., Vihar, U., & Iv, P. (2015). *Fast , Robust and Reliable Method for the Identification and Quantitation of Sildenafil Residue in Honey using LC-MS / MS*. 122015.

Shi, F., Guo, C., Gong, L., Li, J., Dong, P., Zhang, J., Cui, P., Jiang, S., Zhao, Y., & Zeng, S. (2014). Application of a high resolution benchtop quadrupole-Orbitrap mass spectrometry for the rapid screening, confirmation and quantification of illegal adulterated phosphodiesterase-5 inhibitors in herbal medicines and dietary supplements. *Journal of Chromatography A*, *1344*, 91–98. https://doi.org/10.1016/j.chroma.2013.12.030

Singh, S., Prasad, B., Savaliya, A. A., Shah, R. P., Gohil, V. M., & Kaur, A. (2009). Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs. *TrAC - Trends in Analytical Chemistry*, *28*(1), 13–28. https://doi.org/10.1016/j.trac.2008.09.004

Smith, W. B., McCaslin, I. R., Gokce, A., Mandava, S. H., Trost, L., & Hellstrom, W. J. (2013). PDE5 inhibitors: Considerations for preference and long-term adherence. *International Journal of Clinical Practice*, *67*(8), 768–780. https://doi.org/10.1111/ijcp.12074

Steiner, D., Krska, R., Malachova, A., Taschl, I., & Sulyok, M. (2020). *Evaluation of Matrix Effects and Extraction Efficiencies of LC-MS / MS Methods as the Essential Part for Proper Validation of Multiclass Contaminants in Evaluation of Matrix E ff ects and Extraction E ffi ciencies of LC − MS / MS Methods as the Essential . March*. https://doi.org/10.1021/acs.jafc.9b07706

Wang, X. B., Zheng, J., Li, J. J., Yu, H. Y., Li, Q. Y., Xu, L. H., Liu, M. J., Xian, R. Q., Sun, Y. E., & Liu, B. J. (2018). Simultaneous analysis of 23 illegal adulterated aphrodisiac chemical ingredients in health foods and Chinese traditional patent medicines by ultrahigh performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Journal of Food and Drug Analysis*, *26*(3), 1138–1153. https://doi.org/10.1016/j.jfda.2018.02.003

Wang, Y., Wang, J., Cui, Y., Fawcett, J. P., & Gu, J. (2005). Liquid chromatographic-tandem mass spectrometric method for the quantitation of sildenafil in human plasma. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *828*(1–2), 118–121. https://doi.org/10.1016/j.jchromb.2005.09.008