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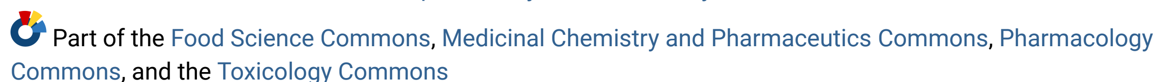

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Original Article

Determination of higenamine in multi-matrix by gas chromatography-mass spectrometry combined with derivatization technology

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ABSTRACT

Higenamine (HG), a cardioactive component of some foods and medicines, has been listed in the doping category by the International Olympic Committee, which may lead to misuse by athletes. We report the development of a gas chromatography-mass spectrometry (GC–MS) method for determination of HG in various matrix samples (biological samples, different forms of Chinese patent medicine, Chinese herbal medicine) based on acylation derivatization of HG by heptafluorobutyric anhydride. Under optimal conditions, the linearity of HG in the range of 5–200 ng mL^{-1} was acceptable ($R^2 > 0.999$), and the limit of detection (LOD) and limit of quantitation (LOQ) for HG was 1.52 ng mL^{-1} and 5 ng mL^{-1}, respectively. Low, medium, and high concentrations (25, 100 and 160 ng mL^{-1}) of HG were added to plasma, urine, oral liquid, capsule, watered bolus, honeyed bolus and Chinese herbal medicine samples, with recovery ranging from 82.70 to 109.80%, intra-day and inter-day precisions were both less than 3.39%. The results indicated that the method had sufficient sensitivity for analysis of biological samples, and Chinese patent and herbal medicine.

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1. Introduction

Higenamine (HG), 6,7,12-trihydroxy-benzyl-1,2,3,4-tetrahydroisoquinoline, is an alkaloid isolated from several plants belonging to the genera *Nandina*, *Aconitum*, *Asarum*, *Galium*, *Annona*, and *Lotus* [1,2]. HG was first separated from aconite root by Kosuge and Yokota in 1976 [3] and has received attention from healthcare professionals for its cardioactive properties since then. HG can stimulate β-adrenergic receptors [4,5] and exert inotropic and chronotropic effects on the cardiovascular system through anti-platelet aggregation, inhibition of iNOS, and upregulation of HO-1 expression [6–10]. HG has also been used in a cardiac stress test in the treatment of acute diseases, including disseminated intravascular coagulation, heart failure, ischemia/reperfusion injury, and spinal cord injury [11,12].

Since HG is a β2-receptor agonist, the International Olympic Committee (IOC) has prohibited its use by athletes [13]. In January 2017, the World Anti-Doping Agency (WADA) included HG in the prohibited list of S3 group (β2-receptor agonists) [14]. Reports in the literatures indicate that HG is widely distributed in plants and even in some food materials and medicines, such as weight loss products, energy drinks, or additives for sports function products [15,16], leading to the potential risk of consumption of HG by athletes. Therefore, athletes must be prevented from eating HG-containing vegetable drugs or food materials in advance. However, judging whether an athlete takes foods, medicines, or illegal drugs containing HG requires urine or blood tests. Therefore, it is important to establish a sensitive and reliable HG detection method suitable for various matrixes. According to the list of HG-containing drugs, nutrients and food products, and the test requirements of biological samples issued by the China Food and Drug Administration [17], different matrixes have been classified (Table 1).

Because the listing of HG in the doping category is issued recently, there are relatively few analytical methods to detect HG at present. The most commonly used detection methods are high performance liquid chromatography (HPLC) [18], capillary electrophoresis (CE) [19], and ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) [11,13,20–22]. Although derivatization methods are used in the HG detection by HPLC and CE, the detection limit has not met the ideal requirements. The UHPLC-MS/MS method has high sensitivity and a low detection limit. Its universal applicability, however, is limited due to its high cost. The methods described in the literature mainly focus on one sample matrix, and there are no sample pretreatment methods for different matrixes in the detection of HG.

Gas chromatography (GC) has high selectivity and separation efficiency. Thus it is suitable for the chromatographic separation and detection of complex sample matrixes. Moreover, the gas chromatography-mass spectrometry (GC–MS) method is simple in operation with a cost lower than liquid chromatography. As HG is a polar molecule and is difficult to be detected directly by GC, very few analyses of HG by GC or GC–MS have been reported [23,24], and the resolution of derivative was not very favorable in practicality [23]. In order to expand the application range of GC, esterification, alkylation, acylation, and silylation reactions [25–28] are often carried out to derivatize matrixes to increase the volatility of samples and to improve the detection sensitivity. In our previous research, a series of derivatives of polar compounds analyzed by GC, were developed and applied to target analysis in various matrixes [29].

In the present study, by combining rational extraction and derivation techniques, a reliable and sensitive GC–MS analytical technique was established to detect HG in different matrixes such as plasma, urine, Chinese herbal medicines, and various formulations of Chinese patent medicines (capsules, bolus, and oral liquids). First, the optimum derivative method was determined by investigating the effects of different derivative reagents. Then the extract and clean up conditions for various samples were optimized, according to matrixes differing properties. The analytical method reported here can satisfactorily detect various types of biological samples, screening medicines and foods for athletes, while applying useful anti-doping information.

2. Materials and methods

2.1. Reagents and materials

Higenamine (HG) was purchased from Sigma (St. Louis, MO, USA). Heptafluorobutyric anhydride (HFBA), *N*-methyl-*N* (trimethylsily) trifluoroacetamide (MSTFA), and ethyl chloroformate (ECF) were bought also from Sigma. HPLC grade methanol and acetonitrile were supplied by Oceanpakalexative chemical., Ltd (Gothenburg, Sweden). Formic

Table 1 – Classification and examples of sample matrixes containing or possibly containing higenamine.

Sample classification		Examples
Biological sample		Plasma, urine.
Chinese patent medicine	Capsules	Lianhuaqingwen capsule, Yaoxitong Capsule, Nanbao Capsules, JiujiXingjun Capsule, Biyuanshu capsule, etc.
	Bolus	Aconite middle-regulating bolus, Zhengtian bolus, Compound Xiaohuoluo bolus, Zhikehuatan bolus, Haimabushen bolus, Zenglizaisheng bolus, etc.
	Oral liquid	ShenxianShengmai oral liquid, Bushentianjing oral liquid, ChaixinGanmao injection, Xiaokepingchuan oral solution, Tongtianoral liquid, etc.
Chinese herbal medicine		*Lotus*, *Lotus plumule*, *Aconitum carmichaeli Debx.*, *Lindera aggregata*, *Asarum*, etc.

acid, chloroform ($CHCl_3$), and dichloromethane (CH_2Cl_2) were purchased from Tianjin Baishi chemical industry Co. Ltd (Tianjin, China) and ethyl acetate (EA) and *n* -hexane from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). Cleanert C18-SPE cartridge (200 mg/6 mL) and Cleanert PEP-2 SPE cartridge (500 mg/6 mL) were purchased from Agela Technologies (Tianjin, China), ProElut C8 cartridge (500 mg/ 6 mL) was bought from DiKMA (Beijing, China), and Oasis@ HLB cartridge (200 mg/6 mL) was supplied by Waters Corporation (Milford, USA). Ultra-pure water was obtained from a HealForce SMART-N system (Heal Force Bio-Meditech Holdings Limited, Shanghai, China).

2.2. *Sample pretreatment*

Representative samples were selected for optimizing sample pretreatment conditions. Biological samples (plasma, urine), Chinese patent medicine (oral liquid: compound radix scutellariae gargle, capsule: Lianhuaqingwen capsule, watered bolus: Zhengtian bolus, honeyed bolus: Zhonghuadieda bolus), Chinese herbal medicine: *Lotus plumule*.

2.2.1. *Biological sample*

Acetonitrile (3 mL) was added to plasma or urine sample (1 mL), and vortexed for 1 min to precipitate proteins. Subsequently, the mixture was centrifuged for 5 min at 15,000 rpm. The obtained supernatant was decanted into a clean centrifuge tube, then dried to approximately 1 mL with a slow stream of nitrogen followed by solid phase extraction (SPE).

2.2.2. *Chinese patent medicine*

2.2.2.1. *Oral liquids*. Fifty microliters of oral liquid sample were diluted with ultra-pure water to 1 mL for SPE.

2.2.2.2. *Capsules*. Soft gel capsules were opened with a scalpel and 5 mg content was accurately weighed into a 20-mL conical flask. Then, 10 mL ultra-pure water was added and the mixture was ultrasonically extracted for 30 min. After centrifugation (5 min, 15,000 rpm), the supernatant was separated for further analysis.

2.2.2.3. *Watered bolus*. Defined amount of bolus were finely ground and sifted. Sifted solid powder (5 mg) was weighed exactly into a 20-mL conical flask, subsequently ultrasonically extracted with 10 mL water for 30 min, and then centrifuged for 5 min at 15,000 rpm. The supernatant (1 mL) was used to SPE.

2.2.2.4. *Honeyed bolus*. A honeyed bolus was weighed accurately (5.9420 g) into a beaker, 500 mL water was added for ultrasonic dissolution and extraction for 30 min. The extraction was centrifuged for 5 min at 15,000 rpm and then 50 μL of supernatant was diluted with ultra-pure water to 1 mL as honeyed bolus extract, followed by SPE.

2.2.3. *Chinese herbal medicine*

Chinese herbal medicine was dried in an oven at 70 °C, ground and sifted. Dried herbs (5 mg of sifted sample) were placed in a 20-mL conical flask, and 10 mL of ultra-pure water were added for ultrasonic treatment for 30 min. Subsequently, the mixture was centrifuged for 5 min at 15,000 rpm and 1 mL of supernatant was used to SPE.

2.3. *Solid phase extraction (SPE)*

In a typical SPE procedure, a PEP-2 cartridge (200 mg/6 mL) was conditioned in turn with methanol and water, each 5 mL. The extract obtained in preceding section was loaded onto the cartridge, and 1 mL of 30% methanol aqueous solution was applied to the cartridge in a wash step. Acidified methanol (5% formic acid; 2 mL) were used to elute the HG. The eluent was collected and dried with a slow stream of nitrogen at 70 °C.

2.4. *Derivatization*

HFBA (100 μL) was added to the SPE dried residues and vortexed thoroughly, at 70 °Cfor 45 min. The derivatized samples were dried by blowing with nitrogen at room temperature, reconstituted in 100 μL CH_2Cl_2, vortexed for 1 min, and then 1 μL of each sample was injected into the GC–MS for analysis.

2.5. *GC–MS analysis*

GC–MS analysis was performed on an Agilent (Little Falls, DE, USA) gas chromatograph 7890A equipped with an electronically controlled split/splitless injection port, a 7683B Series injector/autosampler, and an inert 5975C mass selective detector with electron impact (EI) ionization chamber. An Agilent HP-5 capillary column (30 m × 0.32 mm I.D, 0.25 μm film thickness) was used for separation of analytes.

The initial GC oven temperature was set at 120 °C, ramped at 10 °C/min to 260 °C held for 5 min. A split injection (split ratio, 5:1) and injector temperature of 250 °C were employed. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. In the full-scan mode, preliminary mass spectra of the target analytes were obtained from *m/z* 20 to 750 AMU, and selected ion monitoring (SIM) mode was used for the identification and quantification of HG (*m/z*: 303.1, 330.1, 358.1, 386.1). The MS transfer line temperature was held at 280 °C. Mass spectrometric parameters were set as follows: electron ionization with 70 eV energy, ion source temperature, 230 °C, MS quadrupole temperature, 150 °C and solvent delay 4.0 min.

2.6. *Method validation*

Calibration curve was obtained by spiking ultra-pure water over the concentration range of 5–200 ng mL^{-1} for HG, the SPE and derivatization were carried out according to sections 2.3. and 2.4. The linear equations and correlation coefficients were calculated. Method sensitivity was evaluated by determining limit of detection (LOD) and the limit of quantitation (LOQ) for HG. The LOD and LOQ were determined at signal-to-noise (S/N) ratios of 3 and 10, respectively. Matrix spiked recovery and precision experiments were carried out at low, medium, and high (25, 100 and 160 ng mL^{-1}) concentration levels. The intra-day and inter-day precisions were studied to evaluate the precision of the method. The intra-day precision and inter-day precision were assessed at low, medium and high levels (25, 100 and 160 ng mL^{-1}) within the same day ($n = 6$) and three days ($n = 6$), respectively.

3. Results and discussion

3.1. Selection of chromatographic columns

The target HG was analyzed after being derived with polar (Agilent DB-WAX) and non-polar (Agilent HP-5MS) chromatographic columns. The derivatives on HP-5MS columns were more sensitive and had less matrix interference. The DB-WAX chromatographic column resulted in poor separation of the matrixes and the signal was weak due to the lesser retention. Therefore, HP-5MS non-polar chromatographic columns were selected for use in this study.

3.2. Optimization for sample pretreatment

The presence of proteins may affect the analysis of biological samples (plasma and urine). Typically, protein precipitation was performed in plasma samples with high protein content by methanol during the SPE if the protein is not removed in advance, which will affect the process of SPE. Therefore, for plasma and urine samples, proteins were first precipitated with acetonitrile (plasma: acetonitrile = 1:3) before being treated with SPE to reach a higher recovery rate.

Ultrasonic extraction was used to separate HG from various drugs (capsule, watered bolus, honeyed bolus) and Chinese herbal medicine samples. Since HG is a polar molecule, the effects of different extraction solvents (water, methanol, and ethanol) on the extraction efficiency were first investigated. It was confirmed that the extraction efficiency of water and methanol was equal but that of ethanol is low. In view of the SPE process after extraction, water was selected as the extraction solvent. With the increase of ultrasonic extraction time, the extraction efficiency increased gradually and reached a maximum value after 30 min. Therefore, ultrasonic extraction for 30 min was finally selected as the extraction method of HG.

In order to ensure the recovery rate of HG, the optimized pretreatment methods were different for various drugs. Since content of capsules is water-soluble, the capsule shells can be removed and the contents of capsules can be ultrasonically analyzed directly. The watered bolus can be directly ground, sifted, and ultrasonically extracted. Chinese herbal medicines are mainly botanical (roots, stems, leaves, and fruits), and thus should be dried, ground, sifted, and extracted under ultrasound. Analysis of the role of extractant dosage revealed that the recovery rate was poor when water volume was too low, and late enrichment was difficult if the water volume was too high. Therefore, 10 mL of water was selected for extraction.

The honeyed bolus drug is difficult to dry because of its honey component. A large amount of honeyed bolus was thus dissolved in water, subjected to ultrasound extraction, and properly diluted before SPE treatment. If the oral liquid medicine emitted an aromatic odor, it was heated to remove esters before dilution and SPE to ensure a high recovery rate.

3.3. Optimization for SPE

Derivatization techniques in GC analysis, particularly silanization or esterification reactions, are often required in an anhydrous environment; necessary concentration and enrichment can also improve detection sensitivity and selectivity. The target of the aqueous phase can be transferred to the organic phase by SPE, allowing samples to be blown dry, concentrated, and enriched more easily. Accordingly, SPE has been widely used in sample pretreatments. In this study, optimized SPE was also employed to process the samples. The extraction effects of different SPE columns (C18, C8, PEP-2, and HLB) were first evaluated. HLB exerted the strongest retention effect on the target. Under the experimental conditions, the target was difficult to elute with different types of eluents. C8 exerted the next-strongest retention effect, followed by C18. PEP-2 exhibited a moderate retention effect on the target and was able to remove and purify the sample. Thus, the PEP-2 column was selected for the SPE treatment of the sample in this study.

To ensure that the HG met the detection requirements, the sample loading amount, and types of leachate and eluent were optimized. Given that the column capacity was fixed, HG might not be completely retained if the loading amount of the sample was too large, or the target sample could be affected by the increase in impurities. Therefore, 1 mL was selected as the volume of the sample solution. The effects of using different proportions of methanol/water (v/v = 0/10, 2/8, 3/7, 4/6, 5/5) as leachate on the recovery of HG were analyzed. The optimal recovery rate was obtained at the methanol/water volume ratio of 3/7. Therefore, 1 mL of methanol/water (v/v = 3/7) was selected as the leachate. Elution of the target substance is also an important step in SPE. In this study, 2 mL of methanol failed to completely elute the target substance; if the amount of methanol was further increased, it would be inconvenient for the subsequent drying treatment. Elution with methanol acidified by formic acid was more effective. The addition of formic acid was confirmed to increase the amount of HG in the eluent. The effect of the concentrations of formic acid in 2 mL of methanol (0, 2, 5, 8, and 10%) on HG content in the eluent was optimized. The results showed that with 5% formate methanol solution as the eluent, the HG on the SPE column could be completely eluted. The HG content remained unchanged even with a further increase in concentration. Finally, the optimized conditions for SPE were as follows: loading, 1 mL of the sample; leachate, 1 mL of methanol: water (v/v = 3/7) solution; and eluent, 2 mL of 5% formate methanol solution.

3.4. Optimization for derivatization

Derivatization can effectively improve the sensitivity of GC–MS detection. The HG molecule contains three hydroxyl polar molecules; thus, the derivatizing effects of different derivatization reagents (HFBA, MSTFA, and ECF) on HG (17.9 $\mu g\ mL^{-1}$) were first evaluated. The optimized conditions for silanization were as follows: 100 μL of 17.9 $\mu g\ mL^{-1}$ HG was blow-dried with nitrogen, and 100 μL of MSTFA was added and heated at 70 °C for 1 h. The optimized condition for esterification reaction was as follows: 500 μL of a 17.9 $\mu g\ mL^{-1}$ HG standard solution mixed with 50 μL of pyridine and 200 μL of ethanol at room temperature before adding 50 μL of ethyl chloroformate. After ultrasound reaction for 3 min, the solution was extracted with 200 μL of dichloromethane and then vortexed for 2 min. After centrifugation at 3500 rpm for 3 min,

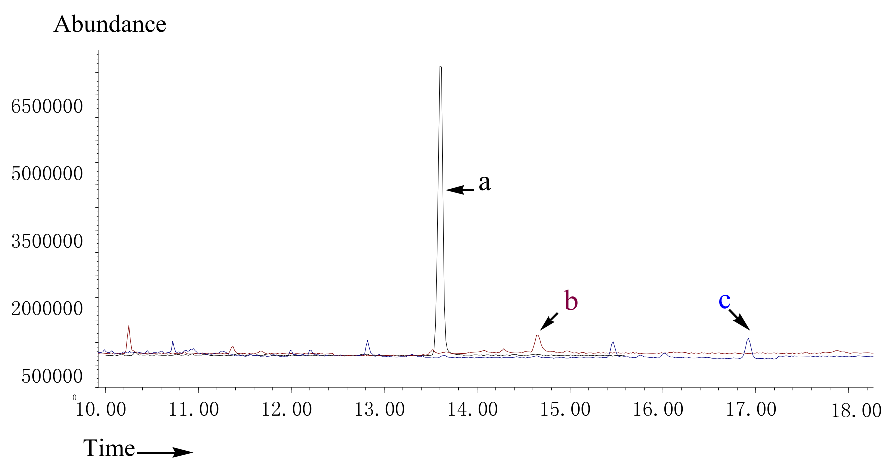

Fig. 1 – Comparison for the derivation efficiency of three different derivatization methods for HG. a: HFBA, b: MSTFA, c: ECF.

the chloroform layer for GC–MS analysis. Acylation reaction was conducted under the following optimized condition: 100 μL of 17.9 μg mL^{-1} HG was blow-dried with nitrogen, 100 μL HFBA was added and then heated at 70 °C for 1 h, and 100 μL of CH_2Cl_2 was redissolved after nitrogen was blow-dried.

Our experimental data indicated that the derivatization effect of the ECF and silylation reagent MSTFA [23] was not optimal and occasionally led to side reactions, whereas HFBA exerted a good derivatization effect on target groups and involved reduced side reactions. The chromatogram of derivatives under the full scanning mode is presented in Fig. 1. Accordingly, HFBA was selected as the derivative reagent.

The reagent amounts, temperature, and time are the most important factors affecting the derivatization reaction.

Table 2 – Recovery and precision of HG in different matrixes.

Matrixes	Spiked concentration (ng mL^{-1})	Measured concentration (ng mL^{-1})	Recovery[a] (%)	RSD (%)		Matrix effect[b] (%)
				Intra-day (n = 6)	Inter-day (n = 6)	
Plasma	0	0	–	–	–	–
	25	26.43	105.73	0.69	0.77	108.58
	100	102.09	102.09	1.22	1.45	101.68
	160	137.10	85.69	0.50	0.88	84.94
Urine	0	0	–	–	–	–
	25	22.21	88.85	1.47	1.52	83.61
	100	102.34	102.34	2.04	2.69	100.26
	160	169.98	106.24	1.21	1.67	105.36
Oral liquid	0	10.63	–	–	–	–
	25	33.37	90.93	0.88	1.72	109.02
	100	97.61	86.98	1.40	1.66	88.73
	160	145.54	84.32	1.92	2.11	90.11
Capsules	0	9.80	–	–	–	–
	25	31.81	88.06	2.71	3.25	107.63
	100	119.60	109.80	1.10	1.98	89.25
	160	177.46	104.79	0.71	1.18	88.75
Watered Bolus	0	22.63	–	–	–	–
	25	45.80	92.69	1.53	3.39	109.07
	100	105.33	82.70	1.07	1.77	107.71
	160	162.05	87.14	0.88	1.72	105.52
Honeyed Bolus	0	0	–	–	–	–
	25	26.66	106.63	0.80	0.96	113.22
	100	107.60	107.60	1.22	1.40	115.52
	160	165.01	103.13	0.98	1.23	110.38
Chinese Herbal Medicine	0	16.18	–	–	–	–
	25	41.85	102.66	2.33	2.68	113.75
	100	118.65	102.47	1.16	1.59	110.18
	160	156.90	87.95	0.87	1.24	97.79

[a] Recovery (%) = (Concentration of analytes in the spiked sample- Concentration in the unspiked sample) × 100/(Spiked concentration).
[b] ME (%) = (Peak area of analytes in the spiked sample- Peak area in the unspiked sample) × 100/(Peak area in methanol solution).

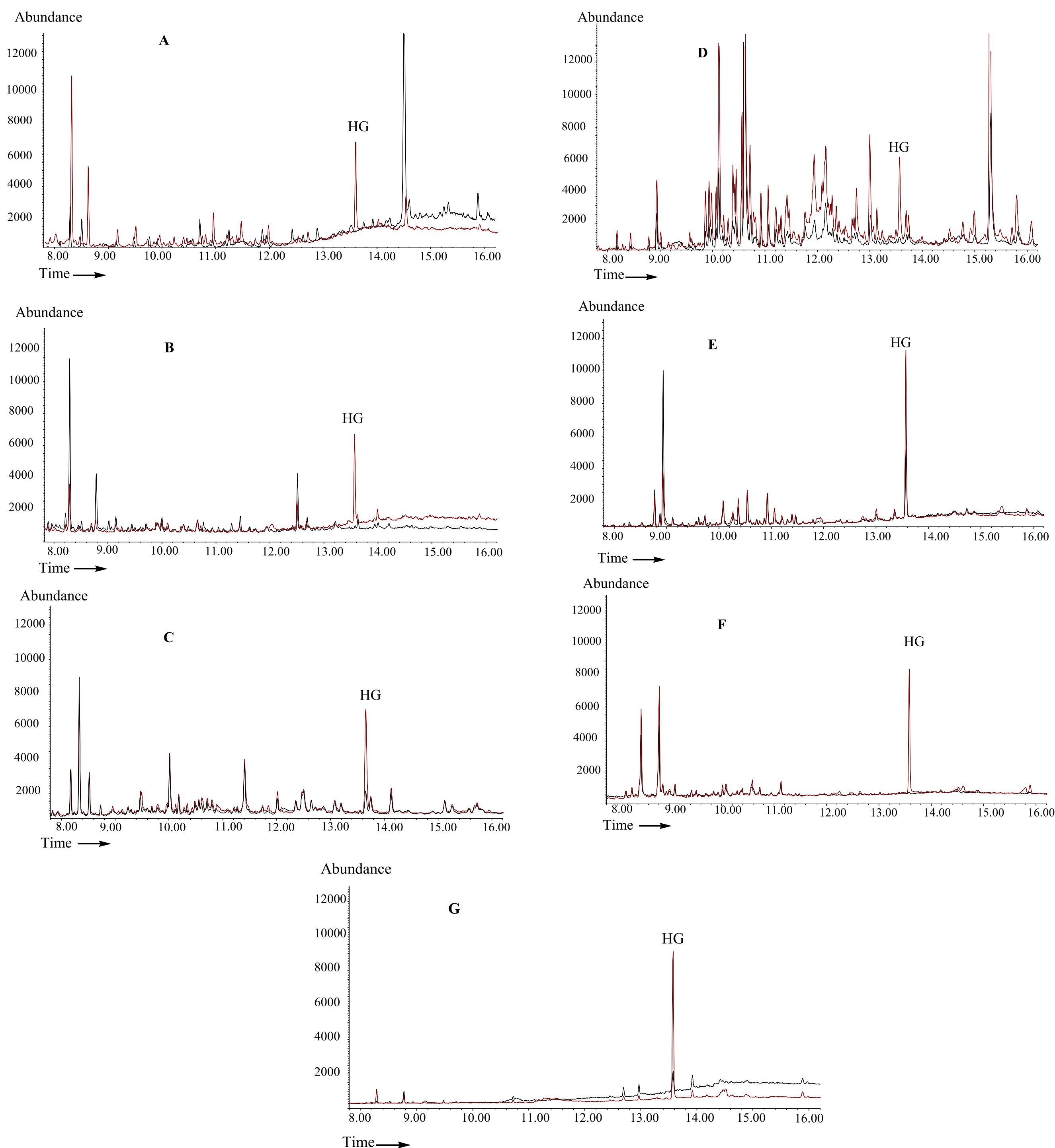

Fig. 2 – Chromatograms of different blank and real samples spiked with HG at the concentration of 25 ng mL^{-1}. A: plasma sample; B: urine sample; C: oral liquid sample; D: capsule sample; E: water bolus; F: honeyed bolus; G: Chinese herbal medicine.

Therefore, the effects of HFBA volume, derivation temperature, and time on derivation efficiency were evaluated. If the amount of the derivatizing reagent was too small, the contact area between the derivatizing reagent and HG would be too low and thus fail to complete the reaction. By optimizing the amount of the derivatizing reagent, the reaction was completed if the amount was set at 75 μL. To ensure complete and thorough derivatization, 100 μL of HFBA was ultimately selected for the subsequent derivatization reaction.

The effects of various derivatization temperatures (40, 50, 60, 70, 80, and 90 °C) on the derivatization reaction were evaluated. The peak area was maximum at 70 °C. With an increase in temperature, the peak area decreased owing to the decomposition of derivatives. Thus 70 °C was selected as the

derivatization temperature. The effects of different derivatization times (5, 15, 30, 45, and 60 min) on the derivatization reaction were assessed. The optimal derivatization effect was achieved under the following conditions: temperature of 70 °C and duration of for 45 min.

Direct entry of acid anhydride into the column could damage the column and lead to irreversible column loss. Thus, the derivative reagent was dried under a nitrogen flow after derivation and was re-dissolved in an organic solvent for analysis. However, different re-dissolving solvents also considerably influenced the results of the analysis. The derivatives exhibited poor stability if re-dissolved in EA and n-hexane. By contrast, the derivatives were stable if re-dissolved in CH_2Cl_2 and $CHCl_3$; moreover, the peak area of the derivatives was not significantly reduced after 12 h. Overall, 100 μL of CH_2Cl_2 was finally selected for redissolution.

3.5. *Method validation and actual sample analysis*

HG in the 5–200 ng mL^{-1} range showed a favorable linear relationship, and the linear equation is expressed as $y = 4764.3x-38051$ ($r = 0.9992$). The LOD of HG was 1.52 ng mL^{-1}, and LOQ was 5 ng mL^{-1}. As mentioned in the WADA Technical Document (TD2018MRPL) [30], the analytical finding of HG should not be reported at levels below 10 ng mL^{-1}. The results indicated that the method exhibited sufficient sensitivity for analysis of biological samples, Chinese patent medicines and Chinese herbal medicines.

The matrix effect (ME) was verified by comparison of the signal response of the pure HG standard dissolved in methanol with that of HG in the different matrixes. HG Standards were dissolved in methanol at three concentration levels (25, 100, and 160 ng mL^{-1}). The different samples were extracted as earlier described and then spiked with the HG standards at the same concentration levels. Unspiked samples were also investigated. The presence (or absence) of matrix effects were calculated.

The three concentration levels showed no particularly obvious enhancing or reducing effect on the matrixes (Table 2).

The HG concentration in various representative samples (plasma, urine, oral liquid, capsule, watered bolus, honeyed bolus, and Chinese herbal medicine) were determined. Matrix spiked recovery and precision experiments were conducted at low, medium, and high concentrations (25, 100 and 160 ng mL^{-1}). The result of intra-day and inter-day precision are listed in Table 2. HG was detected in the other samples at varying concentrations except in the honey pill. The contents were as follows: compound radix scutellariae gargle, 0.213 ng mL^{-1}; Lianhuaqingwen capsule, 19.6 ng mL^{-1}; Zhengtian bolus, 45.26 ng mL^{-1}; *L. plumule*, 647 ng mL^{-1}. The low, medium, and high recovery rates of the sample matrixes were between 82.70% and 109.80%; the intra-day precision ranged from 0.50% to 2.33%; and the inter-day precision ranged from 0.77% to 3.39% these results indicated that the method provided good accuracy and reproducibility. Fig. 2 shows the selective ion (*m/z*: 303.1, 330.1, 358.1, 386.1) detection chromatogram of HG in different sample matrixes. The peak retention time of the target was 13.58 min, and no evident interference peak appeared, suggesting that the method exhibited strong specificity and high selectivity, and was suitable for the qualitative and quantitative detection of HG in various sample matrixes.

The LOD and LOQ of the proposed method were significantly lower than those of HPLC [18] and CE [19]. The proposed method achieved a higher LOD than UHPLC-MS/MS [11,13,21]; however, HPLC, CE, and LC-MS had a single detection matrix, and no CE-MS detection of HG has been reported. GC–MS was established to detect HG in different matrixes such as plasma, urine, Chinese herbal medicines, and various formulations of Chinese patent medicines. Thus, GC–MS is economical widely used and easy to operate. GC–MS is the most appropriate technique under the conditions that the analyzing samples requirements are met.

4. Conclusion

A method of GC–MS detection for HG analysis in different matrixes was established by optimizing the extraction and derivation conditions. GC–MS, combined with HFBA derivation, was used to analyze and determine HG for the first time, and the extraction methods and detection effects of HG in different matrixes were systematically studied. Results showed that the GC–MS method was fast, sensitive, and reliable with high practical value for the administration of athletes' daily food and drug, and doping detection.

Declaration of Competing Interest

The authors have declared that there is no conflict of interest.

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