Detection of adulterated drugs in traditional Chinese medicine and dietary supplements using hydrogen as a carrier gas

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Abstract

Helium, a minor component of natural gas and radioactive minerals, is most commonly used as a carrier in gas chromatography-mass spectrometry (GC-MS). Its scarcity leads to limited availability and higher costs. In this experiment, hydrogen from a safe source of a hydrogen generator was tested as a substitutive carrier gas for the detection of adulterant in traditional Chinese medicine (TCM) and food supplements by GC-MS analysis. We found that the limits of detection (LODs) of using hydrogen were from 10 to 1000 μg/g. The levels of LODs tested among 170 drugs remain the same whether hydrogen or helium was used as a carrier gas with the exception of 7 drugs—benzbrorome, estradiol benzoate, bezafibrate, mefenamic acid, oxymetholone, piperidenafil and cetilistat. The real sample analysis results using hydrogen were as satisfactory as those using helium. In addition, the retention time was shortened after the chromatographic performance was optimized. In summary, it is worth considering hydrogen as a carrier gas due to its affordable costs, energy efficiency, carbon reduction and chromatographic advantages to detect adulterated drugs in TCM and dietary supplement using GC-MS.

Introduction

Currently, TCM and dietary supplements are becoming progressively popular in many countries [1]. The worldwide consumption of TCM and dietary supplements has accumulatively increased. Evidence showed that approximately 80% of the world’s population takes herbal medicines. In general, TCM is generally regarded as possessing few side effects, and users are therefore prone to taking it in excess of the recommended dose for an extended period of time. On the other hand, consumers tend to trust natural products, believing that they are safe and free of side effects. This false belief that TCM and food supplements are all natural with no side effects and that they are not harmful to human health has led to the exponential growth of TCM and food supplement remedies in markets worldwide [2–4].

To enhance their efficacy, TCM and food supplements were often illegally adulterated with western medicine. The quantities of adulterants in TCM and food supplements at times even exceeded the normal dosage range, and in many cases, they were not the ones required or...
responsible for the therapeutic effects advertised on the label [3, 5, 6]. Recently, various scientific and monitoring investigations revealed that undeclared synthetic drugs were found in herbal medicines and dietary supplements. The hidden drugs may cause serious toxic-effects to health. The adulterations of herbal medicine and food supplements are likely to contain indiscernible synthetic medicines, metals, or other toxic substances in high concentrations [7] and has become a problem all over the world. The “Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review, Journal of Internal Medicine” (E. Ernst, 252 (2002) 107–113) showed that 24% of their 2600 Chinese herbal medicines samples contained at least one synthetic medicine [4], and 7% of the 260 Asian patient medicines (one component of TCM) from retail herbal stores in California that were tested were shown to contain an undeclared pharmaceutical [8]. Other than dietary supplements and sexual enhancement remedies, many other traditional herbal products were reported to be adulterated with various types of hidden synthetic chemicals capable of pharmacological activities [2]. These chemicals include the following: steroids (strength enhancers), nonsteroidal anti-inflammatory drugs, PDE-5 inhibitors or their analogues (sexual performance enhancers), antihypertensive agents, sibutramine and its analogues (weight loss products)[9], and many other types of therapeutic synthetic agents [10]. Currently, illegal drug detection in Taiwan is also conducted in accordance with the “Method of Test for Adulterants in Chinese Medicine and Foods,” which is the official inspection protocol by the Taiwan Food and Drug Administration (TFDA) [11]. Therefore, the development of improved analytical methodologies for the detection of adulterants is critically important to protect public health so that the quality of TCM and food supplements can be better insured.

The detection processes of TCM ingredients are complicated and challenging. Several methods are used to determine undeclared adulterants in herbal medicine or food supplements, including the following: LC-PDA [12], GC-MS [13], GC-QQQMS [14], LC-MS [15], LC-MS/MS [12], UPLC-TOF/MS [16], Q-Orbitrap MS [17], NMR [18], X-ray powder diffractometry [19], TLC-image [20], TLC-SERS [21], ATR-IR [22], and CE-MS [23]. The applicability of GC-MS is determined by the volatility and thermal stability of analytes. A faster technique for GC-MS was discovered for the detection of sildenafil, tadalafil, and vardenafil in food and herbal products. A GC-MS method was also successfully used for the screening of 134 pharmaceuticals in patent medications in China. By searching the NIST Mass Spectral Library and comparing the retention times, one can instantly screen out the ingredients and quantify them at the same time [24].

As soon as an unknown substance is found, GC-MS is performed for further verification. The following three gases are commonly used as carriers in gas chromatography (GC): nitrogen (N₂), hydrogen (H₂), and helium (He). Most GC studies commonly use He as the carrier gas. Helium on planet earth is generally found in natural gases and radioactive decay and is a relatively rare-5.2 ppm by volume in the atmosphere [25]. In anticipation of a potential helium-shortage crisis in the future, the price of helium is becoming expensive and vagaries in supply are limited. Thus, we describe the use of hydrogen instead of helium as a carrier gas for the analysis of illegal, adulterated drugs in TCM and food supplements using gas chromatography-mass spectrometry with electron ionization. Illicit drugs are investigated with hydrogen and helium to manifest the utility of hydrogen in the detection of the adulterant.

**Materials and methods**

**Samples**

Samples were taken from drugstores and herbal medicine stores in China, as well as suppliers and manufacturers by the Health Department of the Tainan County Government in Taiwan,
between January 2015 and August 2016. The samples included 83 TCM samples and 40 food supplement samples (Please see S1 Table for sample details including sample name, description of appearance, purchase location and source origin).

**Chemicals and solutions**

One hundred seventy pharmaceutical standards (purity $\geq$95%) were purchased from USP, TLC, Sigma-Aldrich, Cerilliant, European Pharmacopoeia, TCI, AK Scientific, AAPin and Fluka (Please see S2 Table for standard details including compound name, molecular Weight, purity, brand, lot number, storage and source origin). Individual stock solutions (1,000 mg/L) were prepared by the dissolution of 10 mg of each compound in 10 mL of methanol, which were stored at -18˚C. The mixed standard solutions at concentrations of 100 mg/L of each standard were prepared by the additive mixing 1 mL of each stock solution, and diluting it to 10 mL with methanol, respectively. HPLC grade methanol and ethanol were obtained from Merck (Darmstadt, Germany).

**Sample preparation**

Five grams of sample were dissolved in 15–20 mL of ethanol and homogenized in an ultrasonic shaker for 30 minutes, followed by centrifugation at 3000 g for 5 minutes. The supernatant was filtered through a 0.22-$\mu$m PTFE syringe filter prior to being injected into GC-MS.

**GC-MS analysis**

The analysis was performed on an Agilent 7890B GC system coupled to a 5977A MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) and equipped with a Gerstel Multipurpose sampler (Gerstel, Mülheim an der Ruhr, Germany). For the experiments using helium and hydrogen as a buffer gas, a Peak Precision Hydrogen Trace 500cc generator (Peak Scientific Instruments, Inchinnan, Scotland, UK) was used to generate the hydrogen gas. A silica capillary column, Agilent HP-5MS (30 m x 0.25 mm i.d. 0.25 $\mu$m film thickness), was used. The operation conditions were described in Table 1. The compounds’ spectra that were

| Parameter                      | Setting                        |
|-------------------------------|--------------------------------|
| Injector                      |                                |
| Carrier gas                   | Helium                         |
| Flow                          | 1.4 mL/min                     |
| Pressure                      | 9.4 psi                        |
| Injection mode                | Splitless mode                 |
| Injection volume              | 1 $\mu$L                       |
| Injector temperature          | 250˚C                          |
| Oven temperature program      |                                |
| Initial ramp                  | 80˚C at 6˚C/min until reaching 120˚C |
| Final ramp                    | At 8˚C/min until reaching 300˚C for 29 min. |
| Mass spectrometer             |                                |
| Ionization mode               | Electron ionization            |
| Acquisition mode              | Selected Ion Monitoring (SIM)   |
| Dwell time                    | 100 ms                         |
| Source temperature            | 230˚C                          |
| Quadrupole temperature        | 150˚C                          |

Table 1. Gas chromatograph-mass spectrometer settings.

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obtained were compared to the spectra of known compounds using the NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library.

The limit of detection (LOD)

The experiment uses helium as a carrier gas to determine the monitored ion and retention time at 10 μg/mL. If the signal is too low to be detected, we increase the concentration of the drug standard gradually to 100, 500 and 1000 μg/mL until it can be obtained. The same experiment was performed for the comparison using hydrogen as the carrier gas for GC-MS.

Five grams of blank sample powder (Xiao Chai Hu Tong Extract Powder, Sheng Chang, Taipei, Taiwan) was spiked with the abovementioned concentration of drug standard (see 2.5.1) and dissolved in 15–20 mL of ethanol. A sample tube was homogenized in the ultrasonic shaker for 30 minutes and was followed by centrifugation at 3000 g for 5 minutes. The supernatant was filtered through a 0.22-μm PTFE syringe filter prior to being injected into the GC-MS. For the calculation of the method’s LODs, the fortification of three blank samples was performed in a specific concentration of drug standard. The concentration of the standard solution of which the ratio of peak height to noise was over 3 was defined as the LOD.

Proficiency testing TFDA

We have conducted the Proficiency testing using the TFDA standards for the detection of drug-adulterants in traditional Chinese medicine in 2014 and 2015 through the use of hydrogen as a carrier gas by GC–MS. Proficiency testing is another effective tool that can be used to ensure the accuracy and precision of the results when using hydrogen as a carrier gas for the detection of adulterated drugs in Chinese herbal medicine and dietary supplements by GC–MS.

Results

GC-MS analysis

Each drug standard was analyzed with GC using a split-less injection to provide the MS identification of each compound and establish their retention time, LOD and identification ion in both helium and hydrogen as carriers in gas. All the results were obtained using the conditions described in Table 1. According to these results shown in Table 2, the use of hydrogen shortened the analysis time and saved resources. The optimum linear velocity for hydrogen is approximately 40 cm/s, which is about half of that of helium, which leads to a decrease in the analysis time by a factor of four, therefore making it possible to reduce the costs of analysis (less degradation of the capillary columns means a cheaper carrier gas). It is clear that the change from helium to hydrogen as the carrier reduced the run-times in drug analytes. Therefore, according to these results, the use of hydrogen allows for the acceleration of the analysis. The previous study revealed that it should be possible to reduce the length of the capillary column in order to save time and money without a loss of resolution in comparison to a longer column with helium as the carrier [26].

Fig 1 presents the chromatograms of the chlorzoxazone obtained when hydrogen (A) and helium (B) were used as the carrier gas at the retention times of 16.681 and 17.724 min. It can be observed that the use of hydrogen allowed for a reduction in the run-time analysis of 1.043 min and led to a slight peak tailing. However, the same mass spectrum of chlorzoxazone was found by GC-MS when hydrogen (A) and helium (B) were used as the carrier gas in Fig 2. Fig 3 shows a comparison of the chromatograms of sildenafil with hydrogen (A) and helium (B) at the retention times of 45.447 and 49.500 min. An elimination of 4.053 min in the run-time
Table 2. Comparison retention time, signal-to-noise ratio (S/N) and monitored ion of drugs when hydrogen or helium is used as the carrier gas.

| Drug               | Carrier gas | Retention time (min) | S/N   | Monitored ion (m/z) |
|--------------------|-------------|----------------------|-------|---------------------|
| Acetaminophen      | H₂          | 16.227               | 562.29| 151, 109, 80, 83°   |
|                    | He          | 17.045               | 415.15| 109, 151, 43°, 80   |
| Acetildenafil      | H₂          | 41.707               | 529.77| 127, 70, 84, 42, 112, 56, 98 |
|                    | He          | 44.943               | 159.15| 127, 70, 84, 42, 112, 56, 98 |
| Acetohexamide      | H₂          | 19.500               | 177.45| 199, 184, 120°, 104, 91, 76, 64°, 51° |
|                    | He          | 20.254               | 238.09| 184, 199, 76, 121°, 43°, 139°, 91, 104 |
| Allopurinol        | H₂          | 22.872               | 797.93| 136, 52, 109, 120   |
|                    | He          | 20.159               | 18.05 | 136, 52, 29°, 109, 67, 120 |
| Aminopyrine        | H₂          | 19.323               | 2492.34| 231, 97, 77, 56     |
|                    | He          | 20.163               | 3188.28| 231, 97, 77, 56     |
| Aminotadalafil     | H₂          | 46.301               | 131.81| 390, 262, 204, 289, 233, 102, 169, 375, 43°, 405° |
|                    | He          | 49.941               | 133.16| 390, 204, 262, 289, 169, 233, 115°, 169, 102, 375 |
| Amitriptyline      | H₂          | 22.689               | 71.28 | 277, 202, 178, 152, 91, 65, 120 |
|                    | He          | 23.578               | 144.76| 277, 58, 202, 178, 115, 91, 152, 42, 112, 56, 98 |
| Amphetamine        | H₂          | 6.278                | 84.52 | 44, 91, 65, 120     |
|                    | He          | 6.103                | 575.81| 44, 91, 65, 120     |
| Atenolol           | H₂          | 25.113               | 12505.57| 222, 107, 72        |
|                    | He          | 25.693               | 3581.62| 72, 107, 222        |
| Atropine           | H₂          | 22.698               | 1471.07| 124, 82, 94, 289, 140, 67, 103, 42 |
|                    | He          | 23.598               | 1486.90| 124, 82, 289, 140, 67, 42, 103, 91, 115, 72 |
| Barbital           | H₂          | 13.361               | 204.84| 156, 141, 98, 112, 55, 41, 83, 69 |
|                    | He          | 14.160               | 776.86| 156, 141, 98, 55, 112, 41, 83, 69 |
| Benzbromarone      | H₂          | 28.290               | 204.84| 264, 173, 279, 115, 249, 328, 145, 132°, 221 |
|                    | He          | 29.169               | 6448.86| 264, 173, 115, 279, 249, 328, 145, 63°, 221 |
| Benzocaine         | H₂          | 14.195               | 302.25| 165, 120, 92, 65    |
|                    | He          | 15.094               | 381.62| 120, 165, 92, 65    |
| Betamethasone      | H₂          | 29.912               | 273.45| 312, 281°, 207, 160, 122, 91, 55 |
|                    | He          | 30.794               | 381.89| 122, 312, 91, 160, 207, 41, 55, 77° |
| Bezafibrate        | H₂          | 26.950               | 6036.33| 120, 139, 107, 77°, 156 |
|                    | He          | 27.690               | 20.97 | 120, 139, 205°, 107, 156 |
| Bisacodyl          | H₂          | 29.089               | 23138.37| 361, 319, 276, 246°, 199, 154 |
|                    | He          | 29.996               | 18519.59| 361, 276, 277°, 199, 319, 43°, 318°, 183°, 278°, 154 |
| Bromhexine         | H₂          | 24.672               | 240565.29| 376, 293, 264, 112, 70, 374° |
|                    | He          | 25.592               | 1374605.79| 376, 293, 264, 305°, 112, 70 |
| Brompheniramine    | H₂          | 21.654               | 1070326.01| 247, 58, 167, 72, 180, 42, 139 |
|                    | He          | 22.569               | 572.47 | 247, 58, 167, 72, 180, 42, 139 |
| Bromvalerylurea    | H₂          | 12.902               | 18.40 | 137, 44, 100, 55, 83, 69, 120° |
|                    | He          | 13.809               | 43670.57| 137, 44, 183°, 100, 55, 69 |
| Bucetin            | H₂          | 20.613               | 1837.10| 223, 137, 108, 81, 53° |
|                    | He          | 21.466               | 1406.13| 137, 108, 223, 45°, 81 |
| Caffeine           | H₂          | 18.055               | 165.90| 194, 109, 67        |
|                    | He          | 18.878               | 283.12| 194, 109, 67, 55°, 82° |
| Carbetapentane     | H₂          | 23.311               | 1995.44| 86, 144, 115, 100, 58 |
|                    | He          | 24.158               | 4056.80| 86, 144, 115, 100, 58 |

(Continued)
| Drug             | Carrier gas | Retention time (min) | S/N        | Monitored ion (m/z) |
|------------------|-------------|----------------------|------------|---------------------|
| Carbimazole      | H₂          | 15.606               | 417.25     | 186, 114, 72, 81, 42, 56, 127, 141 |
|                  | He          | 16.519               | 1395.15    | 186, 114, 72, 81, 141, 42, 56, 127 |
| Carboxamine      | H₂          | 21.362               | 124.32     | 201, 167, 139°, 71 |
|                  | He          | 22.248               | 181.70     | 201, 167, 58°, 71  |
| Carbodenafil     | H₂          | 43.793               | 249.58     | 84, 56, 70, 381, 452, 339, 311, 42, 113, 136° |
|                  | He          | 47.202               | 96.80      | 84, 97°, 56, 70, 381, 452, 339, 311, 42, 113 |
| Carisoprodol     | H₂          | 18.770               | 36.02      | 245, 184, 158, 97, 83°, 69°, 55 |
|                  | He          | 19.689               | 20.54      | 245, 158, 97, 184, 55, 58°, 43° |
| Chloramphenicol  | H₂          | 27.569               | 2199.59    | 207, 172, 153, 106, 77 |
|                  | He          | 28.303               | 1989.30    | 207, 153, 172, 77, 106 |
| Chlordiazepoxide | H₂          | 29.238               | 124.32     | 201, 167, 139°, 71 |
|                  | He          | 30.310               | 246.65     | 282, 241°, 247, 220, 165, 90, 91 |
| Chlorpromazine   | H₂          | 23.419               | 3689.22    | 208, 174, 152, 98, 70, 381, 452, 339, 311, 42, 113 |
|                  | He          | 24.275               | 364.87     | 152, 208, 174, 98, 125, 69 |
| Chlorpheniramine | H₂          | 20.445               | 377.13     | 203, 58, 167, 72, 180, 42 |
|                  | He          | 21.356               | 660.96     | 203, 58, 167, 72, 180, 42 |
| Chlorpromazine   | H₂          | 25.943               | 393651.65  | 318, 272, 232, 196, 86, 58 |
|                  | He          | 26.863               | 1034722.73 | 318, 58, 86, 272, 232, 196 |
| Chlorpropamide   | H₂          | 16.426               | 413.58     | 190, 174, 127, 111, 75 |
|                  | He          | 17.211               | 640.13     | 190, 111, 174, 127, 75 |
| Chloroxazone     | H₂          | 16.681               | 1126.98    | 169, 113, 78 |
|                  | He          | 17.724               | 428.48     | 169, 113, 78 |
| Cimetidine       | H₂          | 5.722                | 101.59     | 45, 116, 55, 70, 60, 74, 42, 88° |
|                  | He          | 6.651                | 305.40     | 116, 45, 55, 60, 74, 42, 98° |
| Cinnarizine      | H₂          | 31.221               | 393651.65  | 318, 272, 232, 196, 86, 58 |
|                  | He          | 32.229               | 1034722.73 | 318, 58, 86, 272, 232, 196 |
| Clobenzorex      | H₂          | 19.892               | 568.16     | 168, 127, 91, 65 |
|                  | He          | 20.798               | 829.80     | 168, 127, 91, 65 |
| Clofibrate       | H₂          | 13.722               | 657.00     | 242, 169, 128 |
|                  | He          | 14.588               | 1238.64    | 128, 242, 169 |
| Cocaine          | H₂          | 22.792               | 28.65      | 182, 82, 303, 105, 272, 198, 122, 51 |
|                  | He          | 23.671               | 1156.47    | 182, 82, 303, 105, 272, 198, 122, 51 |
| Colchicine       | H₂          | 33.875               | 133.19     | 399, 371, 312, 281, 254° |
|                  | He          | 35.326               | 126.04     | 312, 399, 371, 297°, 281 |
| Cortisone        | H₂          | 27.709               | 131.20     | 122, 300, 91, 256, 105, 77, 147, 161, 55 |
|                  | He          | 28.650               | 328.98     | 122, 300, 91, 256, 161, 147, 105, 55, 77 |
| 7-keto-DHEA      | H₂          | 28.240               | 616.13     | 302, 161, 91, 79, 105, 134, 187, 55, 41, 205 |
|                  | He          | 29.196               | 3160.69    | 302, 161, 91, 79, 105, 134, 187, 55, 41, 205 |
| N-Desmethylsibutramine | H₂     | 18.139               | 8921.42    | 100, 58, 44, 137, 128, 115 |
|                  | He          | 18.993               | 57424.38   | 100, 58, 44, 137, 128, 115 |
| N-Didesmethylsibutramine | H₂   | 18.111               | 4950.37    | 137, 115, 86 |
|                  | He          | 18.989               | 2657.67    | 86, 137, 115 |
| Dexamethasone    | H₂          | 29.940               | 336.90     | 312, 160, 122, 91, 55 |
|                  | He          | 30.655               | 478.25     | 122, 312, 160, 91, 55 |
| Dextromethorphan | H₂          | 22.049               | 684403.48  | 271, 150, 214, 59, 171, 203, 128 |
|                  | He          | 23.199               | 601.70     | 271, 59, 150, 214, 171, 203, 128 |

(Continued)
### Table 2. (Continued)

| Drug              | Carrier gas | Retention time (min) | S/N | Monitored ion (m/z) |
|-------------------|-------------|----------------------|-----|---------------------|
| Diazepam          | H<sub>2</sub> | 25.356               | 191.73 | 283, 256, 221, 165, 77, 51 |
|                   | He          | 26.238               | 790.25 | 283, 256, 221, 165, 77, 51 |
| Dibucaine         | H<sub>2</sub> | 27.963               | 325.46 | 116, 86, 58 |
|                   | He          | 28.855               | 689.64 | 116, 86, 58 |
| Diclofenac        | H<sub>2</sub> | 21.958               | 530.12 | 295, 242, 214, 179, 151 |
|                   | He          | 22.816               | 979.97 | 214, 295, 242, 179, 151 |
| Dicyclomine       | H<sub>2</sub> | 21.505               | 853.46 | 86, 55 |
|                   | He          | 22.375               | 5338.38 | 86, 99, 55 |
| Diphenylpropion   | H<sub>2</sub> | 12.933               | 254.89 | 100, 77, 51 |
|                   | He          | 13.797               | 995.63 | 100, 77, 51 |
| Diethylstilbestrol| H<sub>2</sub> | 23.917               | 2535.31 | 268, 239, 145, 107 |
|                   | He          | 24.749               | 681.06 | 268, 107, 239, 145 |
| Dimethylsildenafil| H<sub>2</sub> | 24.230               | 165.79 | 254, 232, 180, 137, 109, 81, 54 |
|                   | He          | 25.116               | 677.65 | 223, 254, 180, 109, 137, 81, 54 |
| Diphenhydramine   | H<sub>2</sub> | 18.661               | 728.26 | 165, 58 |
|                   | He          | 19.511               | 1728.75 | 165, 58 |
| Diphenylhydantoin | H<sub>2</sub> | 24.361               | 52.21  | 180, 223, 209, 252, 104, 77, 165, 147, 51 |
|                   | He          | 25.460               | 47.80  | 180, 104, 223, 209, 252, 77, 165, 51, 147 |
| Diprophylline     | H<sub>2</sub> | 24.230               | 165.79 | 254, 232, 180, 137, 109, 81, 54 |
|                   | He          | 25.116               | 677.65 | 223, 254, 180, 109, 137, 81, 54 |
| Econazole         | H<sub>2</sub> | 29.123               | 312.59 | 299, 207, 125, 81, 54 |
|                   | He          | 30.013               | 73.59  | 125, 81, 299, 207, 54 |
| Estradiol benzoate| H<sub>2</sub> | 35.659               | 4236.99 | 376, 105, 77 |
| Estriol           | H<sub>2</sub> | 37.513               | 1810.32 | 376, 105, 77 |
| Estrone           | H<sub>2</sub> | 26.785               | 108.53 | 270, 146, 185, 213 |
| Ethinylestradiol  | H<sub>2</sub> | 27.594               | 109.99 | 213, 296, 160, 133, 145, 228, 172, 185, 115 |
| Ethisterone       | H<sub>2</sub> | 27.721               | 21.79  | 124, 312, 91, 229, 245, 79, 107, 148, 189, 67 |
| Ethoxybenzamid    | H<sub>2</sub> | 14.651               | 1578.34 | 165, 150, 120, 92, 65 |
| Ethylestrenol     | H<sub>2</sub> | 24.277               | 3402.39 | 216, 241, 201, 288, 91, 270, 79, 121, 147, 105 |
| Fenfluramine      | H<sub>2</sub> | 7.506                | 4201.59 | 159, 109, 72, 56 |
| Finasteride       | H<sub>2</sub> | 33.484               | 208.98  | 372, 110, 58, 272, 357, 258, 128, 230, 72, 245 |
| Flavoxate         | H<sub>2</sub> | 32.588               | 218.77  | 263, 234, 147, 98 |
| Fluoxymesterone   | H<sub>2</sub> | 18.599               | 4320.42 | 309, 183, 162, 133, 104, 78, 105, 59 |
|                   | He          | 19.489               | 10859.57 | 309, 183, 162, 133, 44, 104, 59 |
| (Continued)       | He          | 30.742               | 145899.76 | 336, 279, 71, 109 |
| Drug                  | Carrier gas | Retention time (min) | S/N          | Monitored ion (m/z) |
|----------------------|-------------|----------------------|--------------|---------------------|
| Gemfibrozil          | H₂          | 19.329               | 1655.20      | 250, 122            |
|                      | He          | 20.183               | 1357.76      | 250, 122            |
| Gendenafil           | H₂          | 31.414               | 520.43       | 354, 326, 339, 136, 166, 282, 43, 311, 297 |
|                      | He          | 31.414               | 514.07       | 354, 326, 339, 136, 166, 282, 43, 311, 297 |
| Griseofulvin         | H₂          | 28.131               | 1108.72      | 352, 310, 284, 254, 214, 171, 138, 95, 69 |
|                      | He          | 28.949               | 3255.28      | 352, 310, 138, 214, 284, 254, 69, 171, 95 |
| Guaifenesin          | H₂          | 28.949               | 1687.05      | 124, 109, 198, 77, 95, 65, 52, 167, 149 |
|                      | He          | 16.375               | 3642.70      | 124, 109, 198, 77, 81, 95, 65, 52, 167 |
| Homatropine          | H₂          | 21.349               | 142.55       | 275, 124, 79        |
|                      | He          | 22.276               | 187.65       | 305°, 163, 123, 91, 55 |
| Homosildenafil       | H₂          | 48.785               | 451.37       | 113, 70, 281°, 56, 42, 207, 355, 341, 309, 253 |
|                      | He          | 53.164               | 463.73       | 113, 404°, 70, 56, 42, 207, 355, 341, 309, 253 |
| Hydralazine          | H₂          | 19.201               | 343.84       | 160, 103, 131, 115, 89, 76, 145, 63, 50 |
|                      | He          | 18.044               | 408.02       | 160, 103, 131, 115, 89, 76, 145, 63, 50 |
| Hydrocortisone       | H₂          | 29.064               | 1090.68      | 206, 161, 117, 91, 65 |
|                      | He          | 29.931               | 674.69       | 161, 206, 117, 91, 65 |
| Ibuprofen            | H₂          | 15.040               | 187.65       | 305°, 163, 123, 91, 55 |
|                      | He          | 15.817               | 3642.70      | 124, 109, 198, 77, 81, 95, 65, 52, 167 |
| Imidazosagatrizinone | H₂          | 27.187               | 9066.54      | 312, 284, 136, 240 |
|                      | He          | 27.969               | 4361.89      | 312, 284, 136, 240 |
| Indomethacin         | H₂          | 30.571               | 1204.53      | 139, 313°, 111, 75 |
|                      | He          | 27.765               | 24.57        | 139, 357°, 111, 75 |
| Ketoprofen           | H₂          | 22.913               | 54.02        | 105, 177, 209, 77, 254, 45°, 194, 131, 165 |
|                      | He          | 23.701               | 674.69       | 161, 206, 117, 91, 65 |
| Lidocaine            | H₂          | 18.667               | 745.66       | 234, 120, 86, 58    |
|                      | He          | 19.537               | 2480.92      | 86, 234, 120, 58    |
| Lorazepam            | H₂          | 25.073               | 80.39        | 239, 274, 302, 75, 138, 177, 111, 203, 163, 100 |
|                      | He          | 25.916               | 448.96       | 239, 274, 302, 75, 138, 177, 111, 203, 163, 100 |
| Mazindol             | H₂          | 24.604               | 3833.98      | 266, 231, 204, 176, 128, 102, 75 |
|                      | He          | 25.835               | 382.97       | 266, 231, 204, 176, 128, 102, 75 |
| Mefenamic acid       | H₂          | 22.179               | 2403.18      | 241, 223, 180, 152, 102° |
|                      | He          | 22.732               | 186.25       | 223, 241, 180, 77°, 152 |
| Melatonin            | H₂          | 24.765               | 855.84       | 232, 172, 160, 145, 130, 117, 102, 89 |
|                      | He          | 25.586               | 834.77       | 160, 172, 232, 145, 117, 130, 102, 89 |
| Mephenesin           | H₂          | 14.300               | 44.99        | 182, 108, 91        |
|                      | He          | 15.027               | 2776.31      | 108, 182, 91        |
| Mephentermine        | H₂          | 9.224                | 3957.33      | 72, 91, 148, 56, 42, 115 |
|                      | He          | 8.883                | 1232.24      | 72, 91, 148, 56, 42, 115 |
| Meprobamate          | H₂          | 17.741               | 904.61       | 83, 55, 43, 71, 62, 96, 114, 144, 101 |
|                      | He          | 18.516               | 2359.60      | 83, 55, 71, 62, 96, 114, 144, 101, 43 |
| Methamphetamine      | H₂          | 6.505                | 2216.40      | 58, 91, 65, 134, 42, 115, 119° |
|                      | He          | 7.195                | 3184.90      | 58, 91, 65, 56°, 134, 42, 115 |
| Methandriol          | H₂          | 26.350               | 74.00        | 253, 213, 271, 304, 105, 145, 286, 228, 119, 159 |
|                      | He          | 27.230               | 305.46       | 253, 213, 304, 105, 145, 271, 286, 228, 119, 159 |
| Methandrostenolone   | H₂          | 27.749               | 76.18        | 122, 91, 161, 147, 105, 134, 77 |
|                      | He          | 28.686               | 360.69       | 122, 91, 161, 147, 105, 134, 77 |

(Continued)
Table 2. (Continued)

| Drug               | Carrier gas | Retention time (min) | S/N                  | Monitored ion (m/z)       |
|--------------------|-------------|----------------------|----------------------|---------------------------|
| Methaqualone       | H<sub>2</sub> | 22.282               | 1208.48              | 250, 91, 132, 65, 77, 217, 117, 50, 104<sup>a</sup> |
|                    | He          | 23.192               | 3143.27              | 235<sup>2</sup>, 250, 91, 132, 65, 77, 217, 117, 50 |
| Metharbital        | H<sub>2</sub> | 12.212               | 2739.29              | 155, 112, 83, 55          |
|                    | He          | 12.953               | 2922.10              | 155, 170<sup>1</sup>, 112, 83, 55 |
| Methimazole        | H<sub>2</sub> | 13.837               | 140.23               | 114, 72, 81, 42, 54, 86, 59 |
|                    | He          | 14.903               | 213.63               | 114, 72, 42, 81, 54, 86, 59 |
| Methylprednisolone | H<sub>2</sub> | 29.813               | 457.10               | 136, 91, 55<sup>1</sup>   |
|                    | He          | 30.677               | 1796.27              | 136, 91, 121<sup>1</sup> |
| Methyltestosterone | H<sub>2</sub> | 27.435               | 26731.78             | 302, 229, 202, 161, 124, 91 |
|                    | He          | 28.384               | 1780.50              | 302, 124, 91, 229, 202, 161 |
| Metoclopramide     | H<sub>2</sub> | 27.217               | 5295.57              | 184, 86, 58               |
|                    | He          | 28.034               | 8438.34              | 86, 184, 58               |
| Metronidazole      | H<sub>2</sub> | 15.394               | 443.85               | 171, 124, 81, 53          |
|                    | He          | 16.174               | 423.83               | 124, 81, 171, 53          |
| Minoxidil          | H<sub>2</sub> | 20.855               | 91.48                | 193, 164, 138, 110, 84, 67 |
|                    | He          | 21.700               | 1697.28              | 193, 164, 110, 138, 84, 67 |
| Morphine           | H<sub>2</sub> | 25.421               | 592.33               | 285, 162, 42, 215, 115, 55, 65, 92, 81 |
|                    | He          | 26.311               | 2101.32              | 285, 162, 42, 215, 115, 55, 65, 92, 81 |
| Nalidixic acid     | H<sub>2</sub> | 25.020               | 2964.06              | 188, 160, 132, 173, 145, 104, 232, 77 |
|                    | He          | 25.602               | 4290.93              | 188, 160, 132, 173, 145, 104, 232, 77 |
| Nandrolone         | H<sub>2</sub> | 25.593               | 112.00               | 274, 215, 173<sup>2</sup>, 147, 119, 91, 67 |
|                    | He          | 27.554               | 413.47               | 274, 110<sup>1</sup>, 91, 67, 119, 215, 147 |
| Naproxen           | H<sub>2</sub> | 21.384               | 479.13               | 230, 185, 170, 141, 115   |
|                    | He          | 31.254               | 21.76                | 185, 230, 170, 141, 115   |
| Nifedipine         | H<sub>2</sub> | 26.652               | 2198.09              | 329, 284, 224, 268, 254, 195, 180 |
|                    | He          | 27.496               | 8691.50              | 329, 284, 224, 268, 254, 195, 180 |
| Noracetildenafil    | H<sub>2</sub> | 39.756               | 143.98               | 113, 70, 42, 56, 98, 207, 311, 452, 136, 354 |
|                    | He          | 42.433               | 144.95               | 113, 70, 42, 56, 98, 207, 452, 311, 136, 354 |
| Norethisterone     | H<sub>2</sub> | 27.236               | 237.22               | 298, 283<sup>3</sup>, 265 |
|                    | He          | 28.102               | 855.06               | 298, 231<sup>1</sup>, 265 |
| Orphenadrine       | H<sub>2</sub> | 19.556               | 345.15               | 58, 73, 165, 178, 45      |
|                    | He          | 20.419               | 1799.00              | 58, 73, 165, 178, 45      |
| Oxethazaine        | H<sub>2</sub> | 26.450               | 198.46               | 114, 86, 213, 56, 133, 72, 304 |
|                    | He          | 27.281               | 289.85               | 114, 86, 56, 213, 133, 72, 304 |
| Oxymetholone       | H<sub>2</sub> | 28.615               | 8872.16              | 174, 275, 332, 43, 161, 91, 81, 71, 216, 107 |
|                    | He          | 29.471               | 16927.40             | 174, 332, 275, 216, 107, 43, 91, 81, 71 |
| Oxyphebutazone     | H<sub>2</sub> | 30.658               | 324.39               | 93, 45<sup>2</sup>, 55, 69, 161, 193<sup>3</sup>, 77, 249<sup>4</sup> |
|                    | He          | 31.419               | 312.73               | 93, 199<sup>1</sup>, 77, 324<sup>2</sup>, 55, 69, 161 |
| Pentazocine        | H<sub>2</sub> | 23.647               | 451.43               | 217, 202, 285, 110, 270, 70, 45, 159, 173 |
|                    | He          | 24.505               | 1600.48              | 217, 202, 110, 285, 270, 70, 45, 159, 173 |
| Phenacetin         | H<sub>2</sub> | 16.184               | 2534.87              | 179, 137, 108, 80, 65, 53 |
|                    | He          | 17.017               | 1248.32              | 108, 179, 137, 80, 65, 53 |
| Phenazopyridine    | H<sub>2</sub> | 23.833               | 1046.17              | 213, 108, 81, 54, 136, 97<sup>5</sup>, 184, 66, 155 |
|                    | He          | 24.670               | 660.83               | 213, 108, 81, 136, 54, 184, 66, 51<sup>6</sup>, 155 |
| Phenformin         | H<sub>2</sub> | 14.011               | 274.32               | 146, 104, 91, 77, 65     |
|                    | He          | 14.844               | 412.63               | 91, 146, 104, 77, 65     |

(Continued)
### Table 2. Using hydrogen as carrier gas to detect adulterated drugs

| Drug              | Carrier gas | Retention time (min) | S/N          | Monitored ion (m/z) |
|-------------------|-------------|----------------------|--------------|---------------------|
| Phenobarbital     | H₂          | 20.141               | 3530.71      | 204, 117, 232, 161, 146, 103, 77, 91, 174 |
|                   | He          | 20.996               | 2851.29      | 204, 117, 232, 161, 146, 103, 91, 174 |
| Phenolphthalein   | H₂          | 31.728               | 255.45       | 318, 274, 225, 181, 152, 104*, 65 |
|                   | He          | 32.746               | 765.90       | 274, 318, 225, 181, 152, 121*, 65 |
| Phentermine       | H₂          | 6.160                | 2271.97      | 70, 91, 105, 58, 65, 115, 41, 115 |
|                   | He          | 6.797                | 3375.10      | 70, 91, 105, 58, 65, 115, 41, 115 |
| Phentolamine      | H₂          | 26.987               | 42.57        | 199, 183, 91, 154, 77, 128, 170 |
|                   | He          | 27.784               | 117.12       | 199, 183, 91, 154, 77, 128, 170 |
| Phenylbutazone    | H₂          | 24.709               | 3014.56      | 308, 252, 183, 152, 107, 77, 51, 91 |
|                   | He          | 25.543               | 5464.75      | 183, 308, 252, 172, 152, 107, 77, 51, 91 |
| Phenylpropylnitrite | H₂     | 9.731                | 236.24       | 44, 77, 105, 51, 117, 91 |
|                   | He          | 10.797               | 1849.14      | 44, 77, 51, 105, 117, 91 |
| Piperidinaplatin  | H₂          | 44.617               | 128.79       | 431, 459, 283, 67, 42, 84, 121, 149, 215 |
|                   | He          | 48.328               | 21.36        | 431, 459, 283, 67, 42, 84, 121, 149, 215 |
| Pirenzepine       | H₂          | 31.102               | 253.75       | 351, 281, 211, 113, 70 |
|                   | He          | 32.009               | 358.14       | 113, 70, 211, 351, 215 |
| Piroxicam         | H₂          | 16.877               | 1640.49      | 104, 76, 43, 152, 69, 118, 386*, 211, 91 |
|                   | He          | 17.665               | 652.90       | 104, 152, 76, 43, 118, 211, 91 |
| Prednisolone      | H₂          | 29.312               | 68.40        | 122, 91, 55 |
|                   | He          | 30.225               | 1015.08      | 122, 91, 55 |
| Prednisone        | H₂          | 27.889               | 417.77       | 298, 245, 226, 186, 160, 131, 91, 115 |
|                   | He          | 28.785               | 534.87       | 298, 160, 91, 245, 226, 186, 131, 115 |
| Primidone         | H₂          | 23.472               | 1344.44      | 146, 190, 117, 161, 103, 91, 77, 174 |
|                   | He          | 24.067               | 1431.77      | 190, 146, 117, 161, 103, 91, 77, 174 |
| Probenecid        | H₂          | 22.941               | 295.85       | 270, 135, 199, 104, 76, 43 |
|                   | He          | 23.788               | 142.22       | 270, 135, 199, 104, 76, 43 |
| Procaine          | H₂          | 20.737               | 341.47       | 86, 99, 120, 65, 56, 164 |
|                   | He          | 21.644               | 523.45       | 86, 99, 120, 64, 65, 56 |
| Progesterone      | H₂          | 28.663               | 54.72        | 314, 272, 229, 147, 124, 91, 67 |
|                   | He          | 29.599               | 358.43       | 124, 314, 272, 229, 91, 67, 147 |
| Propantheline     | H₂          | 24.358               | 735.98       | 86, 181, 310, 99, 152, 44, 58, 325, 127, 71 |
|                   | He          | 25.183               | 3169.32      | 86, 181, 44, 99, 310, 152, 58, 325, 127, 71 |
| Propranolol       | H₂          | 22.279               | 745.35       | 259, 215, 144, 115, 72 |
|                   | He          | 23.188               | 3138.57      | 72, 115, 144, 259, 215 |
| Quinine           | H₂          | 28.967               | 2199.60      | 189, 180, 136 |
|                   | He          | 29.839               | 4577.02      | 136, 189, 160 |
| Ranitidine I      | H₂          | 21.701               | 272.14       | 235, 137, 94, 67 |
|                   | He          | 22.514               | 497.94       | 137, 235, 94, 67 |
| Ranitidine II     | H₂          | 28.971               | 279.25       | 84, 363, 55, 99, 282, 335, 299, 41, 380, 145, 146* |
|                   | He          | 29.960               | 1175.13      | 84, 55, 99, 363, 282, 335, 299, 41, 380, 145, 146* |
| Rimonabant        | H₂          | 35.675               | 601.19       | 84, 363, 55, 99, 282, 335, 299, 41, 380, 145, 146* |
|                   | He          | 37.392               | 1808.45      | 84, 55, 99, 363, 282, 335, 299, 41, 380, 145, 146* |
| Salicylamide      | H₂          | 12.103               | 1129.40      | 120, 137, 92, 65, 53, 44, 80 |
|                   | He          | 12.979               | 457.91       | 120, 137, 92, 65, 53, 44, 80 |

(Continued)
Table 2. (Continued)

| Drug            | Carrier gas | Retention time (min) | S/N                  | Monitored ion (m/z) |
|-----------------|-------------|----------------------|----------------------|---------------------|
| Salicylic acid  | H<sub>2</sub> | 9.100                | 1859.82              | 120, 92, 138, 64, 46 |
|                 | He          | 9.655                | 1343.64              | 120, 92, 138, 64, 46 |
| Scopolamine     | H<sub>2</sub> | 24.172               | 1502.23              | 94, 138, 108, 154, 303 |
|                 | He          | 25.121               | 3631.33              | 94, 138, 108, 154, 303 |
| Secobarbital    | H<sub>2</sub> | 17.809               | 2691.55              | 195, 168, 124, 97, 53 |
|                 | He          | 18.684               | 1204.76              | 168, 195, 97, 124, 53 |
| Sibutramine     | H<sub>2</sub> | 24.172               | 1502.23              | 94, 138, 108, 154, 303 |
|                 | He          | 19.109               | 5010.93              | 114, 72, 58, 101, 128, 137 |
| Sildenafil      | H<sub>2</sub> | 45.447               | 145.57               | 404, 281, 207, 99, 56 |
|                 | He          | 49.500               | 17.60                | 99, 404, 56, 283, 207 |
| Stanozolol      | H<sub>2</sub> | 31.003               | 163.95               | 96, 328, 257, 133, 119, 175 |
|                 | He          | 32.022               | 335.89               | 96, 328, 257, 133, 119, 175 |
| Strychnine      | H<sub>2</sub> | 31.979               | 2444.29              | 334, 167, 130, 107, 77, 55 |
|                 | He          | 33.167               | 296.67               | 334, 167, 130, 107, 77, 55 |
| Sulfadiazine    | H<sub>2</sub> | 26.540               | 512.14               | 185, 92, 65, 108 |
|                 | He          | 27.429               | 756.37               | 185, 92, 65, 108 |
| Sulfadimethoxine I | H<sub>2</sub> | 28.890               | 219.77               | 259, 140, 92, 65, 168, 108, 121, 82, 187 |
|                 | He          | 29.806               | 639.59               | 259, 140, 92, 65, 168, 108, 121, 82, 187 |
| Sulfadimethoxine II | H<sub>2</sub> | 29.269               | 364.43               | 215, 92, 65, 108, 53, 80, 280 |
|                 | He          | 30.114               | 208.79               | 215, 92, 65, 108, 53, 80, 280 |
| Sulfamerazine   | H<sub>2</sub> | 27.012               | 801.84               | 199, 92, 65 |
|                 | He          | 27.910               | 1340.67              | 200, 199, 92, 65 |
| Sulfamethazine  | H<sub>2</sub> | 27.373               | 2275.83              | 213, 92, 65 |
|                 | He          | 28.212               | 1074.36              | 214, 213, 92, 65 |
| Sulfamethoxazole| H<sub>2</sub> | 25.579               | 1070.19              | 92, 108, 65, 156, 119, 162, 174, 43, 140 |
|                 | He          | 26.156               | 910.57               | 92, 156, 108, 65, 162, 119, 253, 174, 189 |
| Sulfamethoxypyridazine | H<sub>2</sub> | 28.799               | 157.78               | 215, 92, 108, 65, 53, 80, 280 |
|                 | He          | 29.673               | 214.35               | 215, 92, 108, 65, 53, 80, 280 |
| Sulfanilamide   | H<sub>2</sub> | 20.112               | 263.20               | 172, 92, 65 |
|                 | He          | 20.718               | 202.79               | 172, 92, 65, 108 |
| Sulfapyrazine   | H<sub>2</sub> | 23.429               | 761.20               | 278, 249, 209, 183, 152, 130, 105, 77, 51 |
|                 | He          | 24.242               | 1169.11              | 278, 77, 105, 130, 51, 249, 209, 183, 152 |
| Sulindac        | H<sub>2</sub> | 29.207               | 198.68               | 233, 297, 312, 248, 67, 123, 47, 133, 220 |
|                 | He          | 30.083               | 516.90               | 297, 233, 312, 248, 123, 220, 67, 47, 133 |
| Synephrine      | H<sub>2</sub> | 15.602               | 43.68                | 135, 44, 107, 179, 160, 77, 51, 91 |
|                 | He          | 16.281               | 35.97                | 44, 77, 108, 107, 65, 51, 91 |
| Tadalafil       | H<sub>2</sub> | 41.729               | 1803.01              | 389, 262, 204, 169 |
|                 | He          | 44.673               | 79.24                | 389, 262, 204, 169 |
| Terbinafine     | H<sub>2</sub> | 23.292               | 289.12               | 141, 276, 234, 115, 291, 196 |
|                 | He          | 24.139               | 1216.53              | 141, 276, 115, 234, 291, 196 |
| Testosterone    | H<sub>2</sub> | 27.168               | 65.84                | 288, 246, 203, 147, 124, 91, 55 |
|                 | He          | 28.115               | 328.53               | 124, 288, 246, 147, 203, 91, 55 |
| Tetracaine      | H<sub>2</sub> | 23.189               | 5752.14              | 58, 71, 176, 150, 105, 193, 92 |
|                 | He          | 24.012               | 3991.85              | 58, 71, 176, 150, 105, 193, 92 |
| Theobromine     | H<sub>2</sub> | 19.022               | 23.33                | 180, 67, 109, 55, 82, 137, 42, 94 |
|                 | He          | 19.157               | 142.02               | 180, 67, 55, 109, 82, 137, 42, 94 |

(Continued)
analysis was observed with the use of hydrogen. Similar mass spectra of hydrogen (A) and helium (B) as the carrier in gas are shown in Fig 4.

**LOD**

In considering the limit of detection (LOD), a signal-to-noise ratio of 3 was defined. The LODs of amitriptyline and 45 other analytes were 10 μg/g, and those of 104 analytes containing acetaminophen were 100 μg/g. The LOD of allopurinol was 500 μg/g, and those of the other 19 drugs—including aminotadalafil—were 1,000 μg/g. Moreover, the monitored ion library of the total analytes was developed for adulterant identification and monitoring. The 170 drugs obtain almost the same level of LODs with the use of hydrogen and helium as a carrier gas as detected by GC–MS for TCM and food supplements. Except for benz bromarone, estradiol benzoate, bezafibrate, mfenamic acid, oxymetholone, piperidenafl and cetilistat, the LODs of those analytes using hydrogen (100, 100, 1000, 1000, 1000, 1000 and 1000 μg/g, respectively) were decoupled as much as those of the previous analytes using helium (10, 10, 100, 100, 100, 100 and 100 μg/g, respectively).

**Proficiency testing TFDA**

Proficiency testing is another effective tool that can be used to ensure the results using hydrogen as a carrier gas for the detection of adulterated drugs in TCM and dietry supplements by GC–MS. Using the abovementioned detection method performed in testing for Chinese herbal analysis was observed with the use of hydrogen. Similar mass spectra of hydrogen (A) and helium (B) as the carrier in gas are shown in Fig 4.

**Table 2. (Continued)**

| Drug                  | Carrier gas | Retention time (min) | S/N   | Monitored ion (m/z) |
|-----------------------|-------------|----------------------|-------|---------------------|
| Theophylline          | H2          | 20.324               | 33.21 | 180, 95, 68, 53     |
|                       | He          | 21.141               | 246.52| 180, 95, 68, 53     |
| Thiodimethylsildenafil| H2          | 40.912               | 33.32 | 113, 70, 42*, 84, 328*, 343, 56 |
|                       | He          | 43.571               | 48.61 | 113, 340*, 70, 84, 283*, 56, 343 |
| Thiohomosildenafil    | H2          | 50.969               | 202.81| 113, 70, 56, 475, 98, 42, 327, 341, 269, 84 |
|                       | He          | 55.952               | 201.35| 113, 70, 56, 475, 98, 42, 327, 341, 269, 84 |
| Thioridazine          | H2          | 31.712               | 366.09| 370, 244, 183, 126, 98, 70 |
|                       | He          | 32.798               | 120.18| 98, 370, 70, 185, 244, 126 |
| Thiosildenafil        | H2          | 47.756               | 296.39| 99, 448, 56, 489, 425, 70, 207 |
|                       | He          | 51.652               | 355.43| 99, 448, 56, 70, 489, 425, 207 |
| Tinidazole            | H2          | 21.175               | 776.12| 201, 123, 80, 68, 93, 107, 53, 154, 247 |
|                       | He          | 21.921               | 3229.62| 201, 123, 80, 68, 93, 107, 53, 154, 247 |
| Tolbutamide           | H2          | 15.612               | 426.74| 91, 171, 155, 65, 107, 77, 197 |
|                       | He          | 16.398               | 684.97| 91, 171, 155, 65, 107, 77, 197 |
| Vardenafil analogue   | H2          | 27.628               | 5472.86| 284, 312, 256, 67, 297, 120, 269, 93, 135 |
|                       | He          | 28.462               | 2912.76| 284, 312, 256, 67, 120, 269, 93, 135 |
| Yohimbine             | H2          | 32.330               | 1464.36| 353, 169 |
|                       | He          | 33.427               | 1587.82| 353, 169 |
| Zolpidem              | H2          | 28.784               | 378.38| 235, 207, 219, 281*, 307, 65, 92, 191 |
|                       | He          | 29.717               | 205.59| 235, 207, 219, 92, 65, 191, 207 |
| Cetilistat            | H2          | 13.943               | 218.91| 177, 160, 133, 55, 104, 77, 401 |
|                       | He          | 14.692               | 234.20| 177, 160, 133, 104, 55, 77, 401 |

*represent the differences between the H2 and He ions.

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medicine adulteration held by the TFDA revealed satisfactory results in 2015 and 2016. The analytical results were obtained using qualitative analysis. This result also provided the opportunity for the present method to verify the performance in testing for Chinese herbal medicine adulteration.
Analytical results of drugs in TCM and supplement foods on the market

In the present study, forty food supplement samples and eighty-three TCM samples were inspected and detected simultaneously with helium and hydrogen as a carrier gas by GC–MS between 2015 and 2016. Out of all 123 samples, 115 were found to be untainted, and the remaining 8 were TCM samples that were found to contain illegal, adulterated drugs (see in Fig 2. Mass spectrum of chlorzoxazone when (A) hydrogen and (B) helium is used as a carrier gas.

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The detected drug adulterants of 123 TCM and food supplement samples are shown in Table 4.

The types, forms and numbers of adulterated drugs in 8 TCM samples were 3 capsules, 3 powders, 1 paste and 1 medicated patch/plaster. The three capsule samples were detected with the kidney-supplement category of sildenafil or tadalafil. The three powder samples were all

Fig 3. Ion chromatogram of sildenafil when (A) hydrogen and (B) helium is used as a carrier gas.

https://doi.org/10.1371/journal.pone.0205371.g003
Fig 4. Mass spectrum of sildenafil when (A) hydrogen and (B) helium is used as a carrier gas.

https://doi.org/10.1371/journal.pone.0205371.g004

| Type of sample      | TCM  | Food supplement | Total |
|---------------------|------|-----------------|-------|
| Number of samples   | 83   | 40              | 123   |
| Number of samples detected | 8    | 0               | 8     |
| Positive rate (%)   | 9.6  | 0               | 6.5   |

https://doi.org/10.1371/journal.pone.0205371.t003
detected with the antirheumatic-analgesics category of acetaminophen, ibuprofen and chlor- 
zoazone. One paste and one medicated patch/plaster sample were detected with the antidote 
category of sulfamethoxazole. The analytical results were consistent with the use of hydrogen 
and helium as a carrier gas by GC-MS in the current study. In brief, the above evidence dem-
strated the availability of the method with the use of hydrogen as a carrier gas for the detec-
tion of adulterated drugs in traditional Chinese medicine and dietary supplements using 
GC-MS for real sample analysis. The previous study also demonstrated the feasibility of using 
hydrogen as an alternative carrier gas, which has been in use for the routine analysis for gov-
ernment regulations for most estrogens, androgens and gestagens of the Belgium national plan 
[27].

**Discussion**

Hydrogen is a highly effective carrier gas because it increases the speed of the analysis and the 
resolution in GC [28]. Hydrogen offers the chromatographer a number of benefits, including 
increased speed, lower temperature separations, longer column life, fewer environmental con-
cerns and greater availability [29]. However, some safety concerns are associated with the use 
of hydrogen cylinders, such as the following: cylinder handling and storage, the flammable 
nature of hydrogen and the variation in quality of the gas selection of the appropriate gas deliv-
ergy equipment to ensure the system’s purity. In addition, hydrogen is flammable over a wide 
concentration range in air from 4% to 74.2% by volume, it has the highest burning velocity of 
any gas, and it can self-ignite due to very low ignition energy when expanding rapidly from 
high pressure [27]. As an alternative to cylinders, hydrogen generators provide a continuous 
source of high purity hydrogen and can eliminate many safety concerns over using hydrogen 
cylinders. Because hydrogen can be generated on demand, the volume of stored gas in a hydro-
genator is very small. Moreover, it has built-in safety features. In the case of a leak, the 
flow of hydrogen will be automatically shut down to ensure that it never reaches to the lower 
explosive limit. In addition to safety concerns, reactions in the ion source, the loss of function-
ality of the pumping system, and high background noise are also disadvantages of using hydro-
gen as a carrier gas [30].

Hydrogen is a reactive gas, and it might react with analytes under certain conditions. The 
major adverse effect of hydrogen is on the GC injector liner activation, which can catalytically 
degrad samples such as certain simple pesticides [31]. Chromatographers should avoid the 
use of chlorinated solvents with the hydrogen carrier gas because of the risk of hydrochloric 
acid (HCl) formation, which can affect the performance of the chromatographic system. All 
situations should be carefully evaluated when changing to hydrogen as a carrier gas. Further-
more, the ability to switch from He to H₂ as a carrier gas will save money and time.

Although hydrogen seems to be an ideal gas for GC/MS and it offers important advantages 
over helium in terms of efficiency, resolution and the speed of analysis, some disadvantages

| Drugs           | Detected frequency |
|-----------------|--------------------|
| Acetaminophen   | 3                  |
| Chloroxazone    | 3                  |
| Ibuprofen       | 3                  |
| Sulfamethoxazole| 2                  |
| Sildenafil      | 2                  |
| Tadalafil       | 1                  |

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should be mentioned. The study suggested that hydrogen as a carrier gas had excellent performance that was comparable to using helium for the nonpolar, nonreactive compounds. Thus, the most polar, reactive compounds displayed significantly lower responses with the hydrogen carrier gas. Furthermore, evidence demonstrated that nitrobenzene, which is one of the most reactive compounds, was reduced to aniline when using hydrogen as a carrier [32]. Hydrogen is a reactive compound that might hydrogenate unsaturated and aromatic compounds under certain conditions. Hydrogen reduces metal oxides at the ion source and exposes bare and highly active metal surfaces at the EI (and CI) ion source. Thus, many compounds are degraded at the ion source, lose their molecular ions and are harder to identify by the library [31]. Previous experiments showed that the baseline of the total ion chromatograms is elevated in hydrogen relative to that in helium. The obtained signal-to-noise ratio is poorer with hydrogen compared with helium via both a lower signal and higher noise. The S/N values are approximately 3-5-fold lower when hydrogen is used as a carrier gas compared to the results using helium. Decreased response factors for some analytes may result from chemical interactions with hydrogen in the MS ion source or other causes [32]. The lower signal-to-noise ratio of hydrogen might lead to the lower LOD in a certain compound. Among the 170 analytes studied, 7 drugs using hydrogen as the carrier gas provided an LOD ten times poorer than those using helium as the carrier gas. The same LOD was found for all other 163 drugs. The 7 drugs were benzbromarone, estradiol benzoate, bezafibrate, mefenamic acid, oxymetholone, piperidenafil and cetilistat. [33]. The structure suggested the potential reactivity of the 7 analytes with the hydrogen carrier gas. Therefore, recent work has reported on the unstable signal and reduced accuracies of the pesticides when hydrogen was used as carrier gas, and moreover some compounds were undetectable rather than those when helium was used as a carrier gas [34]. The research indicated that fragmentation patterns are similar whether helium or hydrogen is used as the carrier gas except nitrobenzene. Nitrobenzene can be reduced in the presence of hydrogen, thus resulting in the different fragmentation patterns and peak tailings [35]. Moreover, in general, more abundant fragmentation is observed, and higher relative abundances of the diagnostic ions for the identification of the components using hydrogen as a carrier gas. In the case of Py-GC, using H₂ as a carrier gas may cause unwanted protonation or hydrogenation reactions, which may lead to difficulties in the library search when using existing MS libraries [36]. One must carefully consider the chemistry of specific analytes when changing to hydrogen as a carrier gas. The potential reactivity of analytes with the hydrogen carrier gas should be evaluated in the early stages of the method’s development.

In this study, an economical analytical method for the determination of adulterated drugs in traditional Chinese medicine and dietary supplements with GC-MS using hydrogen as a carrier gas was developed. In general, helium is considered to be the most widely used carrier gas for GC–MS analysis. However, the cost of helium is increasingly expensive due to its limited supply. Hydrogen is as an alternative GC-MS carrier gas. The hydrogen generator produces ultrahigh purity hydrogen through the electrolysis of deionized-distill water without cost and usage limits; moreover, there were no safety concerns associated with high pressure cylinders. Hydrogen is not only renewable, abundant and economical, but it also offers important advantages in terms of reduced run-times and performance benefits over helium [27]. The screening of all TCM and dietary supplements proved to be necessary for the detection of pharmaceutical substances to protect consumers from adverse reactions and side effects before the products are made available on the market. The screening of illegal drug adulteration using hydrogen instead of helium as a carrier gas has been in use for routine analysis in our laboratory for 2 years now. Eight products adulterated with acetaminophen, ibuprofen, chlorzoxazole, sulfamethoxazole, tadalafil and sildenafil substances that are prohibited in TCM were detected as the result of 123 TCM and food supplements’ screening. Satisfactory consistency
between the hydrogen and helium spectra of illicit drugs in real sample analysis also demonstrates that hydrogen can be used effectively as a buffer gas in GC-MS.

Supporting information
S1 Table. Information of samples.
(DOCX)
S2 Table. Information of standard.
(DOCX)

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