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Characterization of Allegedly Musk-Containing Medicinal Products in Taiwan*

ABSTRACT: As a highly valued ingredient of Chinese medicinal remedies, musk is used as a detoxification agent and for treating fever, inflammation and swelling, and pain. Muscone (3-methylcyclopentadecanone-1), an odoriferous secretion from the ventral glands of male musk deer, is believed to be the active ingredient. A small amount of muscopyridine is also found in the secretion from the ventral glands of male musk deer. Common counterfeit ingredients are musk xylene, musk ambrette, musk ketone, and diphenhydramine. An extraction/GC-MS protocol/data evaluation scheme was developed and applied to study allegedly musk-containing Musk-Tiger Bone Plaster preparations and musk pods (or grains) from Chinese medicine stores and an airport customs. The content of muscone in a specific sample was estimated based on the percentage of the amount recovered from the first extraction. No muscone or counterfeit ingredients were found in all musk pod (or grain) samples from the customs and in the majority of Musk-Tiger Bone Plaster preparations, while muscone (alone or with counterfeit ingredients) was found in most of the musk pod (or grain) collected from Chinese medicine stores.

KEYWORDS: forensic science, muscone, musk deer, endangered species, illegal trade

Similar to bear bile (1), musk is a highly valued ingredient of Chinese medicinal remedies. It is reportedly an effective detoxification agent and can be used to reduce pain, inflammation, and swelling and to treat seizures (2–4). It is also used for some specialty perfumes and is among the most valuable animal products in the world at $45,000/kg (5).

Muscone, reportedly the active ingredient of musk (6), is a secretion of the preputial gland located under the abdomen near the pubic of the male musk deer (genus *Moschus*), of which at least four species have been identified: *M. moschiferus*, *M. berezovskii*, *M. chrysogaster*, and *M. fuscus*. Because of excessive hunting and destruction of forest habitat, the musk deer population has declined dramatically. Some populations now are included in Appendix I of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), while others are listed in CITES Appendix II. This means these deer and their derivatives are banned from (Appendix I) or require permits for (Appendix II) international trade.

Along with our earlier report on bear bile (1), this study was funded by and conducted as part of the Taiwanese Council of Agriculture's initiatives to conform with CITES' regulations and to understand the counterfeit status of musk-containing products in the market. We have conducted a literature review to better understand the ingredients of authentic and counterfeit musk products, collected representative samples, and developed an analytical and data analysis scheme suitable for this study. A consecutive extraction and data evaluation scheme was developed to determine the recovery efficiency of the adapted sample preparation procedure. This procedure was then used in conjunction with gas chromatography-mass spectrometry (GC-MS) protocols for the analysis of representative (allegedly) musk-containing products, and the findings are hereby reported.

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Methods and Materials

Specimens and Reagents

Specimens used in this study included 77 Musk-Tiger Bone Plaster preparations (Fig. 1) and 32 musk pods (or grains) (Fig. 2) collected from various Chinese medicine stores located in different parts of Taiwan and 17 allegedly musk glands obtained from the customs of an international airport. (Gland specimens are referred to as pods, while powder or crystalline materials allegedly resulting from the secretions of the gland are referred to as grains.)

All solvents and reagents were HPLC grade and were purchased from J. T. Baker, Inc. (Phillipsburg, NJ). A muscone standard was purchased from BIOMOL Research Lab (Plymouth Meeting, PA). Imipramine was obtained from Radian, now Cerilliant (Austin, TX).

Sample Preparation

Typically, 20 mg of musk grain or 200 mg of Musk-Tiger Bone Plaster were weighed and placed in a 10 × 75 mm glass test tube. Two mL of ethyl acetate were added. Extraction was carried out by placing the test tube in an ultrasonic system for 15 min, followed by centrifugation for 5 min. One mL of the supernatant was transferred to a 1.8-mL autosampler vial for GC-MS analysis.

Imipramine was used as the internal standard for the quantitation of muscone and muscopyridine. Specifically, 50 μL of a 100 μg/mL standard imipramine solution (in methanol), were added to the test tube before the extraction procedure proceeded. The concentration of the internal standard is equivalent to 5 μg/mL in the test sample.

Recovery of the extraction protocol was evaluated as follows. Following the first extraction, the residual solvent/extract was removed. Another 2-mL aliquot of ethyl acetate was added, and the

FIG. 1—*Various packaging of Musk-Tiger Bone Plaster preparations obtained from the customs of an international airport in Taiwan.*

FIG. 2—(A) *Interior and* (B) *exterior of a musk pod and* (C) *various packaging of muscone-containing products found in Chinese medicine stores in Taiwan.*

same extraction procedure was completed. The same procedure was repeated until a total of six extractions was completed. The concentrations of muscone observed in these six extractions were evaluated (see description presented in the Results and Discussion section) to determine the recovery efficiency of the first extraction.

Gas Chromatography-Mass Spectrometry Analysis

A Hewlett-Packard 5890 gas chromatograph/5972 mass selective detector (GC-MSD) equipped with HP-G1034C Chemstation

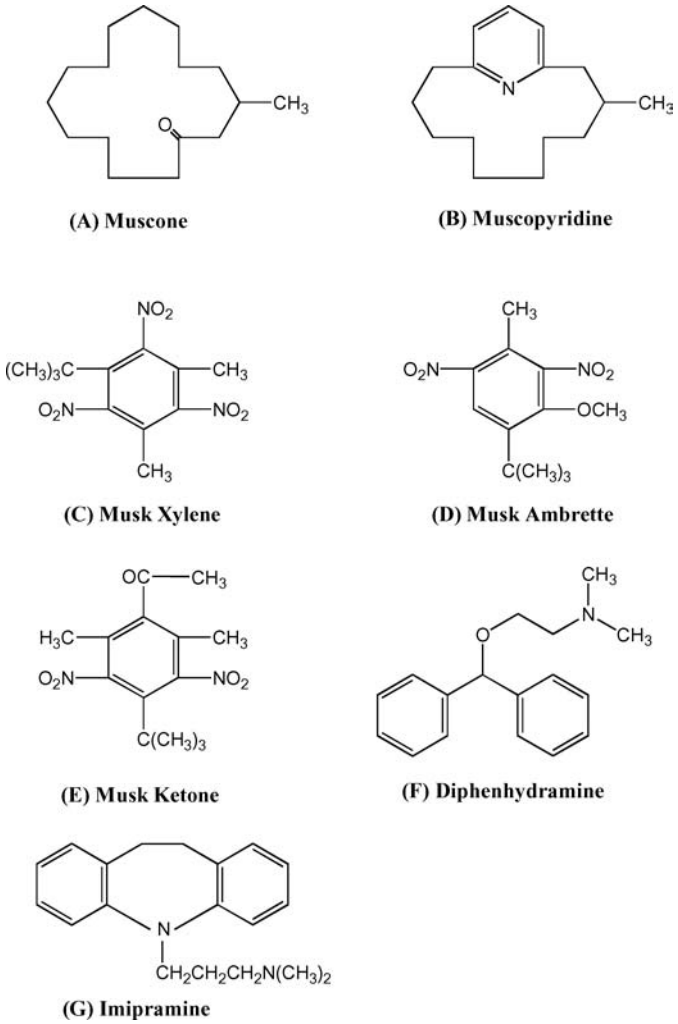

FIG. 3—(A) *Structures of muscone,* (B) *muscopyridine,* (C) *musk xylene,* (D) *musk ambrette,* (E) *musk ketone,* (F) *diphenhydramine, and* (G) *imipramine.*

software was used for this study. The GC was equipped with a 12-m Hewlett-Packard (Andover, MA) HP Ultra-1 (100% dimethyl polysiloxane phase) fused silica capillary column (0.20 mm ID; 0.33 μm film thickness). The injector and interface temperature were maintained at 260 and 280°C, respectively. Oven temperature was held at 100°C for 1 min, then programmed to 280°C at 20°C/min, and held at the final temperature for 10 min. The following parameters were used for injecting samples into the GC-MSD system: sample size, 3 μL; injection mode, splitless; injector purge-off duration, 0.75 min.

Full-scan mass spectrometric data were acquired for the *m*/*z* 50–500 range. Selective ion monitoring (SIM) data were collected with *m*/*z* 238, 223, and 209 designated for muscone; *m*/*z* 231, 120, and 107 for muscopyridine; and *m*/*z* 234, 280, and 193 for imipramine. The first ion listed for each compound was used for quantitation using a five-point (5, 12.5, 25, 50, 100 μg/mL) calibration protocol.

Results and Discussion

Muscone (3-methylcyclopentadecanone-1, Fig. 3*A*), an odoriferous secretion from the ventral glands of male musk deer, is believed to be the active ingredient responsible for the reported

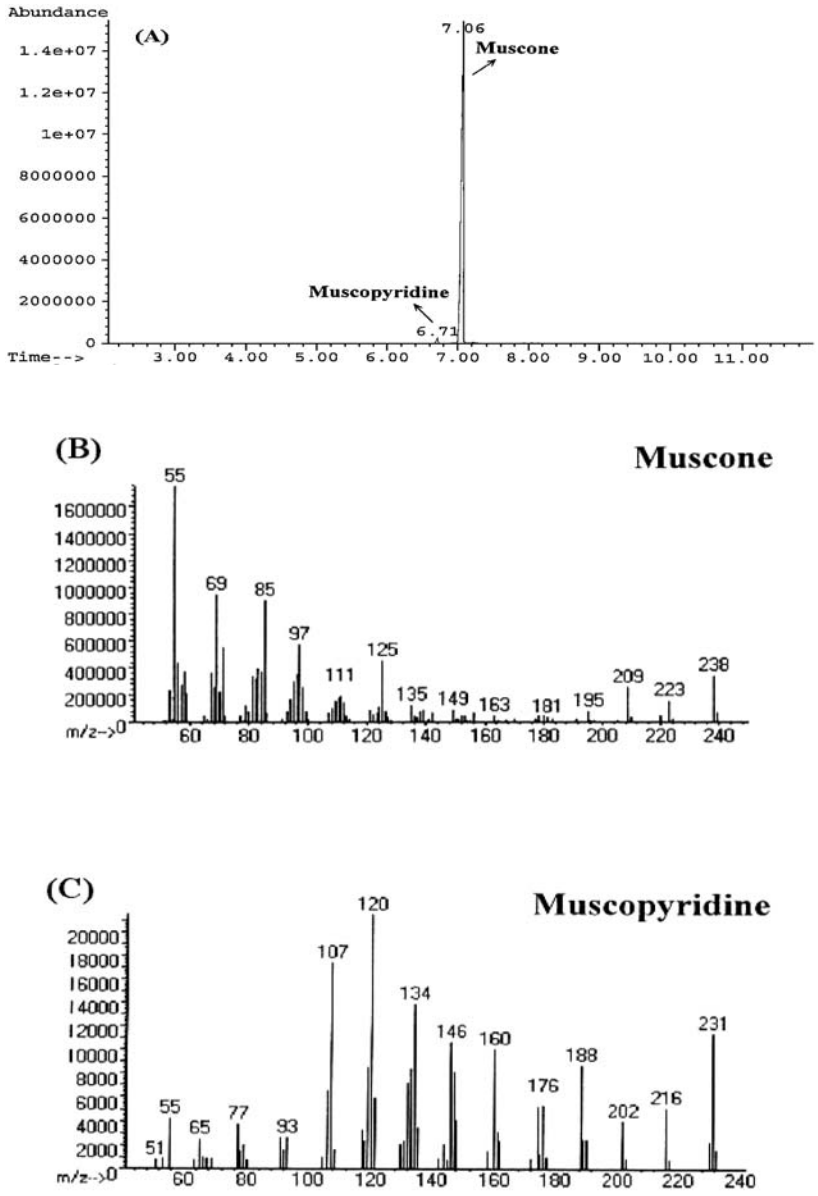

FIG. 4—(A) *A typical full-scan ion chromatogram of extract containing muscone and muscopyridine and* (B) *electron impact mass spectra of muscone and* (C) *muscopyridine.*

medicinal properties. Muscone from the natural source (male musk deer) is *laevo*-muscone, while synthesized muscone typically exists as *racemic* or *d,l*-muscone (7). A small amount of muscopyridine (Fig. 3*B*) is also found in the secretion from the male musk pods.

The following nitro-compounds have been identified in musk counterfeit products (8), presumably for their "look-alike" characteristics: musk xylene (Fig. 3*C*), musk ambrette (Fig. 3*D*), and musk ketone (Fig. 3*E*). Diphenhydramine (Fig. 3*F*), an antihistamine often used to treat inflammation and swelling, is another compound found in musk counterfeit products (8), presumably for its pharmaceutical properties.

Several Chinese articles have reported the analysis of muscone by TLC (9), GC (10–12), and HPLC (13). Recently, the U.S. National Fish and Wildlife Forensic Laboratory (Ashland, OR) adapted GC-MS methodologies to analyze eight items of Musk-Tiger Bone Plaster preparations but failed to find any natural or synthetic muscone in these specimens (14). The Hong Kong Government Laboratory regularly receives samples of alleged musk pods or grains submitted by the Department of Agriculture and Fisheries for authentication. Over 200 suspected musk samples have been examined by GC-MS, 56% of which were found to be fake products (15).

Chromatographic and Mass Spectrometric Characteristics of Common Components Found in Authentic and Counterfeit Musk Grains and Musk-Tiger Bone Plaster Preparations

A typical total ion (full-scan) chromatogram of an extract derived from a musk-containing specimen is shown in Fig. 4*A*, while the full-scan mass spectra of muscone and muscopyridine in this specimen are shown in Figs. 4*B* and 4*C*. Among all musk-containing specimens, the content of muscone is typically high, while the content of muscopyridine is relatively low.

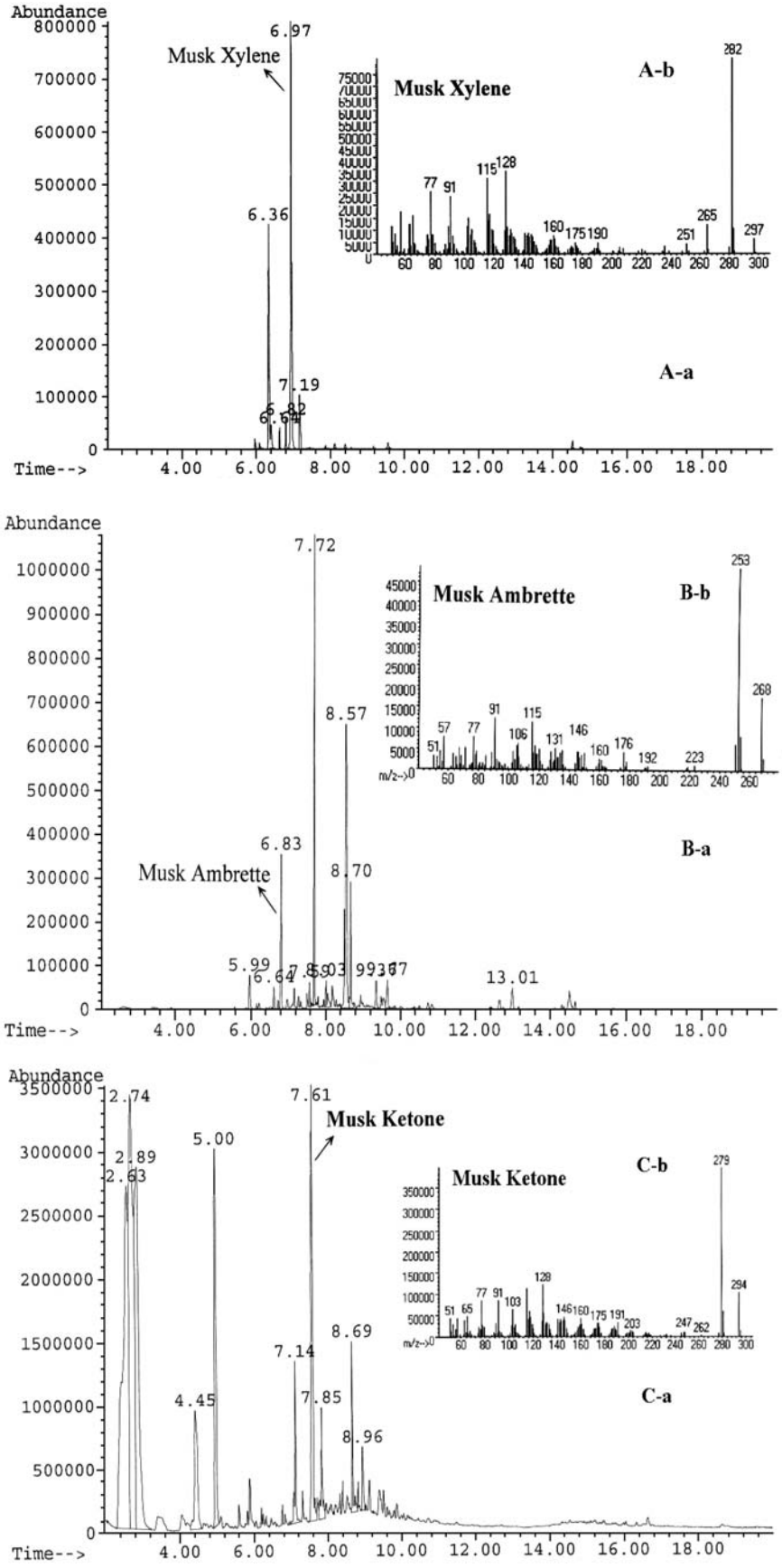

FIG. 5—(a) *Typical full-scan total ion chromatograms and* (b) *mass spectra of extracts containing the following musk counterfeit ingredients:* (A) *musk xylene,* (B) *musk ambrette,* (C) *musk ketone, and* (D) *diphenhydramine*. (A *and* B) *were derived from musk grain samples, while* (C *and* D) *were derived from Musk-Tiger Bone Plaster preparations.*

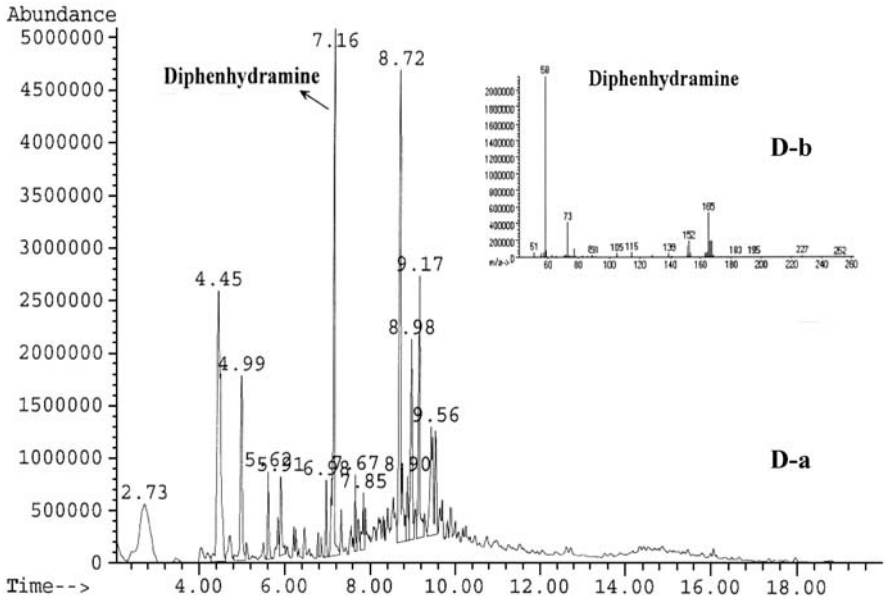

FIG. 5—*Continued.*

Typical total ion (full-scan) chromatograms of extracts derived from samples containing counterfeit ingredients and the mass spectra of these counterfeit compounds are shown in Figs. 5*A*–5*D*. Figures 5*A* and 5*B* were obtained from musk grain samples, while Figs. 5*C* and 5*D* were derived from Musk-Tiger Bone Plaster preparations.

Quantitation of Muscone—Quantitation was based on SIM data using *m*/*z* 234, 238, and 231 for the designations of imipramine, muscone, and muscopyridine, respectively. Since isotopic analogs of the analytes are not available, imipramine, a cyclic compound with some structural similarity (Fig. 3*G*), was adopted as the internal standard.

Five standard solutions (containing 5, 12.5, 25, 50, and 100 μg/mL muscone and 5 μg/mL imipramine) were used to establish a calibration line for the quantitation of muscone in the extracts. Analyte concentrations thereby derived from the extracts were expressed in μg/mL and then converted into μg/mg and % by taking the volume of the extract (2 mL) and the sample sizes (20 mg for musk grain and 200 mg for Musk-Tiger Bone Plaster) into consideration. The limit of quantitation was approximately 0.80 μg/mL in the extract, which is equivalent to 0.080 μg/mg or 0.008% in musk grain.

The amounts of muscone found in the first extracts derived from the 32 musk gland samples are shown in the second column of Table 1. Since muscone cannot be recovered completely by just one extraction, the total amount of muscone in each sample was estimated by dividing the amount found in the first extract by the percentage of recovery achieved by this first extraction. The resulting data are shown in the third column (Table 1).

The following procedure was used to estimate percent recovery achieved by the first extraction. Following the first extraction, five more extractions were conducted. However, muscone was detected only in the first four extracts. Since signals observed in the 5th and the 6th extracts were not distinguishable from the blank, the sum of these four quantities was used as the estimated total quantity of muscone in the sample examined. The percent recovery of the first extraction was estimated by dividing the quantity from the first extraction by the total quantity. Four samples examined to determine the percent recoveries of the first extractions resulted in the following recovery data: 98.77%, 97.53%, 98.08%, and 98.39%. The mean (98.19 ± 0.52%) of these data was used to obtain the total quantities of muscone in all samples.

Musk-Tiger Bone Plaster Preparations and Musk Pods (or Grains) from Various Sources

Our investigation on musk-containing medicinal products has focused on Musk-Tiger Bone Plaster preparations and musk pod (or grain) samples. Seventy-seven allegedly musk-containing Musk-Tiger Bone Plaster preparations and 32 musk pods (or grains) from Chinese medicine stores and 17 musk pods from an international airport customs were extracted and analyzed using GC-MS protocols to identify their components.

Analytical results of the 77 Musk-Tiger Bone Plaster preparations analyzed were: 1 (1.3%) containing muscone, 5 (4.5%) containing muscone mixed with counterfeit ingredients, 15 (19.5%) containing only counterfeit ingredients, and 56 (72.7%) containing neither muscone nor counterfeit products. Analytical findings of the 32 musk pods (or grains) were: 29 (91%) containing muscone (with or without muscopyridine and counterfeit ingredients), and 3 (9%) containing only counterfeit ingredients. Neither muscone nor counterfeit ingredients were found in all 17 (100%) musk pods from the customs.

Authenticity of Musk Specimen

As shown in the 3rd column of Table 1, the concentrations of muscone in the 32 musk gland samples range from 0.028–2.50%. Three of these 32 samples do not show detectable amount of muscone. According to literature data [10], musk normally contains >0.5% of muscone. Thus, samples containing less than 0.5% of muscone are unlikely pure musk dear gland products. Visual inspection also reveals morphological variations among these samples. It is likely that some were prepared by wrapping mixture of various materials with deer skin.

TABLE 1—*Contents (in %) of musk pods or musk grains from Chinese medicine stores in Taiwan.*

Sample no. (designation)	Muscone 1st extra*	Total**	Muscopyridine	Musk Xylene	Musk Ketone	Musk Ambrette	Diphenhydramine
1 (MKS-3)	ND	...	...	+++‡	...	...	...
2 (MKS-5)	ND	...	...	...	...	++‡	...
3 (MKS-25)	ND	...	...	...	...	++‡	...
4 (MKS-28)	0.027	0.028	...	+++‡	...	...	...
5 (MKS-17)	0.074	0.075	...	...	...	...	...
6 (MKS-9)	0.074	0.075	...	...	...	...	...
7 (MKS-19)	0.087	0.089	...	...	...	...	...
8 (MKS-21)	0.111	0.113	...	...	...	...	...
9 (MKS-22)	0.111	0.113	...	...	...	...	...
10 (MKS-14)	0.125	0.127	...	...	...	...	...
11 (MKS-32)	0.16	0.163	...	...	...	...	...
12 (MKS-13)	0.249	0.254	...	...	...	...	...
13 (MKS-11)	0.271	0.276	...	...	...	...	...
14 (MKS-4)	0.372	0.379	...	...	...	...	...
15 (MKS-1)	0.425	0.433	...	...	...	...	...
16 (MKS-18)	0.425	0.433	...	...	...	...	...
17 (MKS-20)	0.527	0.537	+‡	...	...	...	...
18 (MKS-30)	0.55	0.56	+‡	...	...	...	...
19 (MKS-23)	0.57	0.581	...	...	...	...	...
20 (MKS-31)	0.703	0.716	+‡	+‡	...	...	...
21 (MKS-15)	0.704	0.717	...	...	...	...	...
22 (MKS-6)	0.729	0.742	...	...	...	...	...
23 (MKS-12)	0.736	0.75	...	...	...	...	...
24 (MKS-10)	0.813	0.828	...	...	...	...	...
25 (MKS-2)	0.917	0.934	...	...	...	...	...
26 (MKS-16)	0.996	1.014	...	...	...	...	...
27 (MKS-26)	1.228	1.251	+‡	+‡	...	...	...
28 (MKS-27)	1.23	1.253	+‡	...	...	...	...
29 (MKS-24)	1.237	1.26	...	...	...	...	...
30 (MKS-7)	1.651	1.681	+‡	...	...	...	...
31 (MKS-8)	1.785	1.818	+‡	...	...	...	...
32 (MKS-29)	2.455	2.5	...	...	...	...	...

* Concentration based on the amount obtained from the first extraction. The detection limit was approximately 0.008%.
** Concentration based on the total amount extracted (see text for details).
‡ These analyte were detected, but not quantitated. +: Small amount detected; ++: Moderate amount detected; +++: Large amount detected.

Analytical findings of musk-related medicinal products found in Taiwan are hereby reported, with emphasis on conformation with CITES' regulations. It is well beyond the scope of this article to unambiguously determine the authenticity of these specimens for two reasons. First, the test specimens were not fully dissolved, and repetition of extraction was stopped when no detectable muscone was found. It is possible that additional muscone still remained in the interior of the solid specimens. Second, the presence and quantity of muscone and counterfeit components found in a specimen do not necessarily determine its authenticity. Traditional practice in Chinese medicine allows for the use of substitutes possessing similar medicinal activities (8). It is not known whether the detected counterfeit components meet this criterion or whether other components possessing similar medical activities are included in these preparations. With the interest of CITES' regulations in mind, this study simply provides data on the estimated amount of compounds (muscone and muscopyridine) derived from musk deer.

Conclusions

Effective extraction and GC/MS protocols were established for the detection of authentic and counterfeit components found in allegedly musk-containing samples collected from various sources in Taiwan. The total amount of muscone in a specific sample was estimated based on the percentage of the amount extracted in the first extraction. Consecutive extractions and a statistical approach were developed to establish the percentage of the amount recovered from the first extraction. Since *l*- and *d*-forms of muscone cannot be differentiated by the procedure adapted for this study, it is not known whether the detected muscone was enantiomerically pure (*l*-muscone) or in racemic form. However, in samples where muscopyridine was found, the muscone detected is most likely derived from male musk deer. We are currently developing procedures that can be used for enantiomeric analysis of muscone.

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