

CHARACTERIZATION OF MUSKDEER HAIR & IDENTIFICATION OF MUSCONE USING GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Jiwan pd. Rijal*, Birendra R. Shakya*, Dinesh K. Jha*, Hirdaya R. Shakya* and Nirajan Thapa*

*National Forensic Science Laboratory, Khumaltar, Nepal.

Abstract: Muscone is a precious product of Musk deer. The identification of alleged muscone in illegal trade is a demanding job for expert. Muskdeer hair characteristics were observed and GC/MS based muscone identification effective protocol was established in this study.

Keywords: *Illegal trade; Muskpod; Musk deer hair; Muscone identification; GC/MS.*

INTRODUCTION:

The illegal trade in Wildlife parts & products is the second largest illegal international trade next to narcotics. The global wildlife trade is estimated US \$ 20 billion per annum and half of this trade is illegal. Trade includes skins, bones, antlers, rhino horn, bear bile, muscone etc. Muskpod (Fig: 1) is hairy pouch which lies under the skin of the abdomen⁸ in front of the sexual organ of male Musk deer (genus *Moschus*). It is oval to circular in shape 3 X 6 cm diameter containing musk grains (Fig: 1) which is brownish semi-liquid when fresh or dark powdery crystalline when dried. Normally 30–45 grams of musk grains present inside the muskpod of adult musk deer which normally contains >0.5% of muscone¹⁰. Muscone (3-methylcyclopentadecanone-1, $\text{CH}_3\text{C}_{15}\text{H}_{27}\text{O}$, Fig: 5) is an odoriferous secretion² of the preputial gland located between the sex organs and the naval of male musk deer. It is considered as highly priced wildlife products in the world worth about \$ 45,000/kg¹. Muscone normally exhibit a strong, warm, sensual and long lasting odor which makes them invaluable ingredients in perfumes, in fragrances for shampoos, fabric softeners, detergents, soaps and others. It is a highly valued ingredient in Chinese medicinal remedies used as a detoxification agent and for treating fever, inflammation, swelling and pain³. The musk deer is a high altitude deer distributed over a wide area of central and north – east Asia like India, Nepal, Bhutan, Tibet, Northern Burma, Pakistan and southern China⁸. Less than 50,000 known to exist are reported⁴. The numbers are declining rapidly due to poaching and trade. The musk deer is listed as endangered by IUCN, in schedule – I of WPA and Appendix – I of CITES⁴. This means these species and their derivatives are banned for international trade³.

Appliance of methods for the identification of muscone depends on the availability of seized material. Morphological and microscopic examination of hair is effective if muskpod is available. Serological test becomes particularly useful to rule

out the fake Muskpod. Detection of 3-methylcyclopentadecanone-1 by TLC¹¹, HPLC⁹, GC¹⁰ and GC/MS³ is a decisive in characterization of natural muscone from counterfeit products like Musk xylene, musk ambrette, musk ketone etc. More than 50 samples of alleged musk pods and musk grains were received in the laboratory from National parks and wildlife conservation department and other sources for their authentication. Previously examinations of these samples were based on the morphological, microscopic and chemical comparison with authentic sample.

METHODS & MATERIALS:

Specimens: Specimens used in this study comprises 10 seized suspected and one known muskpod sample obtained from Central Zoo, Jawalakhel, Lalitpur, Nepal. These suspected samples were previously concluded as a genuine after attained the similarity in morphological and microscopic characteristics of hair with authentic musk hair. Ketonic compound of a suspected musk grains were too detected as a part of confirmation of muscone using IR spectrophotometer.

Sample preparation:

a) For hair examination: Colors of hair specimens were examined macroscopic, their texture observed and length measured. Cuticular scale caste of hair were developed using transparent nail polish method. Wet mount of samples were made by D.P.X. for medullary pattern study. Transverse sections of shaft of individual hair were prepared after placing hair in straw pipe containing melted wax. Numerical measurements were taken using calibrated ocular micrometer.

b) For musk grains analysis: 20 mg of musk grain from each sample were taken in individual 4 ml GC/MS autosampler vial. Extraction was carried by adding one milliliter of ethyl acetate followed by vortex and centrifugation for 5 minutes. 500 μl of the supernatant was transferred to autosampler vial for GC/

MS analysis.

Gas Chromatography/Mass Spectrometry Analysis: The analysis of muscone is performed using Gas chromatography in combination with mass spectrometry (GC/MS). A Shimadzu gas chromatograph Mass spectrophotometer GC/MS – QP2010 plus equipped with GC/MS solution Ver. 2.5 software was used for this study. The GC was equipped with Rtx – 5 ms (5% diphenyl 95% dimethyl polysiloxane) fused silica capillary column length of 30 m, 0.25 μ m film thickness and 0.25 mm ID. The injector, interface and detector temperature were maintained at 280, 300 and 300°C, respectively. Firstly oven temperature was held at 50°C, increased to 200°C at the rate of 35°C/minute and the final temperature 250°C at the rate of 15°C and hold for 1 minute. One μ L of the sample was injected manually in split mode setting at split ratio of 40. Full-scan mass spectrometric data were acquired for the m/z 40 – 450.

RESULTS & DISCUSSION:

Hair observation: Hairs are composed of three anatomical regions i.e. the Cuticle, the Cortex and when present Medulla. Attempted identification of animal hairs requires the fine detail associated with these regions⁶. Hence, examination of detail morphological and microscopic characteristics of hair becomes highly advantageous when muskpod is available. Hair was multicolored and respectively from proximal to tip white, light black, yellow and black color was observed. Major part of hair was white in color and that portion was straight. Rest portion were wavy in appearance. Shaft length comprises of 1.7 – 6.2 cm. Observed Cuticular scales pattern (Fig: 2) were regular wave with near and smooth margin⁷. Continuous and wide lattice type medulla (Fig: 3) was observed in the hair. The diameter of cortex and Medulla were ranged from 150 – 335 μ m and 140 – 325 μ m respectively. Medullary index (MI) is the width of the medulla by the width of the cortex and average medullary index obtained was 0.940. The cross sectional shape of shaft was more or less Oval (Fig: 4) with nearly filled by Medulla.



Fig 1: Musk pod (left) and musk grains (right)

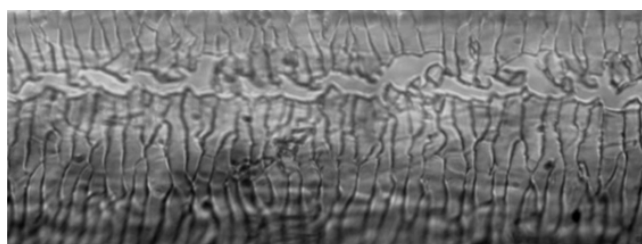


Fig 2: Cuticular Scale (400X)

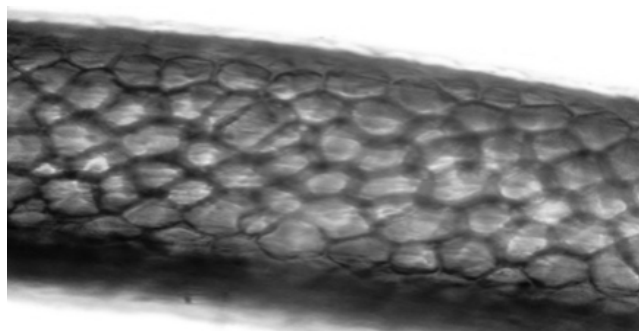


Fig 3: Medulla (400X)

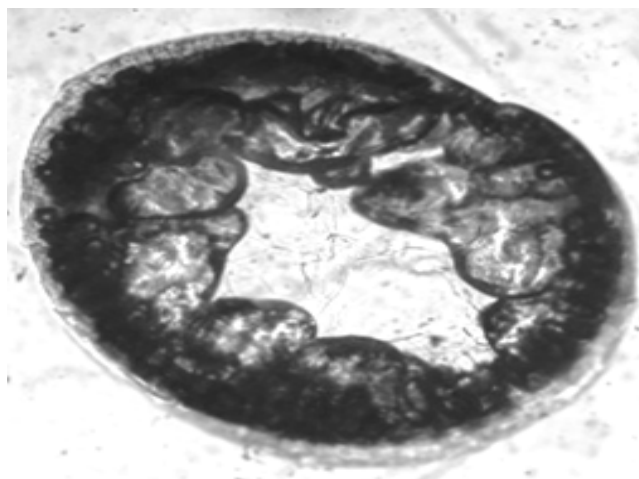


Fig 4: T.S. of Shaft (400X)

Chromatographic and Mass Spectrometric Characteristics of Muscone: Natural muscone secreted from male musk deer is *laevo*-muscone, while synthesized muscone usually exists as *racemic* or *d,l*-muscone⁵. The identification of muscone in the GC/MS was based on a comparison of mass spectra in a NIST05.LIB Mass spectral library. A distinctive total ion chromatogram of an extract derived from a musk grain is shown in Fig: 6. The full – scan mass spectra of muscone of authentic and seized sample (Fig: 7) were similar with muscone mass spectra of NIST05.LIB and with work carried out by Lin et.al. Muscopyridine is also found in the secretion from the male musk pods. Since the content of muscopyridine is relatively lower than the content of muscone³ and no reference mass spectra was accessible in the NIST05.LIB, therefore no Muscopyridine mass spectra were distinguished in the study.

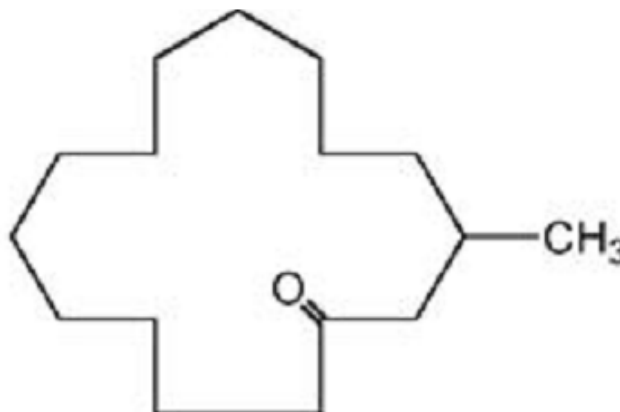


Fig 5: Structure of muscone

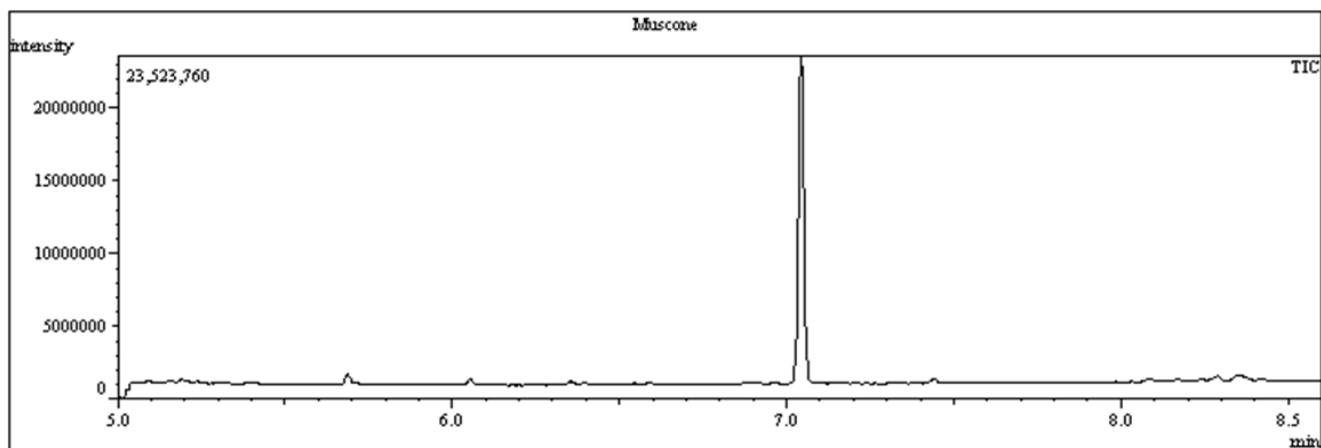


Fig 6: Total ion chromatogram of muscone

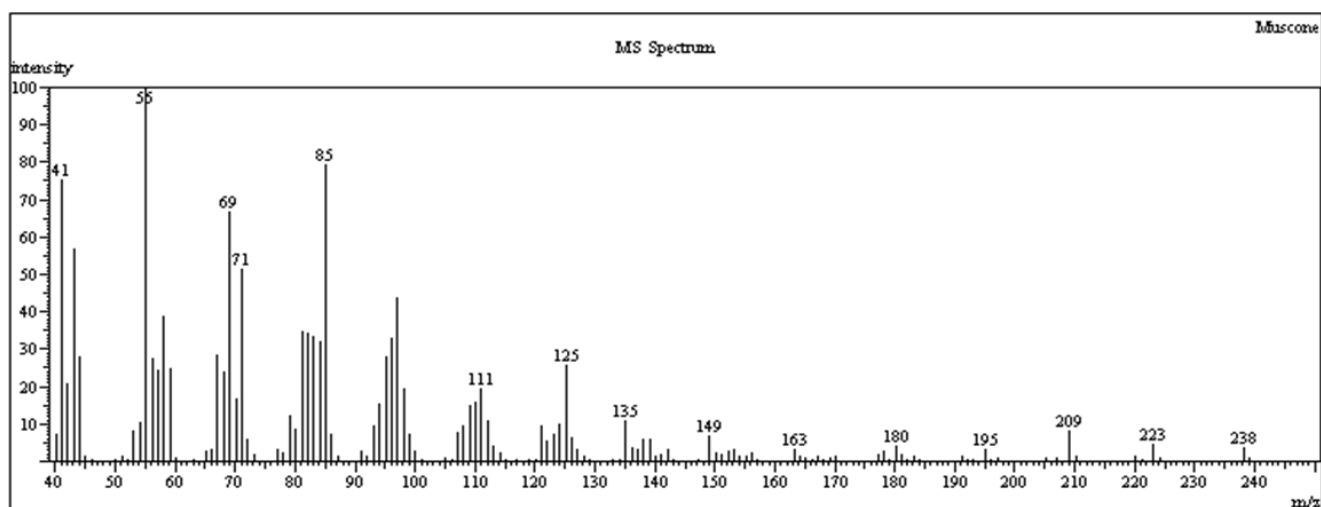


Fig 7: Mass spectra of muscone

CONCLUSION:

Morphological and microscopic distinctiveness of musk hair were noticed. The standard and effective extraction and GC/MS protocols were established for the detection of muscone. Because *l*- and *d*-forms of muscone cannot be distinguished by the procedure adapted for this study, it is not known whether the detected muscone was enantiomerically pure (*l*-muscone) or in racemic form.

ACKNOWLEDGEMENT:

The authors are thankful to Ms Bhinu Shova Tuladhar, Executive Director, National Forensic Science Laboratory, Nepal for her fundamental idea in identification of muscone using GC/MS.

REFERENCES:

- Green, M.J.B., Kattel, B. 1997. Musk deer: Little understood, even its scent. The First International Symposium on Endangered Species Used in Traditional East Asian Medicine: Substitutes for Tiger Bone and Musk. Hong Kong.
- Hawley, G.G. 1984. The condensed chemical Dictionary, tenth edition. Galgotia book source, New Delhi, India. P. 709.
- Lin, D.L., Chang, H.C., Huang, S.H. 2004. Characterization of Allegedly Musk-Containing Medicinal Products in Taiwan J Forensic Sci. **49**(6): 1-7.
- Menon, V., Kumar, A. 1999. Wildlife crime, An enforcement guide, second edition, Natraj publishers, Dehradun, India. Pp. 55 - 56.
- Nelson, K.A., Mash, E.A. 1986. Homochiral ketals in organic synthesis. Enantioselective synthesis of (R)-muscone. J Org Chem. **51**:2721-24.
- Robertson, J. 1999. Forensic examination of Hair. CRC Taylor & Francis group, London. P. 80.
- Seta, Sueshige, S. and Miyake, B. 1998. Forensic hair investigation. Forensic Science progress, Vol II, Springer - verlag Berlin, Heidelberg.
- Sharma, B.D. 1994. High altitude wildlife of India, Oxford and IBH publishing Co. pvt ltd. India. Pp. 136-141.
- Wu, Y., Wu, P., Xiao, X. and Yu, D.Q. 1989. Method of analysis for musk, artificially bred musk and original musk by HPLC. Yao Hsueh Hsueh Pao, Pub Med. **24**(4): 308-11.
- Zhang, Z.H., Yue, R.F. 1982. Quantitative analysis of muscone in musk by GC. Pub Med, Yao Hsueh Hsueh Pao. **17**(12): 922-27.
- Zhang, G.Q., Liang, S.W., Du, TX. 1987. Determination of muscone contents in Chinese drug preparations containing Moschus by the TLC scanning method. Chung Yao Tung Pao, Pub Med. **12**(11): 24-26.

