

## Solid-Phase Extraction of L-Muscone from Aqueous Samples with Amberlite XAD-4 for Gas Chromatographic Assay

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An efficient analytical method was devised for the accurate L-muscone assay in aqueous samples. It involves solid-phase extraction of L-muscone in adsorption mode using XAD-4 as the sorbent and dichloromethane modified with 10% (v/v) methanol as the eluting solvent. The gas chromatographic analysis of the eluate residue dissolved in toluene on a DB-5MS capillary column provided complete resolution of L-muscone from the co-extracted interferences. The overall method showed excellent linearity ( $r^2 \geq 0.9994$ ) in the range of 0.1 to 2.0  $\mu\text{g/mL}$  with good intra- and inter-day precisions (% RSD = 2.5–7.3) and with high extraction recovery rates ( $\geq 98.1\%$ ). When the present method was applied to a L-muscone herbal drink product, the within-batch RE (%) in the labeled concentration (1.5  $\mu\text{g/mL}$ ) for the three randomly chosen bottles were -2.4, -1.3 and -3.3 with high precision (% RSD  $\leq 3.1$ ). The present method is considered to be suitable for quality control evaluation on liquid drinks and other complex formulations fortified with L-muscone.

**Key words:** L-Muscone, Solidphase extraction, XAD-4, Gas chromatography

### INTRODUCTION

L-Muscone (3-methylcyclopentadecanone) used as a cardiac and general stimulant, aphrodisiac, or anti-spasmodic agent (Lee *et al.*, 1997; Kamat *et al.*, 2000; Jacob *et al.*, 2002) is an odorous principle of musk. It is a macrocyclic ketone containing 15 carbons in the ring structure and thus very slightly soluble in water, but miscible with alcohol. However, in recent years, it is increasingly marketed as the active ingredient of herbal liquid preparations. Therefore, its accurate assay for the quality control purpose became an important task, mainly being analyzed by gas chromatography (GC) (Zhang and Yue, 1982; Hong and Wang, 1991; Zou and Su, 1994) and high performance liquid chromatography (HPLC) (Wu, *et al.*, 1989). GC is generally more preferred over HPLC because of its high resolving power, high sensitivity and precision, and high speed of analysis.

An appropriate sample preparation of L-muscone from complex aqueous matrices is required prior to chromatographic analysis. It has been exclusively recovered by

solvent extraction (Zou and Su, 1994). For most organic analysis in aqueous samples, the conventional solvent extractions due to its inherent drawbacks have been replaced by solid-phase extraction (SPE) employing using a suitable solid sorbent (Kim *et al.*, 1994, 1997, 1998; Jung *et al.*, 2001). In contrast to solvent extraction, SPE allows the treatment of larger sample volumes employing much smaller volumes of solvent without emulsion problems with fewer steps. Moreover, it provides highly purified extracts with excellent precision and satisfactory recovery, and offers the possibility of automation (Enoch, *et al.*, 1998). The SPE of L-muscone was, however, rarely attempted to date in the literature.

Of the diverse solid sorbents developed for the SPE, XAD-4 resin that is a styrene-divinylbenzene copolymer with hydrophobic character and porous structure (Lepane 1999; Vera-Avila *et al.*, 1999; Vilar *et al.*, 2003; Filho *et al.*, 2004), has gained widespread acceptance for the extraction of trace amounts of organics in water. They are chemically stable at a wide range of pH conditions and also mechanically strong. Moreover, the sorbent surface with aromatic nature has a higher affinity for hydrophobic macrocyclic compounds through the interaction by the intermolecular dispersion force.

The present study was undertaken to develop an

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efficient SPE of L-muscone from aqueous solution employing Amberlite XAD-4 resin as the sorbent prior to its GC analysis. Under the optimal conditions, the present method was validated to demonstrate its suitability for the quantitative assay for L-muscone in complex liquid preparations.

## MATERIALS AND METHODS

### Chemicals

L-Muscone and cyclopentadecanone were obtained from Cho Seon Pharmaceutical & Trading Co., Ltd. (Kyunggi-do, Korea) and Sigma-Aldrich (St. Louis, MO, USA), respectively. The solvents were of HPLC grade from Merck (Darmstadt, Germany) and all other chemicals were of analytical reagent grade. XAD-4 resin (80-100 mesh) obtained from Supelco (Bellefonte, PA, USA) was purified by sequential extraction with methanol, acetone, and dichloromethane in a Soxhlet extractor, followed by activation (50 °C, 3 h) under vacuum prior to being used as a sorbent.

### Preparation of standard solutions and aqueous calibration solutions

The stock solutions of L-muscone and cyclopentadecanone used as an internal standard (IS) were made up at 10 mg/mL in toluene. The working standard solutions were then prepared by diluting aliquots of the stock solution to 50 µg/mL with toluene. The three calibration samples containing L-muscone in the range of 0.1-2.0 µg/mL with constant concentration of IS at 1.5 µg/mL were prepared by dilution of the working standard solution with water.

### Gas chromatography and gas chromatography-mass spectrometry

GC analysis was performed with a Hewlett-Packard HP model 6890 gas chromatograph equipped with flame ionization detectors (Hewlett-Packard, Avondale, PA, USA). A DB-5MS (SE-54 bonded) fused-silica capillary column (15 m × 250 µm I.D., 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA) was used in the pulsed splitless injection mode (purge delay time 42 sec) for samples (ca 1.0 µL). The oven temperature was maintained at 80 °C for 2 min, raised to 190 °C at a rate of 30 °C/min and to 210 °C at 10 °C/min, and finally at 30 °C/min to 300 °C (3 min). The injector and detector temperatures were 260 and 300 °C, respectively. The flow rate of helium as the carrier gas was 0.8 mL/min in constant flow mode.

Gas chromatography-mass selective detector (GC-MSD) analysis was performed with HP 6890 series Gas Chromatograph, interfaced to HP 5973 Mass Selective

Detector (70 eV, electron impact mode) (Hewlett-Packard, Avondale, PA, USA). An Ultra-2 (SE-54 bonded phase) fused silica capillary column (Hewlett-Packard, Avondale, PA, USA; dimensions 30 m × 0.20 mm I.D., 0.11 µm film thickness) was used in the split injection mode (10:1). The oven temperature was initially 120 °C for 2 min and then raised to 300 °C (5 min) at a 10 °C/min. The injector and interface temperatures were 260 and 300 °C, respectively. The flow rate of helium as the carrier gas was set to 0.5 mL/min in constant flow mode.

### Solid-phase extraction

The SPE columns were prepared by packing 0.2 g of pre-washed XAD into each of 3 mL polypropylene empty tubes secured with polypropylene frits (Supelco, Bellefonte, PA, USA). And they were washed sequentially with methanol, acetone, and dichloromethane followed by methanol and then equilibrated with distilled water before use. An aliquot (1 mL) of aqueous samples after addition of IS at constant amount (1.5 µg/mL) was sonicated for 5 min and immediately loaded onto the activated SPE column, followed by washing with distilled water (5 mL) and applying low vacuum to remove remaining water from the column. It was then slowly passed with dichloromethane (5.0 mL) modified with 10% (v/v) methanol as the eluting solvent. After moisture removal with sodium sulfate, the eluate was evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 1 mL of toluene for the direct analysis by GC and GC-MS. All samples were individually prepared in triplicate.

### Validation of SPE method for assay of L-muscone

The aqueous calibration solutions containing L-muscone at increasing amounts (0.0, 0.1, 0.5, and 2.0 µg/mL) and IS at constant amount (1.5 µg/mL) were used for the method validation. An aliquot (1 mL) of each sample was subjected to SPE with subsequent GC analysis as described in the previous section. The peak area ratios relative to IS were used for all the quantitative calculations. Linearity for quantitative assay for L-muscone was tested by least-squares regression analysis on the peak area ratios against increasing concentration ratios to plot calibration curves. The intra- and inter-day precisions expressed as a percentage of the relative standard deviation (% RSD) and accuracy as a percentage of the relative error (% RE) of the method were determined from calibration samples at the three different concentrations in triplicate. The recovery rates were assessed by comparing the percentages of peak area ratios of extracted samples prepared by the SPE to those of non-extracted counterparts (representing 100% recovery) dissolved in toluene at the same nominal concentrations.

### Sample preparation for L-muscone assay in a L-muscone drink product

Aliquots (1.0 mL) from each bottle of a locally purchased L-muscone drink product were subjected to the SPE procedure after addition of IS (1.5  $\mu\text{g}$ ) as described in the previous section, followed by GC and GC-MS analysis. The drink product tested was labeled as being fortified with L-muscone at 1.5  $\mu\text{g}/\text{mL}$ . The within-batch accuracy in the labeled concentration was expressed as the percent relative error (% RE) of the measured concentrations.

## RESULTS AND DISCUSSION

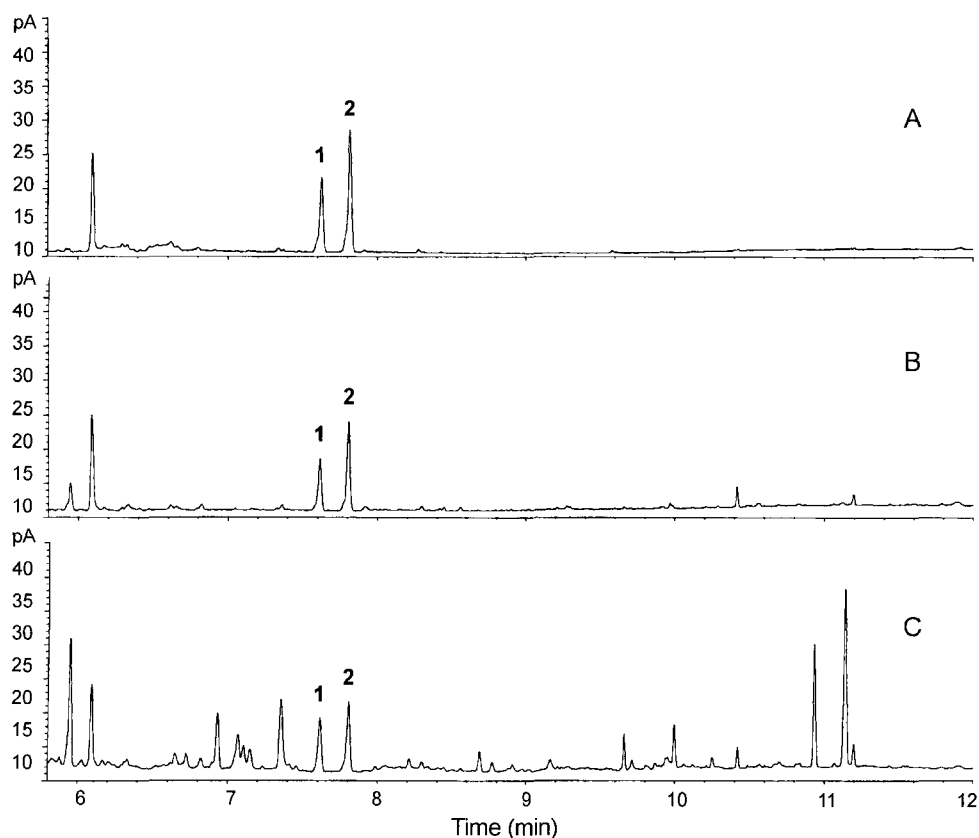
### GC profiling analysis

When the standard solution of L-muscone and IS in toluene was analyzed under the present GC condition, they were well resolved as symmetric peaks along with an unknown peak within 8 min (Fig. 1A). The SPE eluate of an aqueous standard sample showed several minor unknown peaks that might be originated from XAD-4, solvents or water (Fig. 1B). However, they did not interfere

with the quantitation of L-muscone and IS. When the SPE eluate of a commercial L-muscone product was analyzed, much more increased numbers of co-extracted compounds were detected (Fig. 1C). L-muscone and IS were, however, completely resolved with no interfering peaks nearby, indicating that the present method was specific for L-muscone assay.

### Solid-phase extraction and method validation

Prior to loading into the activated SPE column, each aqueous solution required thorough sonication due to the insolubility of L-muscone and IS in water. No breakthrough of L-muscone and IS from the column was observed during the rinsing of the column with water (5 mL), indicating strong adsorption of L-muscone on the hydrophobic XAD resin surface. The less-polar dichloromethane modified with 10% (v/v) polar methanol was found to be optimal as the eluting solvent to recover L-muscone from the column with small solvent volume (5 mL). The present three-step SPE procedure took only 30 min including solvent evaporation from the eluate.



**Fig. 1.** Gas chromatograms for neat standard sample (A), samples solid-phase extracted from an aqueous standard solution (B) and from a L-muscone drink product (C). GC conditions: DB-5MS fused-silica capillary column (15 m  $\times$  250  $\mu\text{m}$  I.D., 0.25  $\mu\text{m}$  film thickness), initially at 80  $^{\circ}\text{C}$  for 2 min, raised to 190  $^{\circ}\text{C}$  at a rate of 30  $^{\circ}\text{C}/\text{min}$  and to 210  $^{\circ}\text{C}$  at 10  $^{\circ}\text{C}/\text{min}$ , and finally at 30  $^{\circ}\text{C}/\text{min}$  to 300  $^{\circ}\text{C}$  (3min); 1.0  $\mu\text{L}$  sample injected in the pulsed splitless injection mode (purge delay time 42 sec); helium as carrier gas at 0.8 ml/min in constant flow mode; injector and detector temperatures at 260 and 300  $^{\circ}\text{C}$ , respectively. Peak: 1 = IS (cyclopentadecanone); 2 = L-muscone.

The calibration curve measured at the concentration range of 0.1-2.0 mg/mL with the present method was linear ( $r^2 = 0.9994$ ) with the following regression equation:  $y = 1.17x + 0.04$ , where  $y$  was peak area ratio and  $x$  was amount ratio of L-muscione relative to IS. The excellent overall linearity proved suitability of the present method for quantitative measurements of L-muscione in unknown samples. The ranges of intra- and inter-day precisions (% RSD) of the overall procedure measured at three different concentrations (0.1, 0.5 and 2.0  $\mu\text{g/ml}$ ) varied from 2.5 to 6.2 and from 6.1 to 7.3, respectively (Table I). These values of the present method indicated that the level of L-muscione could be measured with acceptable precision. The recovery rates examined at 0.1, 0.5 and 2.0  $\mu\text{g/mL}$  were 98.1, 98.4 and 99.2%, respectively (Table II). These high recovery rates with good precision (% RSD  $\leq 6.4$ ) ensured the correct quantitation.

### Precision and accuracy in quantitation of L-muscione in a drink product

**Table I.** Intra- and inter-day precisions for the assay of L-muscione

Concentration ( $\mu\text{g/mL}$ )	Mean area ratio $\pm$ SD <sup>a</sup> (% RSD) <sup>b</sup>	
	Intra-day	Inter-day
0.1	0.102 $\pm$ 0.006 (6.2)	0.100 $\pm$ 0.007 (7.0)
0.5	0.452 $\pm$ 0.011 (2.5)	0.406 $\pm$ 0.030 (7.3)
2.0	1.601 $\pm$ 0.045 (2.8)	1.586 $\pm$ 0.097 (6.1)

<sup>a</sup> Mean area ratio of L-muscione to IS (n=3)

<sup>b</sup> Relative standard deviation

**Table II.** Efficiency of solid-phase extraction for L-muscione assay

Concentration ( $\mu\text{g/mL}$ )	Mean area ratio $\pm$ SD (% RSD) <sup>a</sup>		Recovery <sup>b</sup> (%)
	Non-extracted	Extracted	
0.1	0.077 $\pm$ 0.005 (1.0)	0.075 $\pm$ 0.005 (6.4)	98.1
0.5	0.418 $\pm$ 0.010 (2.0)	0.412 $\pm$ 0.018 (4.4)	98.4
2.0	1.577 $\pm$ 0.020 (1.2)	1.565 $\pm$ 0.052 (3.3)	99.2

<sup>a</sup> Area ratio of L-muscione to IS (Relative standard deviation for n=3)

<sup>b</sup> (Extracted / Non-extracted)  $\times$  100

**Table III.** Accuracy for L-muscione assay in a drink product fortified with L-muscione

Batch No.	Concentration ( $\mu\text{g/mL}$ )		Accuracy RE (%) <sup>b</sup>
	Nominal	Mean measured $\pm$ SD (% RSD) <sup>a</sup>	
1	1.500	1.464 $\pm$ 0.046 (3.1)	-2.4
2	1.500	1.481 $\pm$ 0.042 (2.8)	-1.3
3	1.500	1.451 $\pm$ 0.045 (3.1)	-3.3

<sup>a</sup> Relative standard deviation for n = 3

<sup>b</sup> Relative error; {(measured mean value - nominal value)/nominal value}  $\times$  100

When the present method was applied to a L-muscione drink product, the within-batch RE (%) values in the labeled concentration (1.5  $\mu\text{g/mL}$ ) for the three randomly chosen bottles were -2.4, -1.3 and -3.3 respectively with high precision (% RSD  $\leq 3.1$ ) (Table III). These high accuracy values suggested that the drink products examined in this study were prepared under good quality control.

## CONCLUSIONS

The present SPE employing XAD as the sorbent with minimal volume (5.0 mL) of dichloromethane modified with 10% (v/v) methanol as the eluting solvent provided quantitative recovery of L-muscione from complex aqueous solution with rapidity and minimal labor. The overall method linearity ( $r^2 \geq 0.9994$ ), intra- and inter-day precisions (% RSD = 2.5~7.3), extraction recovery rate ( $\geq 98.1\%$ ) and accuracy (% RE = -3.3~-1.3) were satisfactory for the precise and accurate quantitation of L-muscione in unknown samples. The GC analysis provided complete separation of L-muscione from the co-extracted interferences. Therefore, the method is considered to be suitable for quality control evaluation tests on liquid preparations and other complex formulations fortified with L-muscione.

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## REFERENCES

- Enoch, P., Putzler, A., Rinne, D., and Schlüter, J., Automated solid-phase extraction on-line coupled to gas chromatography. *J. Chromatogr. A*, 822, 75-82 (1998).
- Filho, I. D. N., Schosler, P., Freitas, L. S., Melecchi, M. I. S., Vale, M. G. R., and Caramão, E. B., Selective extraction of benzoic acid from landfill leachate by solid-phase extraction and ion-exchange chromatography. *J. Chromatogr. A*, 1027, 167-170 (2004).
- Hong, X. and Wang, Z., A preliminary research on the quality of musk. *Zhongguo Zhong Yao Za Zhi.*, Apr; 16 (4), 230-232 (1991).
- Jacob, S., Garcia, S. Hayreh, D., and McClintock, M. K., Psychological effects of musky compounds: comparison of androstadienone with androstenol and muscone. *Horm. Behav.*, 42, 274-283 (2002).
- Jung, M. W., Ahn, K. H., Lee, Y., Kim, K. P., Paeng, I. R., Rhee, J. S., Park, J. T., and Paeng, K. J., Evaluation on the adsorption capabilities of new chemically modified polymeric adsorbents with protoporphyrin IX. *J. Chromatogr. A*, 917,

- 87-93 (2001).
- Kamat, V. P., Hagiwara, H., Katsumi, T., Hoshi, T., Suzuki, T., and Ando, M., Ring closing metathesis directed synthesis of (R)-(-)-muscone from (+)-citronellal. *Tetrahedron*, 56, 4397-4403 (2000).
- Kim, K. R., Shin, Y.J., Shim, W. H., and Myung, S. W., Rapid gas chromatographic profiling and screening of acidic non-steroidal anti-inflammatory drugs in biological samples. *Arch. Pharm. Res.*, 17 (3), 175-181 (1994).
- Kim, K. R., Paik, M. J., Kim, J. H., Dong, S. W., and Jeong, D. H., Rapid gas chromatographic profiling and screening of biologically active amines. *J. Pharm. Biomed. Anal.*, 15, 1309-1318 (1997).
- Kim, K. R., Park, H. G., Paik, M. J., Ryu, H. S., Oh, K. S., Myung, S. W., and Liebich, H. M., Gas chromatographic profiling and pattern recognition analysis of urinary organic acids from uterine myoma patients and cervical cancer patients. *J. Chromatogr. B*, 712, 11-22 (1998).
- Lee, S. M., Cho, T. S., Shim, S. H., Park, S. K., Hong, C. Y., Kim, S. S., Shin, D. H., Kim, Y. K., and Park, D. K., General pharmacology of L-Muscone. *J. Appl. Pharmacol.*, 5, 292-298 (1997).
- Lepane, V., Comparison of XAD-4 resins for the isolation of humic substances from seawater. *J. Chromatogr. A*, 845, 329-335 (1999).
- Vera-Avila, L. E., Gallegos-Perez, J. L., and Camacho-Frias, E., Frontal analysis of aqueous of phenol solutions in amberlite XAD-4 columns, Implication on the operation and design of solid-phase extraction system. *Talanta*, 50, 509-526 (1999).
- Vilar, R. B. C., Silva, V. R. da., Schossler, P., Veses, R. C., Piatnicki, C. M. S., Samios, D., and Caramão, E. B., Preliminary characterization of anhydrous ethanol used Brazil as automotive fuel. *J. Chromatogr. A*, 985, 367-373 (2003).
- Wu, Y., Wu, P., Xiao, X., and Yu, D. Q., Method of analysis for musk, artificially bred musk and original musk by HPLC. *Yao Xue Xue Bao*, 24 (4), 308-311 (1989).
- Zhang, Z. H., and Yue, P. F., Quantitative analysis of muscone in musk by GC. *Yao Xue Xue Bao*, May; 16 (5), 371-378 (1982).
- Zou, Q. G. and Su, J. J., Quantitative analysis of muscone in shexiang baixin pills by gas chromatography. *Zhongguo Yao Za Zhi*, Jul; 19 (7), 418-419 (1994).