[Chem. Pharm. Bull. 34(2) 906—908 (1986)]

Rapid Analysis of Muscone in Musk by High-Performance Liquid Chromatography

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(Received July 6, 1985)

Muscone in musk was successfully determined by high-performance liquid chromatography using a refractive index (RI) detector after extraction with ether.

Keywords——muscone; musk; quantitative analysis; high-performance liquid chromatography

Musk, which is well-known as the basis of numerous perfumes, is one of the most important and expensive Chinese crude drugs. Since its active principle has not yet been elucidated, both the analysis of muscone, the characteristic aromatic component, and organoleptic examinations such as odor, color, and taste, are still indispensable for the quality evaluation of musk. Several studies on the analysis of muscone have been reported.[1-4] Recently, a reliable gas chromatographic method combined with a preparative thin-layer chromatography was also reported by Kubo *et al.*[5] On the other hand, little work has been done on the high-performance liquid chromatography (HPLC) of muscone. This is probably because the commonly used method of ultraviolet (UV) detection is inappropriate in the case of muscone, which lacks UV absorption.

This note deals with the rapid analysis of muscone in musk by HPLC using a refractive index (RI) detector. The method is sufficiently sensitive to analyze commercial musk.

Experimental

Apparatus——The analyses were carried out on a Waters ALC/GPC 206D high-performance liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) equipped with an RI detector (Showa Denko model Shodex SE-11), a UV detector (Shimadzu model SPD-1), and a stainless-steel column, Zorbax ODS (4.6 mm i.d. × 25 cm).

Materials——Muscone was isolated by the use of preparative YMC gel ODS (20 mm i.d. × 25 cm) (mobile phase: CH_3CN) from the ether extract of Chinese musk, and its identity was checked by proton and carbon-13 nuclear magnetic resonance (^1H- and ^{13}C-NMR) spectral examinations. Cyclopentadecanone (CPD) was purchased from Aldrich Chemical Co., Inc.

Recommended Procedure for Sample Analysis——Mix a musk sample (0.200 g) with sea sand (*ca.* 0.5 g) in an alumina mortar. After addition of CPD (3.5 mg), extract with ether (150 ml) for about 6 h in a Soxhlet extractor. Evaporate the ether solution in the flask of the Soxhlet extractor nearly to dryness under reduced pressure. Add 0.8 ml of CH_3CN to the residue and dissolve it by heating on a water bath. After allowing to stand for a while, transfer the CH_3CN solution into a small volumetric flask (1 ml). Add a further 1.0 ml of CH_3CN to the flask of the Soxhlet extractor, dissolve the residue by heating on a water bath, and concentrate to about 0.2 ml under reduced pressure. After allowing to stand for a while, transfer the solution to the same volumetric flask (1 ml) and combine it with the former CH_3CN solution (0.8 ml). Dilute the combined solution to the volume of 1 ml with CH_3CN if its volume is less than 1 ml due to evaporation of the solvent during the preparation. Inject 20 μl of each sample solution into the column with a microsyringe. Calculate the quantity of muscone by reference to a previously prepared calibration curve (Fig. 3).

Recovery Test for Muscone——Muscone (2.0 mg) in ether (1 ml) and CPD (3.5 mg) were added to a powdered mixture of a musk sample (No. 3, 0.200 g) and sea sand (0.5 g), and then extracted with ether (150 ml) for about 6 h in a Soxhlet extractor. The following analysis of muscone was done according to the recommended procedure.

Results and Discussion

The reversed-phase partition mode is suitable for the analysis of muscone by HPLC because of the high lipophilicity of the compound. Thus, analytical conditions were examined using a Zorbax ODS column with the CH_3CN–H_2O system. Figure 1 shows the relationship between the k' values of muscone and CPD and the water concentration in the mobile phase. CH_3CN–H_2O (95 : 5) was chosen by taking into account both the rapidity of analysis and the separation from CPD. Muscone was monitored using the RI detector because of its lack of absorption in the UV region. Figure 2 shows chromatograms of the ether extract from musk (No. 3) obtained with the RI and UV detectors. RI detection was clearly more effective than UV detection at 210 nm. Muscone was successfully separated from other components such as steroids and choresterol.[6] The calibration curve was constructed by plotting the ratio of the peak area of muscone to that of CPD, added as an internal standard. Good linearity was observed in the concentration range of muscone in musk from 0.6 to 3.1% (Fig. 3).[7] Next, the extraction conditions were investigated. Several workers have successfully extracted muscone with ether from musk.[1,2,5] In fact, a known amount of muscone added to a musk sample (No. 3) was satisfactorily recovered, as shown in Table I. Reproducibility of the

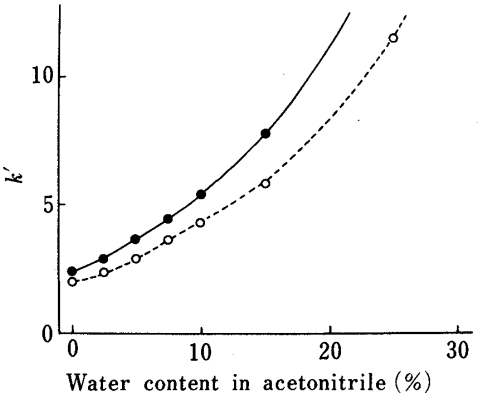

Fig. 1. Plot of k' Value against Water Content of Acetonitrile

Mobile phase: CH_3CN–H_2O system. —●—, muscone; ---○---, CPD.

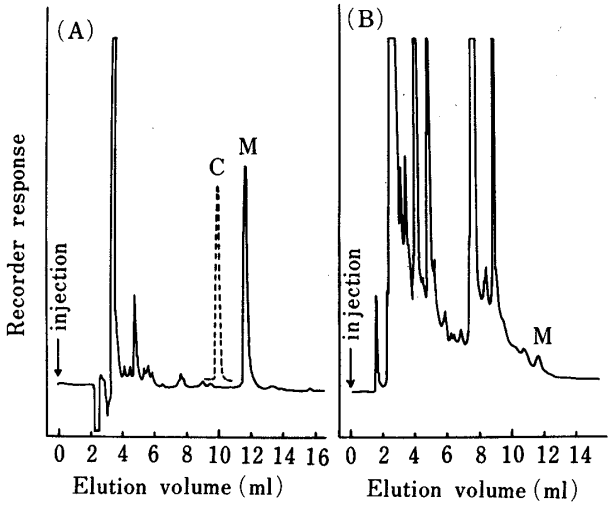

Fig. 2. Chromatograms of the Ether Extract of Musk

(A) Column, Zorbax ODS (4.6 mm i.d. × 25 cm); mobile phase, CH_3CN–H_2O (95 : 5); flow rate, 1.0 ml/min; detection, RI; temperature, 25 °C.
(B) Detection, UV (210 nm). Other chromatographic conditions were the same as in (A).
M, muscone; C, CPD.

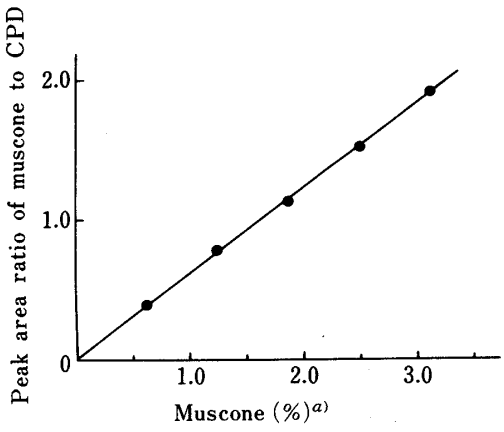

Fig. 3. Calibration Curve for Muscone Analysis Using CPD as an Internal Standard

a) Muscone content (%) of musk sample.

Table I. Recovery Test for Muscone Added to Musk

In musk[a] (mg)	Added (mg)	Found (mg)	Recovery rate Mean ± S.D. (%)
4.20	2.00	6.15, 5.99, 6.08	98 ± 1.3

a) Figures represent the amount of muscone in 0.200 g of the musk sample (No. 3).

Table II. Analytical Results for Muscone in Some Musk Samples by HPLC

Musk Sample No.	Muscone content in musk (%)
1	2.9
2	1.6
3	2.1
4	1.0
5	<0.02

overall procedure using sample No. 3 was 2.0% (CV) for 8 measurements.

The above results led us to propose the recommended procedure described in Experimental for the determination of muscone in musk by HPLC.

Some musk samples purchased in Osaka market were analyzed by the established procedure. The results are listed in Table II. The analytical values are in the range of less than 0.02 to *ca.* 3.0%. Samples No. 1, 2, and 3 are in order of quality from a particular company according to the labelling. Kubo *et al.* reported that the content of muscone was correlated to the grade of musk in the products of each company.[5] In this work, however, the cheapest sample (No. 3) contained muscone at a higher level than the medium grade sample (No. 2). Analysis for muscone in pharmaceutical preparations such as "Rokushingan" was attempted, but it was difficult because of the appearance of peaks due to other crude drugs at the positions close to the peak of muscone.

In conclusion, an HPLC method for the determination of muscone in musk was developed using an RI detector after ether extraction. This procedure does not require complex pretreatment, and in addition, the time required for analysis is shorter than that in the method of Kubo *et al.*[5] This method is cosidered to be suitable for the analysis of muscone in musk in order to evaluate the quality of musk, at least until the active principle of musk is identified.

References and Notes

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7) When the ratio of the peak heights was used, the calibration curve was linear in the concentration range of muscone up to 2.0%, but not at higher concentrations, probably due to slight tailing of the peak of muscone.