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Analysis of Patchouli Alcohol by HPLC using Core-Shell Column

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Abstract

Objective: The aim of this study was to determine the conditions for rapid quantitation of Patchouli Alcohol (PA) by Liquid Chromatography (LC) using a core-shell technology column whose porous layer is formed by copolymerization of styrene and divinylbenzene.

Method: PA was dissolved in ethanol and serially diluted. A calibration curve was prepared from the ratio of peak areas obtained on each chromatogram using an absolute calibration curve method. The calibration curves were used to calculate the detection limit (DL) and quantitation limit (QL).

Results: PA was measured using HPLC with the mobile phase CH_3CN/Hexane/H_2O (67/3/30) at a wavelength of 205 nm. The PA peak was observed at a retention time of 10.8 minutes and the degree of separation was 4.5. The calibration curve prepared under this condition had a correlation coefficient of R>0.997 and sufficient linearity. The quantitation limit and detection limit calculated using these calibration curves were 2.08 μg/mL and 6.31 μg/mL, respectively.

Conclusion: Comparing HPLC to GC for the speed of quantifying PA, HPLC is more useful than GC because it takes half the time to measure. Improvement of the quantification method of PA will allow research aimed at expanding the use of PA to proceed more rapidly.

Keywords: Patchouli alcohol; Liquid chromatography; Core-shell column; Quantification

Introduction

Patchouli (*Pogostemon cablin*) is a plant of the Lamiaceae family and is used as a fragrance. The main component of patchouli essential oil used for fragrance and cosmetics is patchouli alcohol (PA; Figure 1) which is a tricyclic sesquiterpene alcohol [1]. In recent studies, it was reported that PA has an anti-inflammatory effect by inhibiting mediators of inflammation [2], an anti-photoaging effect by inhibiting matrix metalloproteinase (MMP)-1 and MMP-3 [3], and antibacterial activity against Helicobacter pylori by inhibiting urease [4].

Quantification of the primary component of a fragrance is generally performed by gas chromatography (GC) GC is also used to analyze volatile organic compounds such as PA [5]. GC is unsuitable for the analysis of nonvolatile drugs and substances sensitive to heat. However, for the analysis of volatile drugs, it is a general-purpose analytical method with high resolution and high detection sensitivity. However, the measurement time of PA by GC take about 25 minutes [6], so as a simple analysis method it could be improved upon.

Recently, quantitative analysis has been performed in liquid chromatography (LC) with a core-shell column [7]. The filler in the core-shell column is not entirely porous, but rather has a solid core at the center of the column surrounded by a porous layer. Because the core-shell column has a hard core at the center, longitudinal diffusion of the solute is inhibited. The porous layer on the outside of the column produces a short diffusion distance because it is thin. The features of core-shell columns produce higher resolution, shorter analysis time, and lower backpressure than conventional all-porous columns. Core-shell columns are used for analysis of carbohydrates and amino acids, and are expected to be particularly applicable to the fields of food and fragrance.

In order to quantitate PA, ingenuity is necessary, because PA lacks a chromophore to measure. On the other hand, it has been reported that amantadine, a terpene drug contained in meat, can be quantitatively measured using LC with a core-shell column [8]. Therefore, the objective of this study was to investigate the conditions for quantitation of PA using LC with a core-shell column whose porous layer is formed by copolymerization of styrene and divinylbenzene.

Materials and Methods

Reagents

PA was donated by Malya Optima Indonesia. HPLC grade ethanol (Lot TWN2116) and acetonitrile (Lot TWN2635), were purchased from Wako Pure Chemical Co., Ltd. Special grade hexane reagent was purchased from Wako Pure Chemical Co., Ltd. Other standard reagents were purchased from Wako Pure Chemical Co.

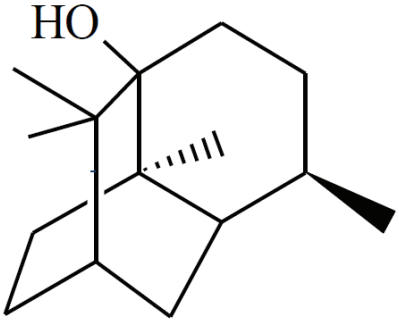

Figure 1: Structure of PA

Quantitative condition

GC measurement condition: GC measurements were performed using a GC-2010 Plus instrument (SHIMADZU Corp.) with an Rtx-1 (30 m × 0.25 I.D. × 0.25 μm) column. The column temperature was raised from 55°C to 134°C (15°C/min), held for 5 minutes, and then raised from 134°C to 143°C (1°C/min). After holding again for 5 minutes, it was raised from 143°C to 230°C (10°C /min) and held for 1 minute. Injector and detector temperatures were both set at 250°C. The carrier gas was nitrogen with a flow rate of 1.3 mL/min. Flame ionization detector (FID) was used for detection.

HPLC measurement condition: HPLC measurements were performed using an Alliance Separations Module e2695 instrument (Waters) with a core-shell type porous polymer (4.6 mm (I.D.) × 150 mm (L)) column. The porous layer is formed by copolymerization of styrene and divinylbenzene on the surface of the core with no exchange group. The polymerization ratio of styrene to divinylbenzene is 60/40. Column temperature was set at 40°C. The mobile phase was composed of acetonitrile/hexane (97/3, v/v) or acetonitrile/hexane/water (67/3/30, v/v/v). Flow rate was set at 1 mL/min. A UV/Visible Detector 2489 was used for detection at a wavelength of 205 nm and 210 nm.

Calculation of detection limit and quantitation limit by GC and HPLC

PA was dissolved in ethanol and serially diluted from 56.5 μg/mL for HPLC and from 61.5 μg/mL for GC. The calibration curve was prepared from the peak area ratio obtained from each chromatogram using an absolute calibration curve method. Detection limit (DL) and quantitation limit (QL) were calculated with their respective calibration curve.

DL = 3.3s/a (eq.1)

QL = 10s/a (eq.2)

s; SD of blank sample

a; The slope of the calibration curve near the detection limit

Results and Discussion

Measurement of PA by GC

Measuring PA by GC, the measurement time was 34 minutes and the PA peak was observed at 23 minutes (Figure 2). This result was similar to those reported previously [9].

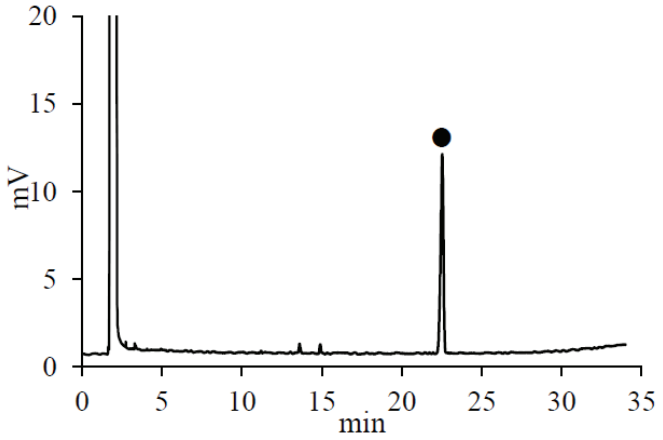

Figure 2: Calibration curves
a) HPLC; CH_3CN/Hexane/H_2O (67/3/30), 205 nm
b) GC

Investigation of HPLC conditions (Figure 3)

With the mobile phase acetonitrile/hexane (97/3) and wavelength 210 nm on HPLC, the peak of PA was observed at 7.6 minutes and the degree of separation was 2.0 (Figure 3a). The solvent peak appeared broadly near the PA peak creating an overlap causing a poor degree of separation. To improve the separation between PA and other peaks, the mobile phase and wavelength were considered.

The mobile phase was changed to acetonitrile/hexane/water (67/3/30) and measurement was carried out at a wavelength of 210 nm. The PA peak was observed at 10.1 minutes and the degree of separation was 4.4 (Figure 3b). The addition of water increased the polarity of the mobile phase and the appearance of the PA peak was delayed. However, under these conditions the baseline was disturbed, so it was considered unsuitable for quantification. Next, using the mobile phase acetonitrile/hexane (97/3), the wavelength was changed to 205 nm. The PA peak was observed at 8.1 minutes and the degree of separation was 2.2 (Figure 3c). The PA peak was sharper than when the wavelength was 210 nm, but the solvent peak continued to overlap the PA peak. This was also considered unsuitable for quantification. Therefore, measurements were carried out with the mobile phase acetonitrile/hexane/water (67/3/30) measuring at a wavelength of 205 nm. The PA peak was observed at 10.8 minutes and the degree of separation was 4.5 (Figure 3d). This condition was preferable because the baseline was undisturbed and the PA peak did not overlap with solvent.

Calculation of detection limit and quantitation limit of GC and HPLC (Figure 4)

The calibration curves were prepared using PA solutions of known concentration. The GC calibration curve had a correlation coefficient of R>0.987 with sufficient linearity (Figure 4a). DL and QL were estimated to be 1.63 g/mL and 4.94 g/mL, respectively, using this calibration curve (Table 1). The HPLC calibration curve from a mobile phase of acetonitrile/hexane/water (67/3/30) at a wavelength of 205 nm had a correlation coefficient of R>0.997 with sufficient linearity (Figure 4b). DL and QL were estimated to be 2.08 g/mL and 6.31 g/mL, respectively, using this calibration curve (Table 1).

The quantification time for PA was 34 minutes by GC and 15 minutes by LC. The quantitative conditions for PA in LC are a mobile phase of acetonitrile/hexane/water (67/3/30) measuring at a wavelength of 205 nm. In terms of sensitivity and range, GC and LC are equivalent based on similar DL and QL values. Quantitative analysis of PA by LC showed the same detection power as GC, which is generally thought to have excellent detection capability. The LC analytical method was made simpler than GC by using a core-shell column.

When comparing the performance of the core-shell column to that of the monolithic and standard 3.5-μm-particle-size columns in order to qualitatively and quantitatively 18 common adulterants in herbal medicine and food samples, the results show that the core-shell column provides the best separation [10]. The main polyphenols in grapes can be quantified rapidly by using core-shell columns, which are used for quantitation and quality control [11]. Determination of aflatoxin B_1 contained in raw milk was more efficient and sensitive using a core-shell

Table 1: Detection limits and quantitation limits

	Detection limit (μg/mL)	Quantitation limit (μg/mL)
Condition	3.3 s/a	10 s/a
GC	1.63	4.94
HPLC (CH_3CN/Hexane/H_2O = 67/3/30)	2.08	6.31

s: SD of blank sample
a: The slope of calibration curve near the detection limit

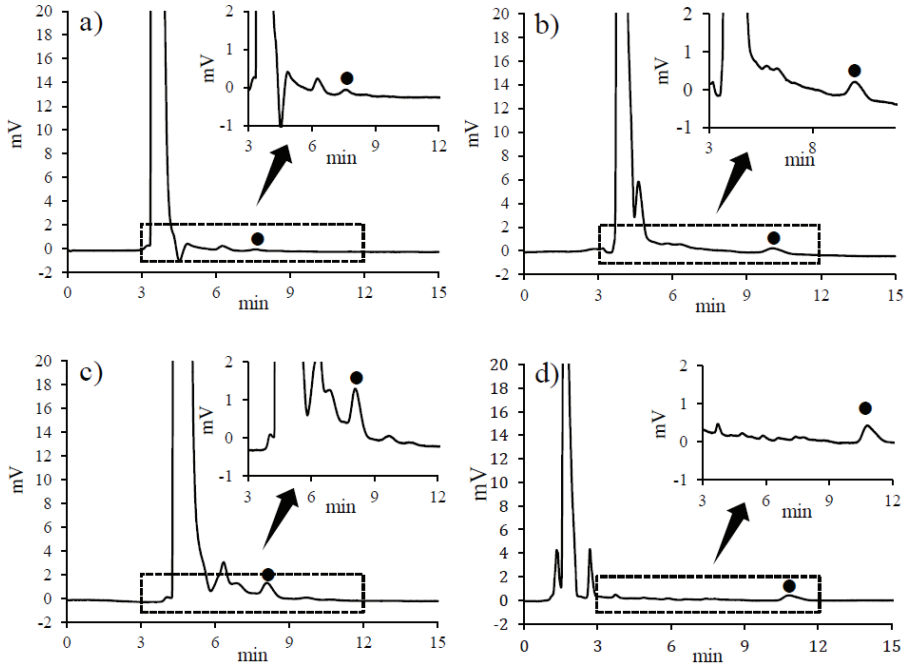

Figure 3: Chromatograms obtained using HPLC
a) CH_3CN/Hexane (97/3), 210 nm
b) CH_3CN/Hexane/H_2O (67/3/30), 210 nm
c) CH_3CN/Hexane (97/3), 205 nm
d) CH_3CN/Hexane/H_2O (67/3/30), 205 nm

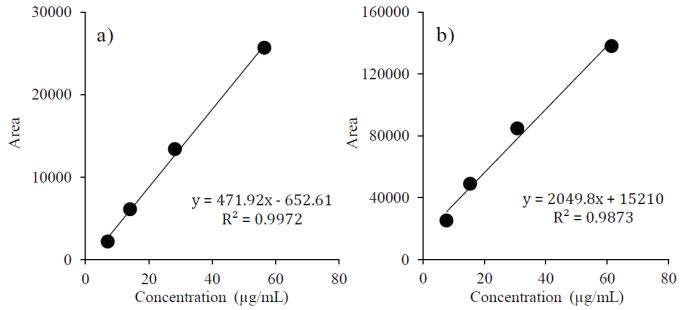

Figure 4: Calibration curves
a) HPLC; CH_3CN/Hexane/H_2O = 67/3/30, 205 nm
b) GC

column, which made processing of raw milk easier [12]. Based on this quantitative determination of PA, a core-shell column can be widely applied for quantitation of drugs having no specific chromophore. When HPLC and GC are compared in terms of speed, HPLC required half the measurement time, making it more useful than GC.

However, in this LC condition, hexane is included in the mobile phase, and it is possible that the LC pump is exposed to it. Compared to LC, the quantification of PA by GC is advantageous in that preparation of a mobile phase is unnecessary. Therefore, there is a need to continue exploring simple and easy quantitative methods.

Conclusion

LC measurement using the core-shell column shortened measuring time significantly, making it possible to quantitate PA in a short time. By improving the quantification method of PA, research aimed at expanding the use of PA should proceed more rapidly.

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Conflicts Of Interest

The authors declare that there are no conflicts of interests regarding the publication of this paper.

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