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Comparison of continuous subcritical water extraction and hydrodistillation of marjoram essential oil

M.M. Jiménez-Carmona[a], J.L. Ubera[b], M.D. Luque de Castro[a,*]

[a]*Analytical Chemistry Division, Faculty of Sciences, University of Córdoba, E-14004 Córdoba, Spain*

[b]*Department of Plant Biology and Ecology, Faculty of Sciences, University of Córdoba, E-14004 Córdoba, Spain*

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Abstract

Continuous subcritical water extraction (CSWE) and hydrodistillation were compared for the extraction of essential oil from marjoram leaves. Ground marjoram leaves (0.4 g) were subjected to dynamic extraction with water at 50 bar, 150°C and 2 ml/min for 15 min. Hydrodistillation was performed treating 140 g of marjoram leaves with 1000 ml of water for 3 h. When CSWE was used the compounds were removed from the aqueous extract by a single extraction with 5 ml of hexane, detected by gas chromatography–flame ionization detection (GC–FID) and identified by mass spectrometry, electronic impact. The CSWE method is quicker (15 min versus 3 h), provides a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes) and allows substantial savings of costs, in terms both of energy and plant material. The efficiency (in terms of volume of essential oil/1 g of plant) of CSWE is 5.1 times higher than that provided by hydrodistillation. The precision of the overall method (CSWE combined with GC–FID) is good (RSD less than 7.5%) for $n=5$.

Keywords: Continuous subcritical water extraction; Subcritical water extraction; Extraction methods; Marjoram; *Thymus mastichina*; Essential oils; Pinenes; Alcohols

1. Introduction

Essential oils are currently being extracted from natural products either by hydrodistillation or solvent extraction. Losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds through thermal or hydrolytic effects and toxic solvent residue in the extract may be encountered using these extraction methods. These shortcomings have led to the consideration of the use of supercritical fluids in essential oil extraction processes. CO_2 is the most commonly used supercritical fluid because of its modest critical conditions. Thus, the supercritical CO_2 extraction of essential oils from spices is well documented [1–8]. The green house effect caused by the emissions of carbon dioxide, the cost of the fluid with the required purity and specially its low dielectric constant (thus giving rise to a non-polar character that hinders or makes difficult the extraction of polar compounds) make mandatory the searching for new solvents. The use of subcritical water (SW) (pressure high enough to maintain water under liquid state and temperature

*Corresponding author. Tel.: +34-957-218-615; fax: +34-957-218-606.

E-mail address: qa1lucam@uco.es (M.D. Luque de Castro)

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between 100 and 374°C) under dynamic conditions is a promising trend in this context.

Subcritical water has been used in a continuous fashion to extract pollutants within a wide range of polarities from environmental samples [9–13]. The partitioning of organic compounds from gasoline and diesel fuel has been studied [14]. The coupling of continuous subcritical water extraction (CSWE) with inmmunoassay has provided fast and efficient extraction of pesticides [15] and that of polycyclic aromatic hydrocarbons in soil [16]. Subcritical water has also been used for the development of reversed-phase chromatographic separations [17,18]. The use of subcritical water for the extraction of essential oils from natural products is proposed in this work.

The aim of this research was to develop a rapid, efficient and inexpensive method for the extraction of the essential oil from marjoram. The proposed CSWE method has been applied prior to liquid-liquid extraction coupled to gas chromatography–flame ionization detection (GC–FID) or gas chromatography–mass spectrometry (GC–MS) for detection and identification of the extracted compounds, respectively.

2. Experimental

2.1. Instruments and apparatus

CSWE was performed using the following assembly: a Shimadzu LC10AD pump was used to propel the water used as extractant through the system. An extractor (a prototype designed and patented by Salvador and Merchán [19]), consisting of a stainless steel cylindrical extraction chamber, (8 cm×3 mm I.D.), closed with screws at either end that permit the circulation of the leaching fluid through them, was used. The screw caps also contain stainless steel filter plates (2 μm in thickness and 1/4 in. I.D.; 1 in.=2.54 cm) to ensure that the plant material remains in the extraction chamber. This chamber, together with a stainless steel preheater, is located in an oven, designed to work up to 300°C and controlled using a Toho TC-22 temperature controller. A cooler system, (consisting of a coil coupled to an Ultraterm 6000383 P-Selecta recirculation bath) was used to cool the fluid from the oven to a constant temperature close to 25°C, thus avoiding the losses of volatiles caused by the hot water. The outlet of this coil was coupled to a stainless steel home-made variable restrictor that was used to control the pressure in the system in order to maintain the extractant water in liquid state.

A Varian Star 3400 gas chromatograph equipped with a flame ionization detector was used for the analysis of the hexane extracts. A Clevenger extractor was used for the hydrodistillation. Finally, a Fisons VG Autospec (Micromass Instruments) mass spectrometer was used to identify compounds in the extracts.

2.2. Materials

Marjoram (*Thymus mastichina*) leaves were collected from a wild population growing in the South of Spain (Vadofresno, Córdoba). Stock standard solution of 3400 μg/ml of *n*-nonane (Sigma, St. Louis, USA) in HPLC grade hexane (Scharlau, Barcelona, Spain) was prepared. NaCl and Na_2SO_4 (both from Merck, Darmstadt, Germany) and HPLC-grade hexane were used as demulsifier, drying agent and extractant, respectively, in the liquid–liquid extraction step of the aqueous extracts. Bidistilled degassed water purified through a Milli-Q deionizing unit (Millipore, Milwaukee, USA) was used as extractant.

2.3. Sample preparation

Marjoram leaves stored in the dark at 4°C (0.4 g for subcritical water extractions and 140 g for hydrodistillations) were ground immediately prior to extraction in order to avoid losses of volatiles.

2.4. Procedure

2.4.1. Continuous extractions with subcritical water

Extractions were performed using the assembly described above. Degassed Milli-Q water stored in a reservoir was pumped to the oven, where it reached the preheater and passed through the 1 ml extraction chamber, which contained 0.4 g of ground marjoram leaves. The aqueous extract was cooled in the refrigerant at 25°C and, after passing through the

variable restrictor, collected in a vial. For kinetic experiments, the extraction was performed under optimum conditions and replacing the vial at preset intervals (5 min). 5 ml of hexane and 0.1 ml of *n*-nonane stock solution were added to each extract in a separating funnel and about 1 g of NaCl was added to facilitate the breaking of the emulsion. The hexane layer was then separated and dried with 0.1 g of anhydrous sodium sulphate before GC analysis.

2.4.2. Hydrodistillation procedure

One hundred and forty g of marjoram leaves was placed in the flask of a Clevenger extractor and extracted with 1000 ml of water steam for 180 min (until no more essential oil was obtained). 6.7 ml of essential oil was obtained after hydrodistillation. A 1:260 hexane dilution prior to GC analysis was required in order to compare the chromatograms of the oil obtained by subcritical water extraction with that from hydrodistillation.

2.4.3. Chromatographic separation–detection

Aliquots (1 μl) of the hexane extracts obtained after either subcritical water extraction plus liquid–liquid extraction or hydrodistillation, were injected into an SGL-5 fused-silica capillary column (25 m×0.25 mm I.D., 0.25 μm film thickness). The flow-rate of the carrier gas (helium) was 0.7 ml/min. The detector temperature was 310°C. The oven temperature was 50°C for 5 min, then increased to 250°C at a rate of 5°C/min.

2.4.4. Mass spectrometric identification

Fractions of the hexane extracts corresponding either to subcritical water extraction under the optimum working conditions or hydrodistillation were collected individually in order to identify components by mass spectrometry. Mass spectra (electronic impact) of the compounds were obtained by a Fisons VG Autospec instrument (70 eV) by direct insertion of 1 μl of solution at an ionization chamber temperature of 250°C.

3. Results and discussion

3.1. Optimisation of variables

The experimental variables were optimized in order to maximize the yield of essential oil in a time as short as possible. The ranges over which the variables were studied, and the optimum values found are listed in Table 1. The univariate method was used in all instances.

Table 1
Optimisation of Variables

Variable	Range studied	Optimum value
CSWE		
Temperature, °C	100–175	150
Pressure, bar	20–200	>5
Flow-rate, ml/min	0.5–3.0	2.0
Liquid–Liquid extraction		
Extractant volume, ml	5.0–15.0	5.0
Number of extraction steps	1–2	1
Chromatographic system		
Type of carrier		Helium
Injection volume, μl		1
Kind of column		SGL-5
Split/splitless ratio	0–1:4	1:4
Carrier flow-rate, ml/min	0.5–1.0	0.7
Rate of temperature gradient, °C/min	3.0–5.0	5.0

3.1.1. Variables of continuous extraction with subcritical water

An amount of 0.4 g of marjoram and an extraction time of 15 min were used for this study.

The *temperature of the extraction chamber* is the key variable when subcritical water is used as extractant. Its influence was studied between 100 and 175°C at a constant flow-rate of 1 ml/min and a pressure of ~50 bar. The yield increased with temperature up to 150°C. For the extraction carried out at 175°C, only one of the peaks corresponding to the major compounds in the extract was slightly higher than that obtained at 150°C. Therefore, the coextraction of undesirable compounds (as it can be inferred from the fact that a great deal of additional peaks appear in the chromatogram), such as paraffins and cuticular waxes, makes the solvent extraction step difficult and longer at the highest temperature. These are the reasons for selecting 150°C as optimum temperature.

The *pressure* was a key variable to maintain water under liquid state at temperatures over 100°C. To study the influence of this variable (once surpassed the minimum value require to ensure the liquid state of the extractant) a variable restrictor whose scheme is shown in Fig. 1was used. The internal diameter of the restrictor was hand-modified by using a pair of wrenches to screw or unscrew the upper part of the restrictor in order to raise or decrease the pressure of the system, respectively. A flow-rate of 1 ml/min and a temperature of 150°C were used. As pressures of 20, 90 and 200 bar (obtained by a progressive sealing of the variable restrictor) gave rise to chromatograms with no significant differences, an intermediate value of ca. 50 bar was used in further experiments.

The *flow-rate* was studied in the range 0.5–3 ml/min at a constant temperature of 150°C and pressure of about 50 bar. The height of the peaks in the chromatogram (and thus, the yield) increased with the flow-rate up to 2 ml/min and kept constant above this value. So, 2 ml/min was selected as optimum value.

3.1.2. Liquid–liquid extraction variables

Volumes of extractant (hexane) between 5 and 15 ml were tested in order both to remove the compounds from the aqueous extract and achieve a preconcentration effect prior to GC analysis. From the chromatograms, 5 ml appeared to be the optimum value, as it gave rise to the highest peaks.

Number of extraction steps. A second 5 ml hexane extraction was performed in order to ensure quantitativeness of this step. A flat chromatogram was obtained after injection of the second extract. Thus, a single 5 ml hexane extraction was enough for total removal of the compounds from the aqueous extract prior to GC analysis.

3.1.3. Chromatographic variables

The *split/splitless ratio* was studied by performing a previous injection in the splitless mode which provided out of scale peaks. The highest peaks within the suitable scale were obtained for a 1:4 split/splitless ratio.

A *flow-rate of the carrier* (helium) of about 0.7 ml/min (measured at the oulet of the column) provided a pressure at the head of the column of 188.5 bar and gave rise to suitable chromatographic signals.

A *temperature program* consisting of an initial temperature of 50°C held for 5 min and then increased to 250°C at a rate of 5°C/min provided the separation of the major compounds in about 30 min.

3.2. Features of the CSWE–chromatographic method

The precision of the chromatographic step was calculated by injecting one of the organic extracts from CSWE of 0.4 g of marjoram under the optimum working conditions. The average RSD ($n=5$) for the eleven identified compounds in Fig. 1 was of 3.2%. The precision of the overall method was studied by CSWE of 0.4 g of marjoram under the optimum conditions and injecting the hexane layer obtained after liquid–liquid extraction in the GC, obtaining an average RSD ($n=5$) for the eleven compounds of 7.1%. The efficiency of the method was studied by extracting 1 g of marjoram under the optimum conditions and measuring the volume of essential oil in the extract, which was 5.1 times higher than that obtained by hydrodistillation.

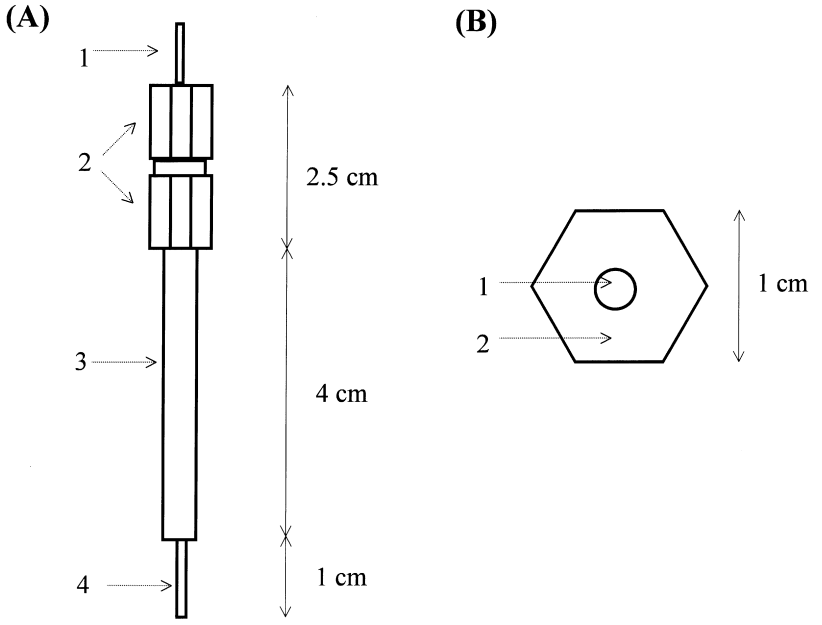

Fig. 1. Schematic diagram of the variable restrictor coupled to the extraction system from a side view (A) and upper view (B). 1: 1/16 in. O.D. stainless steel tubing; 2: adjustable pieces; 3: restrictor body; 4: outlet piece.

3.3. Kinetics of the leaching process

The kinetics of the CSWE under the optimum conditions for the identified compounds is shown in Fig. 2. The extraction rate for each compound can be qualitatively inferred from the plot. While compounds such as α-terpineol, eucalyptol and geraniol reach the plateau at 7.5 min (15 ml of water), other such as geranylacetate and 4-ethenyl-α,α, 4-trimethyl-3-(1-methylethenyl) cyclohexanemethanol show a slower extraction rate, following an increasing kinetic curve even up to 15 min. The monoterpenes are the most slowly extracted compounds and they show indistinguishable kinetic curves. Basicly, a good correlation between the solubilities of the compounds in water and the extraction rates can be observed. The kinetics of the extraction is strictly dependent on the amount of water passed through the extraction chamber, as can be proved by the fact that the cumulative area ratios for each compound at 150°C, about 50 bar and 1 ml/min after 15 min of extraction are fairly similar to those obtained under optimum conditions at 2 ml/min after 7.5 minutes of extraction (an amount of water of 15 ml passed through the extraction chamber in both cases). All these features show that the rate of extraction is determined by the partition of the compound between the plant material and the water and not by the rate of diffusion of the compound out of the plant material.

3.4. Comparison of CSWE and hydrodistillation methods

Subcritical water extraction combined with GC–FID, as well as hydrodistillation combined with GC–FID as methods for the extraction of essential oil from marjoram, are compared in terms of time, quality of the oil, efficiency and costs. The gas chromatograms of the extracts from hydrodistillation (A) and CSWE (B) are shown in Fig. 3. The comparison of composition for each extract is reported in Table 2. Substantial higher amounts of oxygenated compounds and lower amounts of terpenes are present in the CSWE extract as can be inferred from this table.

3.4.1. Time

One of the greatest advantages of the CSWE method is rapidity. An extraction time of 15 min

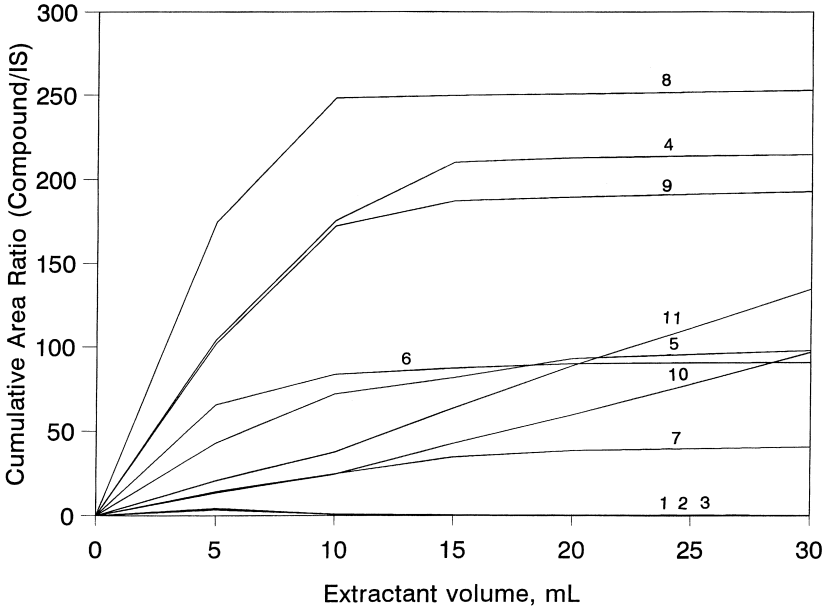

Fig. 2. Kinetic curves obtained by SWCE under optimum working conditions for (1) α-pinene; (2) β-pinene; (3) β-myrcene; (4) eucalyptol; (5) linalool; (6) 2-methyl-6-methylen-7-octen-2-ol; (7) terpinen-4-ol, (8) α-terpineol; (9) geraniol; (10) geranyl acetate and (11) 4-ethenyl-α,α, 4-trimethyl-3-(1- methylethenyl) cyclohexanemethanol (11). Eucalyptol peaks were divided by 8 in order to fit them into the scale.

provides comparable yields (as can be inferred from the chromatograms) to those obtained after 3 h of hydrodistillation.

3.4.2. Quality of the oil

Monoterpene compounds are less valuable than oxygenated compounds as they contribute to the fragrance of the oil in a minor extension. Conversely, the latter are highly odoriferous and, hence, the most valuable. The monoterpene hydrocarbons (α-pinene, β-pinene and β-myrcene) are present in larger amounts (22 times, 11 times and 22 times, respectively) in the hydrodistilled oil than in the CSWE oil, but the extract obtained by continuous extraction with water is more concentrated in oxygenated compounds. Therefore, the oil produced by CSWE gives a better reproduction of the natural aroma of the marjoram oil than the hydrodistilled oil.

3.4.3. Efficiency

The CSWE method is 5.1 times more efficient (that is, 6.7 ml of essential oil from 140 g and 244 μl of essential oil from 1.0 g of plant were obtained after hydrodistillation and CSWE, respectively) than hydrodistillation; this means a substantial saving of energy and plant material.

3.4.4. Cost

A higher amount of extractant is required for performing CSWE (extractant/mass of plant ratios of 10:1 and 1:1 for CSWE and hydrodistillation, respectively) as compared with hydrodistillation but this fact is clearly compensated by the energy saving allowed by the CSWE method. Thus, the energy cost required for water evaporation surpassed that required for reaching subcritical conditions (>5 bar and 150°C) about twenty times.

4. Conclusions

The proposed method consisting on continuous extraction with subcritical water combined with GC–FID is quicker than hydrodistillation coupled to GC–FID, provides a more valuable essential oil (since the oxygenated fraction is larger than the terpenic one) and allows substantial savings of both energy and investment cost. Its high precision makes it a good

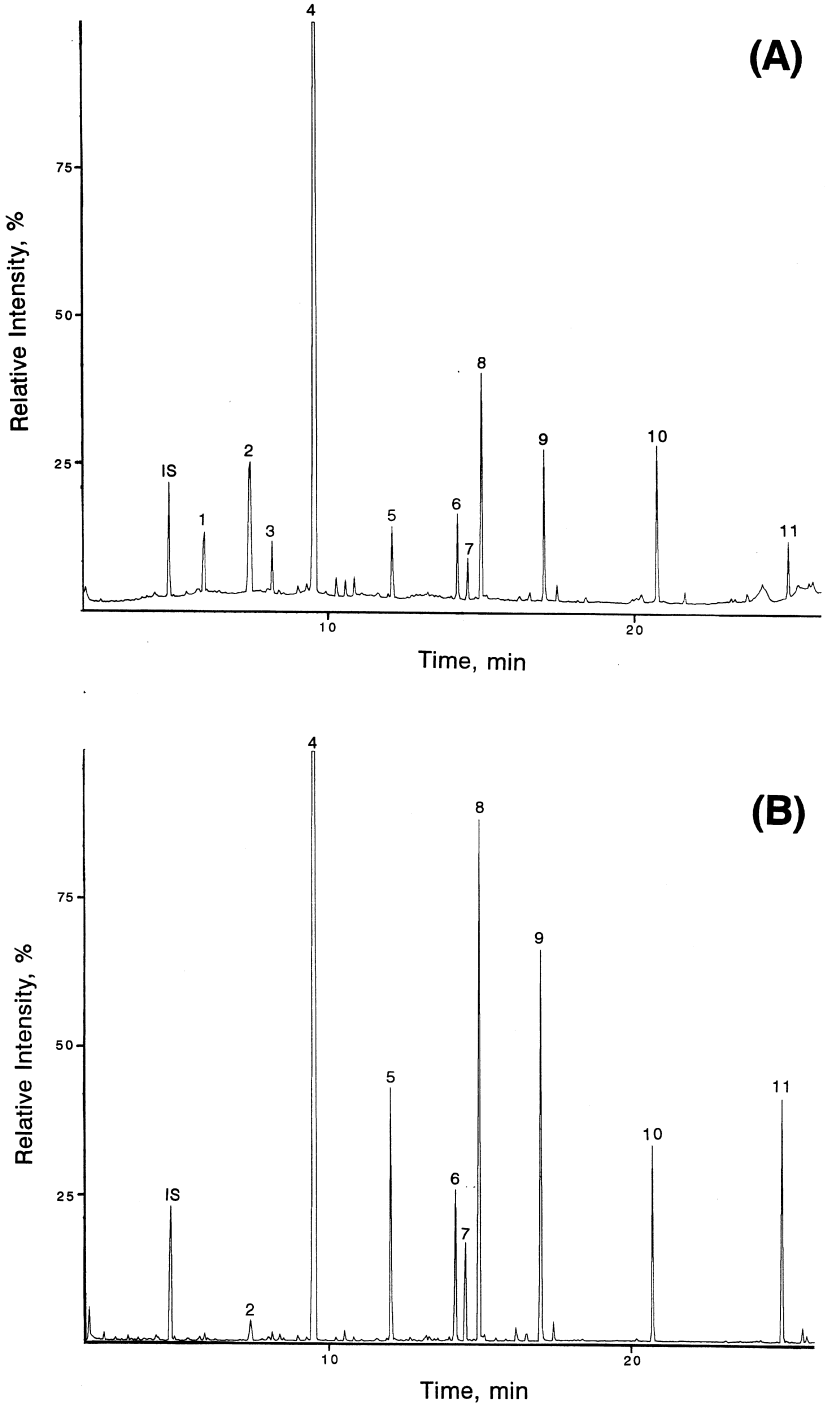

Fig. 3. Comparison of gas chromatograms of the extracts from hydrodistillation (A) and SWCE (B) of marjoram leaves under optimum working conditions. Peak identification: I.S., internal standard; (1) α-pinene; (2) β-pinene; (3) β-myrcene; (4) eucalyptol; (5) linalool; (6) 2-methyl-6-methylen-7 octen-2-ol; (7) terpinen- 4-ol; (8) α-terpineol; (9) geraniol; (10) geranyl acetate and (11) 4-ethenyl-α,α, 4-trimethyl-3-(1-methylethenyl) cyclohexanemethanol.

Table 2
Comparison of area compound/internal standard ratio for GC of extracts obtained by hydrodistillation and SWE under optimum working conditions

Compound	Hydrodistillation	SWE
α-Pinene	0.912	0.041
β-Pinene	1.616	0.149
β-Myrcene	1.853	0.083
Eucalyptol	24.810	18.865
Linalool	0.755	1.361
2-Methyl-6-methylen-7-octen-2 ol	0.748	0.873
Terpinen-4-ol	0.386	0.713
α-Terpineol	2.203	2.916
Geraniol	1.309	1.771
Geranyl acetate	1.342	0.755
ETMC[a]	0.853	1.487

[a] 4-Ethenyl-α,α 4-trimethyl-3-(1-methylethenyl)-cyclohexanemethanol.

alternative for the extraction of essential oils from aromatic plants.

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