Thermo Fisher Scientific
Molecular Spectroscopy
5225 Verona Rd, Madison, WI 53711
(608) 276-6100
www.thermoscientific.com

picoSpin™ 45/80: Extraction of Eugenol from Cloves

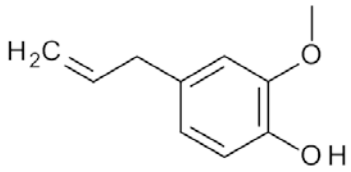
Dean Antic, Ph.D., Thermo Fisher Scientific, Boulder, CO, USA

1. Introduction

Essential oils are highly aromatic compounds extracted from a variety of botanical materials, including tree bark, flowers, stems, leaves, needles, plant roots, fruits and grasses. They are used in the production of perfumes, cosmetics, drinks, food flavoring, air fresheners, household cleaning products and aromatherapy oils. Essential oils also have a long history of use in traditional medicines. In health remedies, they find use as treatment oils, pastes and salves for minor aches, pains, congestion and coughing, and in therapeutic applications for anxiety, insomnia and relaxation.

The taste and aroma of natural essential oils are familiar to us. Oil of Wintergreen, an extract from the wintergreen group of plants, which contains 98% methyl salicylate, is a familiar fragrance and flavor associated with toothpaste and chewing gum. While we may relate a particular aroma with a single compound, the composition and aroma profile of essential oils is complex. Extracted oils are mixtures of varying concentrations of many compounds. In addition, essential oils are not oils in the conventional sense, that is, they are not long-chain hydrocarbon compounds, and their strong aroma does not always arise from the presence of an aromatic (phenyl) group in the oil's chemical composition. Instead, essential oils are complex mixtures of low viscosity fluids containing a surprising variety of molecular species and functional group chemistry.

Eugenol, the main ingredient in clove oil, is a familiar fragrance in many dental offices as it is often mixed into a paste and used in dentistry as a local antiseptic and anesthetic. It is a pale yellow oil with a warm, pungent, yet pleasing aroma, the smell of bay leaves and clove. Eugenol concentration in clove oil is as high as 90%. Eugenol is also found in bay leaves and allspice, and other botanical oil, but in lower concentrations.



Eugenol

Eugenol is a substituted methoxy phenol compound, a compound structurally similar to vanillin, but with an allylic functional group in place of the aldehyde in the *para* position. The allylic chain gives eugenol its characteristic strong odor.

Essential oils are extracted, depending on the nature of the botanical material, by a variety of techniques, including expression (cold-press), solvent, enfleurage (cold-fat) and supercritical fluid (SCF) extraction, and distillation. For instance, citrus essential oils are often extracted by cold pressing, a technique similar to household juicers. Solvent, SCF and enfleurage extraction are used with delicate materials, such as flower petals, and for making absolutes. Water distillation is employed with flowers, and indirect steam distillation is often used with leafy materials, seeds and pods. Most essential oils are extracted by direct or indirect steam distillation.

Steam distillation is a co-distillation technique that uses live steam to separate components of a mixture. It is effective at extracting high boiling-point components of essential oils, where boiling points are as high as 200°C. Yet the oil vapors themselves are closer to 100°C, thus helping to preserve the structural integrity of the compounds. It allows distillation to be performed at temperatures below the boiling points of the individual components. Indirect steam distillation is a technique used for generating steam in situ, where the water level is kept below the plant material.

Co-distillation is distillation of components of a mixture that are immiscible with water. Steam vaporizes the high-boiling essential oils and the hot vapors condense back into a liquid, along with water, as they pass through a cooling system. Since the oils are immiscible in water a two-phase distillate is produced, a water layer and an oil layer. The oil is usually less dense and floats atop the water. The aqueous layer can then be siphoned off using a separatory funnel. If an emulsion develops, as is often the case in small-scale distillation, the immiscibility of the hydrophobic oil makes extraction and isolation of the product with a non-polar organic solvent highly effective.

2. Purpose

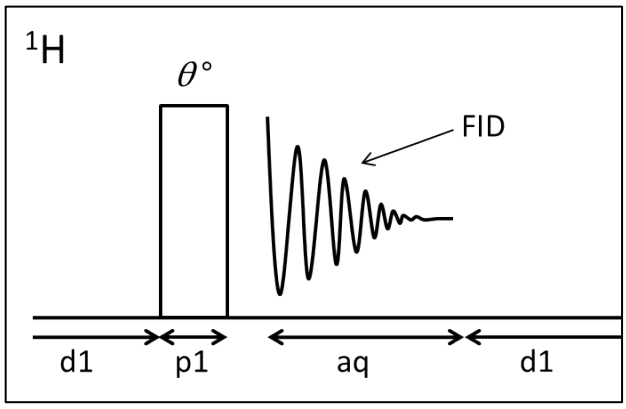
The purpose of this experiment is to extract eugenol from whole cloves by co-distillation with indirect steam, steam that is generated in situ. Eugenol will be extracted from the distillate with dichloromethane and analyzed using the Thermo Scientific™ picoSpin™ 45 or 80 NMR spectrometers.

3. Literature

Adapted from Williamson, K. L.; Minard, R.; Masters, K. M. Macroscale and Microscale Organic Experiments, 5th ed., Houghton Mifflin Co., 2007.

4. Pulse Sequence

In this experiment, we use a standard 90° single pulse experiment. The recycle delay time (d1) is adjusted to maximize signal intensity prior to signal averaging the next FID.



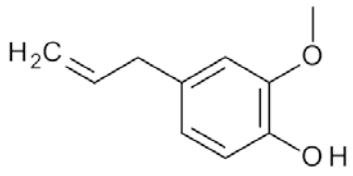
Sequence: d1–[θ°–aq–d1]$_{ns}$
θ°: Pulse rotation angle (flip angle)
FID: Free induction decay
d1: Recycle delay (µs) for spin-lattice relaxation
p1: R.F. transmitter pulse length (µs)
aq: Acquisition time (ms)
ns: # of scans (individual FIDs)

5. Procedures and Analysis

Time requirements: 5 hrs
Difficulty: Moderate
Sample: Eugenol
Equipment/materials:

- Thermo Scientific™ picoSpin™ 45 or 80
- Cloves, whole
- Dichloromethane (CH_2Cl_2)
- Tetramethylsilane (TMS; $(CH_3)_4Si$)
- Steam distillation apparatus
  - 250 mL round bottom flask
  - 100 mL Erlenmeyer flask
  - Claisen adapter
  - Condenser w/water jacket
  - Three-way adapter
  - Vacuum adapter
  - Clamps (flask or Keck)
  - Ring stand, ring clamp, iron ring
- Thermometer
- Thermometer adapter
- Boiling chips
- Mnova NMR Processing Suite
- picoSpin accessory kit:
  - Port plugs
  - Syringe port adapter
  - Drain tube assembly
- 50 mL beaker
- 1 mL polypropylene syringes
- 22 gauge blunt-tip dispensing needles
- 7 mL vial

Molecules:



Eugenol

Physical data:

Substance	FW (g/mol)	Quantity	MP (°C)	BP (°C)	Density (g/mL)
cloves, whole		25 g			
dichloromethane	84.93	45 mL	-96.7	39.6	1.3266
sodium sulfate, anhydr.					
tetramethylsilane (TMS)*	88.22	3 drps	-99	26-28	0.648
chloroform-d ($CDCl_3$) w/1%TMS*	120.384	1 mL	-64	61	1.50
acetone-d_6 (Ac-d_6) w/ 1%TMS*	64.12	1 mL	-94	56	0.872

*Optional NMR solvents and chemical shift reagent

Safety Precautions

CAUTION Eye protection should be worn at all times while using this instrument.

CAUTION Avoid shock hazard. Each wall outlet used must be equipped with a 3-prong grounded outlet. The ground must be a noncurrent-carrying wire connected to earth ground at the main distribution box.

Experimental

Distillation procedure

- Set up a steam distillation apparatus (Figure 1). In this setup, steam is generated in situ.

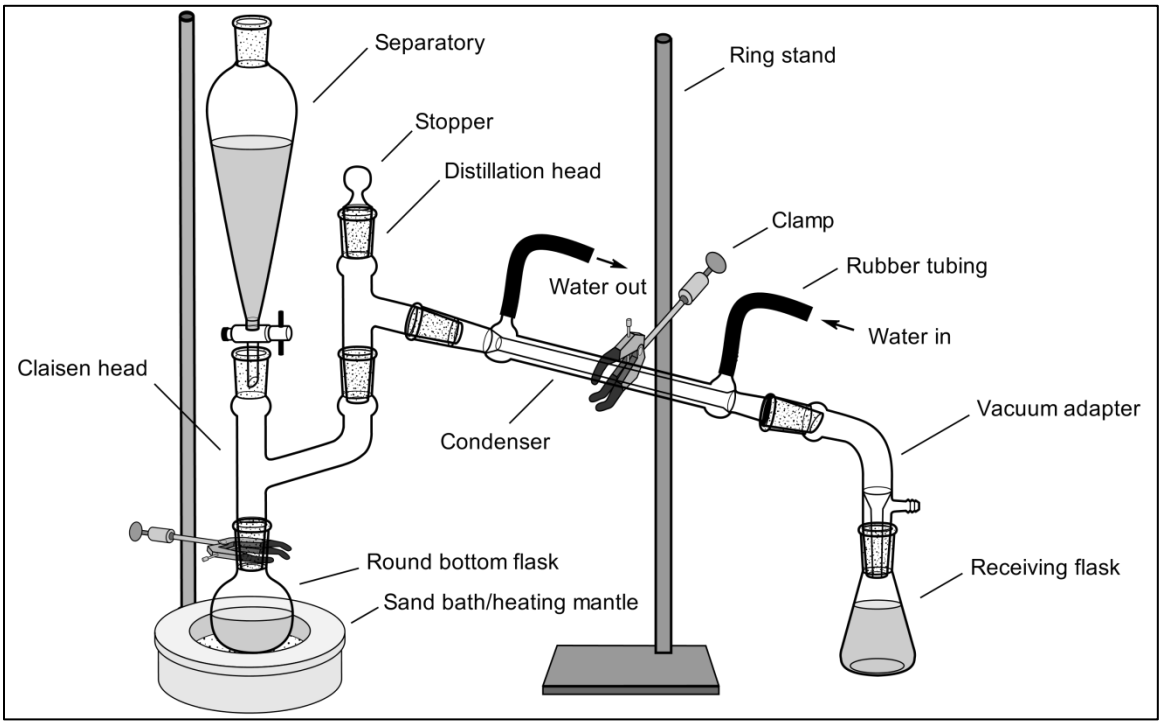


Figure 1 Steam co-distillation apparatus

- Use a sand bath of heating mantle as a heat source.

  If using a sand bath, the amount of heat transfer to the flask can be controlled by piling up or removing heated sand from around the flask.

- Place 25 g of whole cloves in a 250 mL round-bottom flask, add 100 mL of water.
- Heat the flask strongly until boiling starts, then reduce the heat enough to prevent foam from being carried over into the receiver.
- Use an Erlenmeyer flask as a receiver.
- Do not heat the solution to dryness; periodically add a few mL of water from the separatory funnel.

  Keeping the round-bottom flask about 1/2 to 2/3 full throughout co-distillation will yield the best results. Be careful not to allow the distillate from boiling over into the Claisen head and contaminating the distillate.

- Distill off approximately 60 mL of distillate.

- Turn off the sand bath and let the system cool.

Solvent extraction procedure

Eugenol will be extracted from the distillate using dichloromethane.

- Place the 60 mL of distillate in a 250 mL separatory funnel.
- Extract with three 15 mL portions of dichloromethane.

For the first two portions, shake the separatory funnel vigorously. This will create an emulsion; draw off the clear lower layer to the emulsion line for the first two extractions. Shake the third portion less vigorously; allow a longer period for the layers to separate.

- Combine the dichloromethane extracts (discard the aqueous layer).
- Add enough anhydrous sodium sulfate so that it no longer clumps together. It should appear to settle as a dry powder
- Swirl the flask for a couple of minutes.
- Decant the solvent into a clean, dry Erlenmeyer flask.
- Add a wood boiling stick.
- Evaporate the solvent on a steam bath in a hood. The residue of crude clove oil will be used in the NMR analysis.
- Disassemble and clean the distillation apparatus and all glassware.
- Prepare samples for NMR analysis.

Preparing Samples

One sample of eugenol will be prepared for analysis. The sample can be analyzed as a neat liquid. The ^{1}H NMR chemical shift from the methoxy singlet (3.72 ppm) in eugenol can be used as an internal chemical shift reference. Alternatively, a few microdrops of TMS (0 ppm) can be added to the sample, or eugenol can be diluted up to 50% with $CDCl_3$ containing 1% TMS. The sample preparation guide and spectra presented are for spectra acquired from a neat sample; spectra are internally referenced.

- Sample 1: To a labeled vial measure about 0.20 mL of eugenol. (*Optional*: Add a couple microdrops of TMS). Cap and save for NMR analysis.

Instrumental procedure

The general procedure for sample analysis using a picoSpin NMR spectrometer is as follows:

Shim

- Ensure the NMR spectrometer is shimmed and ready to accept samples.

Pre-sample preparation

- Displace the shim fluid from the picoSpin capillary cartridge with air.
- Flush the cartridge with 0.1 mL of chloroform, and then displace the solvent with an air push. A small signal in your sample spectrum may appear at 7.24 ppm due to residual chloroform, it can be used to shift reference the spectrum.
- Set up the *onePulse* script according to parameters listed in the Pulse Script table.

Injection

- Using a 1 mL disposable polypropylene syringe fitted with a 1.5" long, 22-gauge blunt-tip needle, withdraw a 0.2 mL aliquot of sample.
- Inject about half the sample. Ensure all air bubbles have been displaced from the cartridge by examining the drain tube.
- Cap both the inlet and outlet ports with PEEK plugs.

Acquire

- Execute the onePulse script according to the values in the table of parameters provided
- Once the onePulse script has finished, prepare the cartridge for the next user by displacing the sample from the cartridge according to the following protocol: air, solvent, air.

Pulse Script: onePulse

Acquisition parameters apply to both the picoSpin 45 and picoSpin 80 spectrometers. Use the tx frequency (tx) and pulse length (p1) appropriate for each system.

Parameter[a]	**Value**
tx frequency (tx)	proton Larmor frequency (MHz)
auto tx	✓
auto tx offset	270 Hz (pS45), 380 Hz (pS80)
scans (ns)	4, 10 or 16
pulse length (p1)	Instrument specific 90° pulse length
acquisition points (aq)	3000
rx recovery delay (r1)	500 µs
recycle delay (d1)	6 s
bandwidth (bw)	4 kHz
post filter attenuation (pfa)	11 (10)[b]
zero filling (zf)	8192
align-avg. data	✓
phase correction (ph)	0 degrees (or any value)
exp. apodization (LB)	0 Hz
JCAMP avg.	✓
JCAMP ind.	Unchecked
max time to plot	250 ms

min freq. to plot	-200 Hz
max freq. to plot	+1000 Hz
max plot points	400
live plot	✓

[a] Auto tx and auto tx offset are parameters introduced in picoSpin software version 0.9.0. Parameter names and position in scripts changed slightly in version 0.9.0. [b] Choose the instrument's default pfa value.

6. Processing

Download the experimental JCAMP spectra files and open them by importing into Mnova. The free induction decay (FID) will undergo automatic Fourier transformation and a spectrum will be displayed. To each spectrum, apply the following processing steps using the given settings:

Function	Value
Zero-filling (zf) & Linear Predict (LP)	16 k
Forward predict (FP)	From aq → 16 k
Backward predict (BP)	From -2 → 0
Phase Correction (PH)	PH0: Manually adjust
	PH1: 0
Apodization	
Exponential (LB)	0 Hz
First Point	0.5
Shift reference (CS)	Manually reference
Peak Picking (pp)	Manually Select Peaks
Integration (I)	-
Multiplet Analysis (J)	-

- Import each data file into the same workspace in Mnova. Manually apply Ph0 phase correction to each spectrum.
- Manually shift reference each spectrum using Mnova's TMS tool. Assign the methoxy signal from eugenol (3.72), TMS signal (0 ppm), or residual chloroform signal (7.24 ppm).
- Identify and assign each signal in spectra.
- Save the Mnova document, print each spectrum and paste into your lab notebook.

7. Results

The 45 MHz and 82 MHz ^{1}H NMR spectrum of eugenol (neat) is presented in Figure 2. The spectra show several signal groups. Aromatic proton signals (C_3, C_5 & C_6) appear between 6.5-7.2 ppm. The C_3 proton is uncoupled and gives rise to a singlet (~6.77 ppm); it overlaps other signals in this region thus complicating its assignment. Protons on C_5 and C_6 are spin coupled thus generating a doublet of doublet multiplicity pattern. The downfield doublet of this pair is unobstructed, whereas the upfield doublet overlaps the C_3 signal.

Vinyl protons (H_2C=CH) at C_8 and C_9 shift to 5.16 ppm and 6.05 ppm, respectively. The expected first-order coupling pattern (a doublet of doublet pair) due to *cis* and *trans* coupling of the terminal vinyl protons (C_9; 5.16 ppm) to the C_8 proton is not evident. This is likely to due to the crude nature of the extracted product. In contrast, the C_8 proton signal (6.05 ppm) exhibits a resolvable complex multiple arising from *cis* and *trans* coupling to the terminal vinyl protons (C_9) as well as coupling to the allylic C_7 proton.

The doublet structure, centered at 3.36 ppm, arises from spin-spin coupling of the methylene bridge protons (C_7) to a single internal vinyl proton on C_8. The downfield shift to 3.36 ppm is due to attachment of the C_7 carbon to both a phenyl and a vinyl group.

The final signal group readily assigned belongs to the methoxy methyl functional group (OCH_3); it appears as a strong singlet at 3.73 ppm. One remaining signal appears at 6.36 ppm in the 45 MHz spectrum and, roughly, at 6.53 ppm in the 82 MHz spectrum; this signal is likely due to the phenol proton (PhOH).

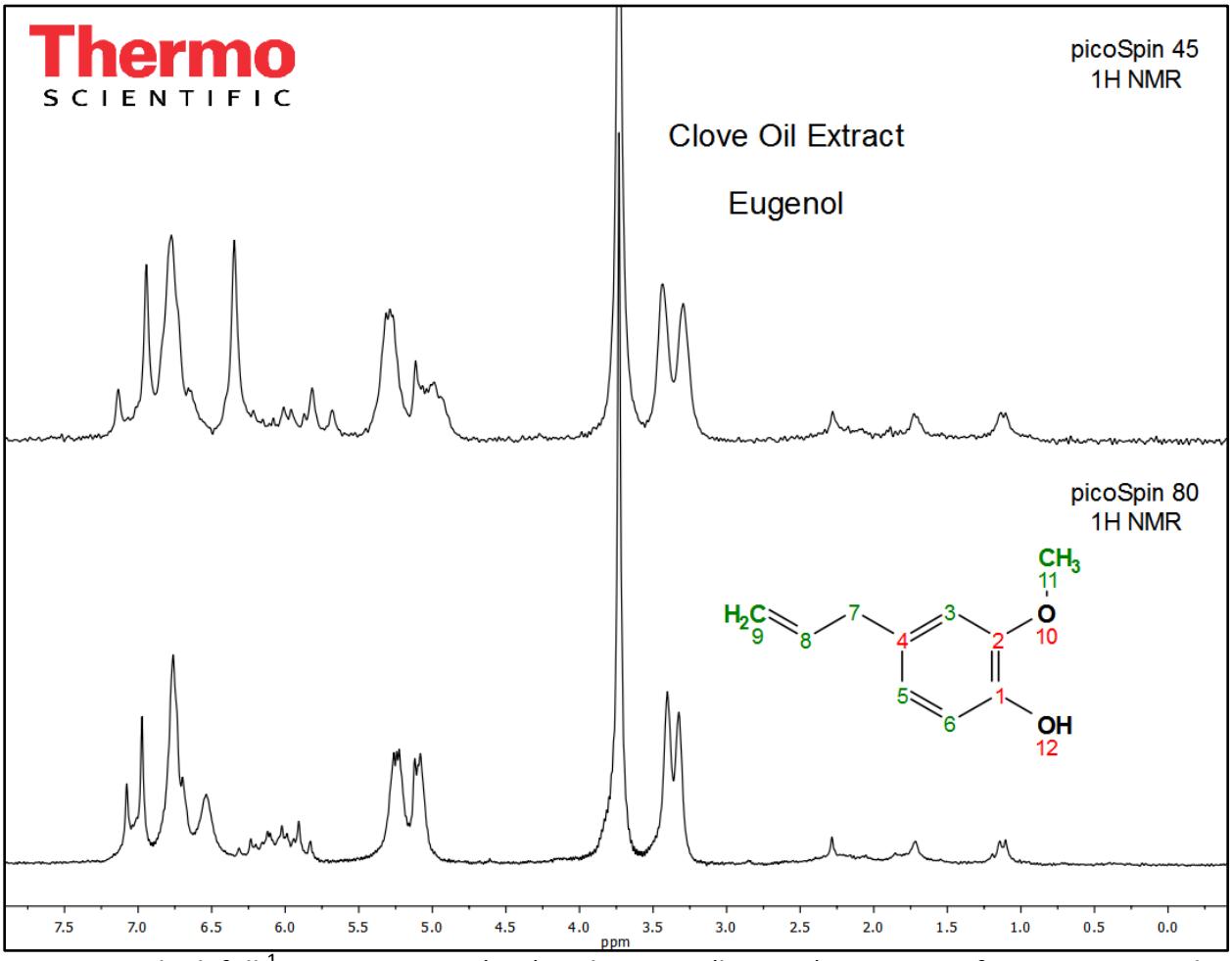


Figure 2 Stacked, full ^{1}H NMR 45 MHz (top) and 82 MHz (bottom) spectrum of steam-extracted eugenol from clove oil (neat).

Table 1. ^{1}H NMR Spectral Data

Figure	Compound	Signal Group	Chemical Shift (ppm)	Nuclides	Multiplicity
2	Eugenol	CH-CH_2-Ar	3.36	2 H	doublet
		CH_3-O	3.73	3 H	singlet
		CH_2=CH	5.16	2 H	multiplet
		CH_2=CH-CH_2	6.05	1 H	multiplet
		-OH	6.35 (pS45), 6.53 (pS80)	1 H	singlet
		Ar	6.77	1 H	singlet
		Ar	6.5-7.2	2 H	doublet of doublets

8. Comments

- The challenge in this lab is extracting sufficient quantities of eugenol.

- Collecting at least 60 mL of distillate can yield up to 2 mL of eugenol after extraction and evaporating off dichloromethane.
- Should students not have sufficient sample after extraction and solvent evaporation, there are two alternatives:
  - Combine samples from larger groups of students,
  - Follow the extraction procedure with store-bought clove oil using approximately 2 mL of the essential oil, then compare pre- and post-extraction eugenol.

9. Own Observations

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China +86 21 6865 4588
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland/Norway/Sweden +46 8 556 468 00
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 45 453 9100
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Russia/CIS +43 1 333 50 34 0
Spain +34 914 845 965
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Part of Thermo Fisher Scientific

FL52575_E_03/14