Optimization of the Analytical Supercritical Fluid Extraction of Cloves via an On-Column Interface to an Ion Trap GC/MS System

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Various densities of supercritical carbon dioxide were evaluated in terms of their ability to selectively extract the components of ground clove buds (*Syzygium aromaticum*). The extracts were recovered by using either a direct on-column interface or solvent recovery, and an ion trap GC/MS system was used for solute separation and identification. The on-column interface was more efficient than the solvent recovery method under all evaluated conditions. Extraction time was also found to have a significant effect on extract composition for both recovery methods. The on-column interface system provided optimal component selectivity with minimum analysis and method development times and greater potential resolution. These capabilities are particularly valuable for trace and volatile constituent analyses of complex natural products.

INTRODUCTION

The complex nature of natural products often requires maximum performance from the sample preparation, separation, and identification methods used. Supercritical fluid extraction (SFE) is valuable as a sample preparation technique because it provides unique selectivities with short extraction times and minimal solvent disposal concerns. The coupling of SFE to high-resolution capillary gas chromatography (GC) provided superior analyses in addition to being valuable as an automatable sample preparation and analysis system (Wright et al., 1987).

Works referring to "directly coupled", "direct interface", or "on-line" SFE/GC do not universally describe closed analytical systems that are specifically designed to maintain sample integrity, however. While these systems have been effectively used to analyze natural products such as cloves (Hawthorne, 1989), the use of flow-splitting GC inlets or hot, flash inlets may be responsible for the selective loss or degradation of some of the extracted components. Temperature-programmable on-column GC injectors have been shown to avoid these potential losses. An "optimal" analytical system thus might include a direct interface to an on-column injector as well as a sensitive information-rich detector for identification of unknowns.

The analytical supercritical fluid extraction of cloves via a direct on-column interface to high-resolution GC and an ion trap mass spectrometer (MS) was undertaken in this work to evaluate the optimum potential of this system for analysis of complex natural products.

EXPERIMENTAL PROCEDURES

A syphon-type cylinder with 1500 psi of helium overpressure supplied SFE grade carbon dioxide (Scott Specialty Gases) to a modified (software and hardware) Varian 9010 LC pump. The pump provided constant control of the carbon dioxide pressure while temperature was established inside the extraction vessel via a temperature-controlled heating block. The extraction vessel consisted of a 3.5-cm length of $^1/_4$ in. stainless steel tubing (0.4 cm i.d.) that was terminated on each end with low dead volume $^1/_4$–$^1/_{16}$ in. compression fittings with fixed 2-μm frits (Varian). Approximately 0.08 g of ground clove buds (*Syzygium aromaticum*) (Schilling) was placed into the 0.44-mL extraction vessel for analysis. A zero dead volume union was used to connect the $^1/_{16}$ in. stainless steel tubing to a restrictor of 10 μm i.d. fused silica capillary (Polymicro Technologies, Inc.). The effluent flow rate was established at approximately 3 mL/min and was inversely proportional to the length of the restrictor (normally 30 cm).

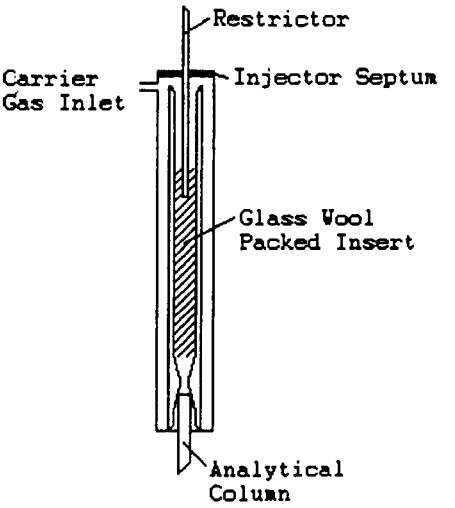

Figure 1. Schematic of the direct on-column interface between the restrictor capillary and the analytical column in the temperature programmable injector.

Table I. Results from Different Density Supercritical Carbon Dioxide Extractions (4.5 h) of Cloves Using Solvent Recovery

	0.959 g/cm³, 400 atm/40 °C	0.521 g/cm³, 200 atm/90 °C	0.124 g/cm³, 82 atm/140 °C
wt % eugenol in ground clove	17	16	15
wt % eugenol in clove oil	71	73	71
clove oil wt % of ground sample	24	22	21

Extract recovery was accomplished by using either solvent recovery or direct interfacing to the temperature-programmable injector (Varian SPI) provided with the GC/MS system (Varian Saturn System). The glass insert acted as an extension of the analytical column because a seal existed between the column's outer polyimide coating and the inner surface of the tapered glass insert (Figure 1). For both traditional injection of the solvent recovery samples and introduction of the direct interface samples, this seal allowed the SPI to provide on-column chromatographic performance.

For either recovery method, the restrictor was inserted through a needle that was used to pierce either the recovery vial septum

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Table II. Retention Time Information and Tentative Identification of the 27 Monitored Compounds

peak	compound (Rt of component/Rt of std)	Rt	P	F	RF
1	2-(acetyloxy)benzoic acid, methyl ester	8.05	648	813	775
2	eugenyl acetate std component (10:23/13:26)	8.59	961	974	976
3	unknown	9.48			
4	unknown	10.10			
5	eugenol (10:42)	11.02	938	974	956
6	α-cubebene	11.22	826	925	875
7	3-hydroxy-4-methoxybenzaldehyde	11.37	851	906	913
8	caryophyllene (11:56)	12.05	906	967	914
9	humulene std component (12:48/12:30)	12.39	896	947	914
10	humulene std component (12:48/12:30)	13.17	886	906	899
11	eugenyl acetate (13:26)	13.38	926	970	936
12	isoeugenol std component (13:23/12:17)	13.48	763	853	822
13	isoeugenol std component (14:28/12:17)	14.14	872	937	906
14	unknown	14.36			
15	caryophyllene std component (14:32/11:56)	14.44	931	980	946
16	humulene std component (14:56/12:30)	15.28	879	972	896
17	caryophyllene std component (14:32/11:56)	15.39	843	917	875
18	caryophyllene std component (15:47/11:56)	15.58	797	926	847
19	caryophyllene std component (14:03/11:56)	16.12	800	894	864
20	eugenyl acetate std component (18:13/13:26)	16.45	687	883	745
21	unknown	16.59			
22	benzoic acid, phenylmethyl ester	17.16	818	936	843
23	unknown	17.34			
24	unknown	17.58			
25	unknown	18.24			
26	unknown	18.55			
27	unknown	18.44			

Table III. Effect of Extraction Time (in Minutes) on the Area Count Percentages of 27 Compounds Monitored via Direct Interface SFE of Cloves[a]

	10	20	30	40	55	65
1	0.10	0.10				
2	0.10	0.40	0.40	0.60	0.40	0.30
3			0.02	0.12	0.20	
4						
5	68.00	69.00	91.00	96.30	25.00	30.30
6	0.40	0.08	0.03	0.02		
7		0.02	0.02			
8	25.10	21.20	1.30	0.70	1.40	0.80
9	2.90	3.40	0.20	0.10	0.20	0.10
10	0.80	0.90	0.10	0.05	0.08	0.01
11	1.80	4.20	6.30	1.90	68.50	64.70
12	0.60	0.60	0.10	0.05	0.08	0.03
13	0.06	0.06	0.03	0.02		
14			0.03	0.03		0.09
15	0.09	0.20	0.20	0.20	1.00	1.50
16			0.05	0.01		0.30
17		0.10	0.10	0.02	1.70	1.30
18			0.06		0.60	0.50
19		0.09			0.70	
20						
21						
22		0.05			0.10	
23						
24		0.08	0.02			
25						
26						
27						

[a] A carbon dioxide density of 0.124 g/cm^3 was used.

Table IV. Effect of Extraction Time (in Minutes) on the Area Count Percentages of 27 Compounds Monitored via Direct Interface SFE of Cloves[a]

	5	10	15	25	35	62
1	0.05	0.06	0.05			
2	1.00	0.90	0.70	0.50	0.40	0.30
3	0.04	0.07	0.07			
4						
5	72.60	75.70	75.80	72.70	72.00	71.50
6	0.50	0.10	0.10	0.10		
7	0.40	0.20	0.20	0.30	0.20	0.20
8	9.00	8.60	7.70	8.70	6.80	9.00
9	1.00	0.90	0.90	1.00	0.80	1.00
10	0.30	0.20	0.20	0.30	0.20	0.20
11	11.90	10.70	11.70	12.80	17.40	16.30
12	0.30	0.20	0.20	0.30	0.20	0.20
13	0.06	0.06	0.05			
14						
15	0.40	0.40	0.40	0.50	0.70	0.50
16	0.08	0.10	0.08	0.10		
17	0.30	0.30	0.20	0.40	0.50	0.40
18	0.07	0.05				
19	0.20	0.10	0.20	0.20	0.30	0.30
20	0.30	0.30	0.30	0.50		
21	0.40	0.09	0.10	0.70		
22	0.30	0.09	0.10	0.60	0.40	
23	0.20	0.20	0.10			
24	0.30	0.20	0.20	0.10		
25		0.07	0.10			
26		0.05	0.08	0.10		
27	0.20	0.20	0.30			

[a] A carbon dioxide density of 0.959 g/cm^3 was used.

or the injector septum. A valve between the extraction vessel and the restrictor allowed short, single "injections" to be made via the direct interface in the following sequence: (1) insertion of the restrictor into the SPI, (2) pressurization of the extraction vessel and opening of the valve, and (3) removal of the restrictor after the chosen injection time had elapsed. Solvent recovery was accomplished by injecting, for much longer times, into sealed 2-mL vials containing a known amount of recovery solvent. Recovery solvent samples were, subsequently, analyzed by GC/MS after a traditional liquid injection into the SPI. 2-Propanol worked well as a recovery solvent, and for direct interfacing, the SPI insert was packed with glass wool to facilitate both analyte recovery and removal of nonvolatile extraction products. With a minimum of triplicate replications, the extraction variability for either technique was generally less than 5% rsd for eugenol.

The SPI was programmed as follows: 0.1-min initial hold at 50 °C, ramp to 250 °C at 150 °C/min, end hold of 23 min. The helium carrier gas pressure established a velocity of approximately 30 cm/s, and the column oven program consisted of a 0.5-min initial hold at 80 °C, a ramp to 250 °C at 8 °C/min, and an end hold of 6 min. The 30 m × 250 μm DB-5 (J&W Scientific) capillary column with a 0.25-μm film was directly interfaced to the ion trap mass spectrometer. Both the direct MS interface and the trap manifold were held at 260 °C. The ion trap was calibrated by using perfluorotributylamine (PFTBA) and the following settings: an automatic gain control (AGC) target value of 15 000, an axial modulation voltage of 4 V, a filament emission current

Table V. Effect of Extraction Time (in Minutes) on the Area Count Percentages of 27 Compounds Monitored via Solvent Recovery SFE of Cloves[a]

	3.00	6.00	8.00	10.00	12.00	14.00	16.00	18.00	21.00	24.00	29.00	34.00	39.00	44.00	54.00	64.00	84.00	104.00	all
1	0.07	0.10	0.10	0.08	0.06	0.06									0.20	2.60	7.40	5.70	0.1
2	0.06	0.06	0.05	0.09	0.01	0.10	0.10	0.20	0.20	0.20	0.50	0.70	0.80	0.40	0.20	0.50			0.2
3																			
4																			
5	60.20	56.90	57.60	65.10	73.30	74.50	82.40	81.30	84.40	90.50	93.50	96.00	97.40	79.50	17.40	10.30	63.60	70.00	71
6	0.40	0.60	0.50	0.30	0.10	0.10													0.2
7																			
8	23.80	28.30	29.10	21.60	14.30	13.00	5.90	6.50	4.90	1.50	0.70	0.50	0.30	0.30	0.40	0.90	6.40	8.80	12
9	2.40	2.50	2.60	2.20	1.70	1.60	1.00	1.00	1.10	0.40	0.20	0.10	0.06	0.03	0.07	0.40	0.60	1.00	1.4
10	0.60	0.60	0.50	0.60	0.50	0.40	0.30	0.30	0.30	0.20	0.07								0.4
11	11.80	10.30	9.00	9.50	9.30	9.60	9.80	10.20	8.50	6.80	4.80	2.60	1.30	18.50	77.50	70.40	7.60	6.80	13.6
12	0.30	0.30	0.30	0.30	0.30	0.30	0.20	0.20	0.30	0.20	0.09	0.06	0.06						0.2
13																			
14																			
15	0.40	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.20	0.10	0.08	0.09	1.00	1.10				0.4
16														0.10					0.01
17														0.20	1.60	4.40	2.50		0.2
18															0.60	2.40	4.40	7.70	
19															0.80	3.50	4.80		0.2
20																			
21																			
22															0.10	4.60	2.60		0.1
23																			
24																			
25																			
26																			
27																			

[a] A carbon dioxide density of 0.124 g/cm^3 was used. Results from a 4.5-h extraction are shown in the column labeled "all".

of 50 μA, and an electron energy of 12 eV. The Saturn data system used both the NIST library and a special library created from analyses of known standards for identity confirmation.

RESULTS AND DISCUSSION

Previously described compressibility factor methods were used to calculate the density of supercritical carbon dioxide for various pressure/temperature settings (Huston and Bernhard, 1989). Different densities were evaluated in terms of both total extraction yield and extraction selectivity.

For exhaustive clove extractions recovered in 2-propanol, supercritical carbon dioxide with a density of 0.959 g/cm^3 yielded 24% clove oil (Table I). The other two major components of clove oil, caryophyllene and eugenol acetate, were present at approximately 12% and 14%, respectively, under these same conditions. System efficiency for recovery of eugenol from a spiked sample (addition at 15 wt %) was greater than 90%.

Optimum supercritical carbon dioxide extractions of cloves have previously given oil yields of 16% (Moyler, 1986) and 19% (Gopalakrishnan et al., 1990). Neither work found a distinct difference between steam distillation and supercritical fluid extraction in terms of oil yield. Extraction with liquid carbon dioxide also gave a maximum yield of 19% oil (Gopalakrishnan et al., 1990).

Factors other than fluid density played a major role in extraction efficiency, however. At any given density (e.g., 0.959 g/cm^3), the total ion chromatogram (TIC) for the direct interface method (Figure 2) showed a greater number of extracted components than did solvent recovery analyses (Figure 3). The coordinate of the TIC for the solvent recovery run (Figure 3) was scaled to show a maximum of 1.56% of the total ion signal (TOT); a scale of 6.25% of the total ion signal was used for Figure 2. When direct interfacing to the on-column injector was used, over 27 components could be recovered and analyzed (Figure 2).

Most SFE methods use at least a 10-min extraction and concentration for optimum recovery. The on-column interface gave maximum recovery of extracted compounds by using only 2 s of effluent flow of analysis. Although longer injection times could be used for trace analyses, the most abundant clove compounds quickly overloaded the stationary phase. To maintain sample integrity, no flow-splitting devices were used anywhere in the SFE/GC/MS system. Previous direct interface SFE analyses of cloves recovered fewer components (relative to this work) even when column oven cryogenics (−30 °C) were used to concentrate the recovered solutes (Hawthorne et al., 1989). Because the temperature-programmable on-column interface avoided flow splitting and thermal degradation losses, it allowed more components to be recovered in greater quantities than for other direct interface works.

In electron ionization mass spectrometry, the detector is typically turned "off" for the initial part of the GC run to increase instrument lifetime by avoiding the intense signal of the solvent peak. This filament/multiplier delay time eliminates a certain amount of chromatographic data. The solvent peak elution time, however, normally provides no useful analytical information even with more common GC detectors (e.g., the flame ionization detector). Solvent contaminants may also be present (e.g., the early eluting peaks in Figure 3) which can interfere with volatile analytes. The direct on-column configuration thus provided the potential for analysis of more volatile constituents because it avoided interference from both the solvent and the solvent contaminants. Injector and column oven cryogenics could be used to further optimize this method for volatile analyses.

The presence of a sample solvent (in solute recovery samples) was also seen to cause a loss of potential resolution when compared to analysis of neat clove extracts (from on-column interfacing). Solutes generally eluted earlier for solvent recovery samples (Figure 3) than for directly recovered analytes (Figure 2). Shorter elution times result when some of the retention characteristics of the analytical column have been lost. For example, peak 16 eluted about 15 min into the solvent recovery analysis, while with the direct interface, it took approximately 16 min. With a

Table VI. Effect of Extraction Time (in Minutes) on the Area Count Percentages of 27 Compounds Monitored via Solvent Recovery SFE of Cloves[a]

	2	4	6	8	10	12	15	18	21	24	29	34	39	44	49	80	all
1						0.05	0.05										
2	0.30	0.20	0.30	0.30	0.10	0.10	0.01	0.10	0.20	0.20	0.40	0.90	1.60	2.70	1.60	1.00	0.20
3																	
4																	
5	77.50	75.60	74.80	67.90	60.90	70.00	69.30	71.00	74.20	78.50	79.60	79.40	77.40	77.40	78.00	76.30	71.40
6				0.30	0.40	0.30	0.20	0.20	0.10	0.10	0.10	0.10	0.10				0.20
7																	
8	9.10	10.50	11.40	16.50	21.70	14.70	12.60	11.60	9.20	7.20	7.10	7.50	8.30	8.70	9.40	10.40	11.80
9	1.00	1.20	1.20	1.60	2.20	1.60	1.40	1.30	1.10	0.80	0.80	0.80	1.00	1.20	1.10	1.60	1.30
10	0.20	0.30	0.30	0.40	0.60	0.40	0.40	0.30	0.30	0.20	0.20	0.20	0.20	0.20			0.40
11	11.10	11.00	11.40	12.00	13.10	14.70	14.90	14.50	13.90	12.00	10.60	10.20	10.30	9.50	9.90	10.70	13.70
12	0.20	0.60	0.20	0.20	0.30	0.30	0.20	0.20	0.20	0.20	0.20	0.20	0.20				0.20
13						0.04	0.03										
14																	
15	0.50	0.50	0.40	0.40	0.50	0.50	0.50	0.50	0.50	0.40	0.40	0.40	0.30	0.30			0.50
16					0.05	0.07	0.07	0.06	0.06								0.06
17						0.05	0.05										
18													0.30				
19				0.30	0.10	0.10	0.20	0.10	0.10	0.30	0.40	0.30	0.20				0.20
20																	
21																	
22				0.10	0.01	0.07	0.09	0.10	0.10	0.10	0.20						0.10
23																	
24																	
25																	
26																	
27																	

[a] A carbon dioxide density of 0.959 g/cm^3 was used. Results from a 4.5-h extraction are shown in the column labeled "all".

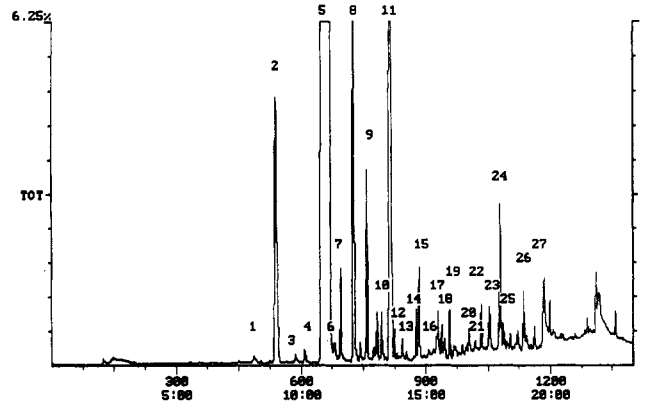

Figure 2. Characteristic total ion chromatogram (TIC) for SFE/GC/MS of cloves using the on-column interface.

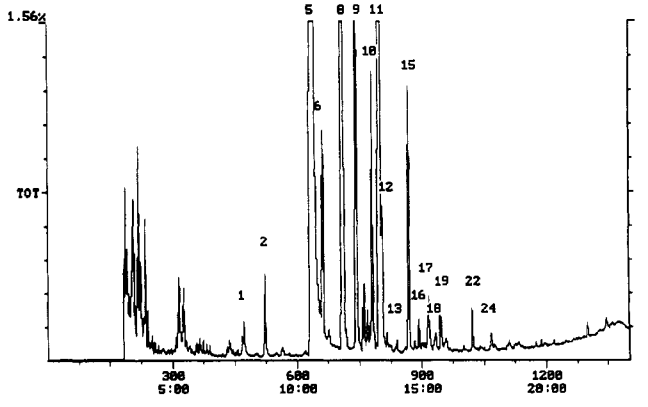

Figure 3. Characteristic total ion chromatogram (TIC) for SFE/GC/MS of cloves using the solvent recovery method.

30-m column and a velocity of 30 cm/s, a 1-min difference for this peak corresponded to a difference of approximately 2 m. Because the solvent deposited the solute 2 m down the length of the column, only 94% of the resolving length of the column was utilized for this compound.

Table II lists the tentative identifications of the 27 peaks selected from the direct on-column interface analysis of Figure 2. The spectra of unknowns were searched against the NIST library (containing over 49 000 compounds) and against a library constructed from various standards. All major chromatographic peaks for each standard analysis (e.g., oxidation products, isomers, etc.) were included in the "user" library and were referred to as a specific "std component". The ion trap gave consistent spectra even for low picogram quantities of compounds and purity (P), fit (F), and reverse fit (Rf) scores were listed to the right of the retention times (Rt) for each peak. Isomer presence was indicated when different peaks gave good library matches to the same standard components (e.g., peaks 9 and 10 have spectra similar to the humulene std component with a retention time of 12:48). This work is incomplete in terms of identity confirmation of the extracted clove components, and continued work (e.g., identifications of terpenoid compounds) is recommended.

Depending on the density of supercritical carbon dioxide, the extraction equilibrium can be dominated by either solute solubilities or solute volatilities (Huston and Bernhard, 1990). Examination of the effects of extraction time gave additional information about the selectivity of supercritical carbon dioxide for the 27 monitored clove components (Tables III–VI). The trials were conducted by sequentially analyzing the extract effluents at various times. For the on-column interface (Tables III and IV), the effluent was allowed to vent to waste unless being recovered as a 2-s injection. The solvent recovery analyses (Tables V and VI) represent sequential accumulation (concentration) of the effluent in different solvent vials (e.g., the 21-min sample in Table V represents 3 min of effluent accumulation following 18 min of total extraction). The tables list the relative area count information from chromatographic analyses of the 27 monitored compounds.

While some compounds were present throughout the extraction (e.g., major compounds 5 and 8), others were predominant in a particular time frame of the extraction (e.g., compound 17 did not elute until 44 min in Table V). Higher density extractions (Table IV and VI) gave more

universal extraction of the 27 compounds in a shorter time, while a more selective recovery was found at various times for lower density extractions (Tables III and V).

Regardless of contact time or fluid density, the on-column interface (Tables III and IV), once again, was more efficient at solute recovery than the solvent recovery method (Tables V and VI). The difference between the two recovery methods is particularly obvious for later eluting compounds such as peaks 23–27. The use of a direct interface to a temperature-programmable on-column GC inlet thus allowed for effective utilization of selectivity factors such as fluid density and extraction time for analysis of clove constituents.

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