Supercritical Fluid Extraction of Moist Snuff

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Commercial moist snuff was extracted by using supercritical carbon dioxide at 60 °C and 8000 psi for a period of 20 min. A wide variety of components were extracted and identified, and several of them were quantitatively assayed. Nicotine was quantified with high precision and reproducibility (RSD 4.1%). The enhancement of the extraction due to addition of a polar entrainer like methanol was investigated.

INTRODUCTION

The isolation of organic components from complex matrices such as tobacco, diesel exhaust, soils and sediments, and lipid-rich foods often requires the development of a rapid, precise, and inexpensive analytical approach to their characterization and quantification. While there has been remarkable progress in methods involving chromatographic separation and detection, the sample preparation methods can be error-prone and time-consuming.

The common methods of extraction, such as multistep, liquid–liquid extraction and Soxhlet extraction, often lack precision and are time-consuming and expensive. They require high temperatures and are often done in bright light so that they are subject to decomposition of heat-sensitive and photosensitive compounds. They are also prone to lead to artifacts.

The supercritical fluids are unique solvating media which have been widely accepted. Supercritical fluids have distinct advantages over liquid solvents in that they have lower viscosity (10^{-4} vs 10^{-3} N·s/m^2) and can more readily diffuse (10^{-4} vs 10^{-5} cm^2/s) than liquid solvents. These characteristics enable better mass transfer than can be achieved with liquids; thus, the process of extraction with supercritical fluids is faster (McHugh and Krukonis, 1985).

Carbon dioxide has been used as a supercritical fluid for the single-step isolation of additives such as anthraquinone from paper and wood pulp (Schneiderman et al., 1987), menadione from animal feed (Schneiderman et al., 1988a), and vitamin K_1 from powdered infant formulas (Schneiderman et al., 1988b). Conventional methods of isolation for compounds like these involve multistep liquid–liquid extractions, sometimes even preceded by hydrolysis (in the case of menadione and vitamin K_1), and followed by a tedious sample cleanup and concentration. The supercritical extraction process has been patented for the removal of nicotine from tobacco (Roselius et al., 1979).

In this paper we report on a fast, reproducible, single-step method for the isolation of organic components from chewing tobacco (commercial snuff) using supercritical CO_2 as the solvent. Nicotine, its major metabolite cotinine, as well as myosmine and other alkaloids, methyl salicylate, a major flavor additive, and dodecanoic acid have been quantitatively determined in the commercial snuff.

EXPERIMENTAL PROCEDURES

Materials. Commercial snuff samples were purchased from retailers in Westchester County, NY. Kentucky reference moist snuff (1S3) was obtained from the Tobacco and Health Research Institute, University of Kentucky, Lexington, KY.

Reagents. HPLC-grade *n*-hexane and acetone were obtained from J. T. Baker Inc., Phillipsburg, NJ. HPLC-grade methanol was purchased from Mallinckrodt Inc., Paris, KY, and CO_2 (99.9% pure) from Liquid Carbonics Specialty Gas Corp., Chicago, IL. Silica gel, grade 60, mesh 230–240, was obtained from Aldrich Chemical Co., Inc., Milwaukee, WI.

Supercritical Fluid Extraction (SCFE) Apparatus. The extractions were performed in a self-assembled apparatus (Figure 1). The required high pressure is generated by a single-ended, motor-driven diaphragm pump (New Port Scientific, Inc., Model 46-13411-2) that can produce pressures of up to 10 000 psi. The extraction chamber is a 12 × $^3/_8$ in. stainless steel threaded tube (Autoclave Engineers Inc., Erie, PA) heated with a column oven heater (Fiatron, Oconomowoc, WI, Model CH-30). The pressure of the system is controlled by two adjustable stem valves (Autoclave Engineers). The extracts are collected after depressurization across a 6-in. stainless steel tube filled with a suitable adsorbent such as silica, Tenex, C-18. To prevent accidental overpressurization, the system is equipped with safety valve 20-RUP9072 (Autoclave Engineers) set at 15 000 psi.

Extraction Procedure. A moist snuff sample (50–200 mg) in the extraction chamber was held in place by glass wool plugs. Air was removed from the system by flushing CO_2 through the chamber at ambient temperature and at <45 psi for about 2 min. The system was heated to the desired temperature with valve 2 closed; then the required pressure was attained. For equilibration, the system was maintained at the constant high pressure and temperature for 20 min. Afterward, the pressure was released slowly by opening valve 2. The fittings and the trap (filled with 1.5 g of silica) were rinsed with 15 mL of *n*-hexane/acetone (9:1). The solvent was first rotoevaporized slowly at 30 °C to approximately 2 mL and then reduced to 0.5 mL under a gentle flow of nitrogen.

For quantitative determination, 50–200 mg of moist snuff was placed into the extraction chamber and was extracted twice for an equilibration period of 15 min at 8000 psi and 60 °C.

To assess the qualitative effect of a polar entrainer, 1.0 mL of MeOH was added directly to 100 mg of moist tobacco in the extraction chamber and supercritical fluid extraction was performed as described above.

Chromatography and Identification. The tobacco extracts were analyzed on an HP 5890A gas chromatograph equipped with a split injector (split ratio 1:10, injection port 200 °C) and a FID (250 °C). Helium was used as carrier gas (flow rate 1 mL/min) and as makeup gas for the FID (flow rate 35 mL/min). The chromatographic separation was performed on a 30-m DB-5 bonded phase (0.25-μm thickness, 0.25 mm i.d.) capillary column (J&W Scientific, Folsom, CA). The initial temperature was kept at 70 °C for 1 min and was raised at increments of 2 °C/min to a final temperature of 250 °C, which was held for 14 min.

The peaks were identified by determining retention times of pure components and by mass spectral analyses using an HP 5890 GC–MS system equipped with an HP 1000 data system.

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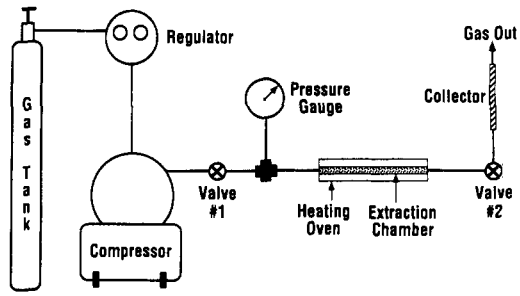

Figure 1. Schematic of the supercritical fluid extraction apparatus.

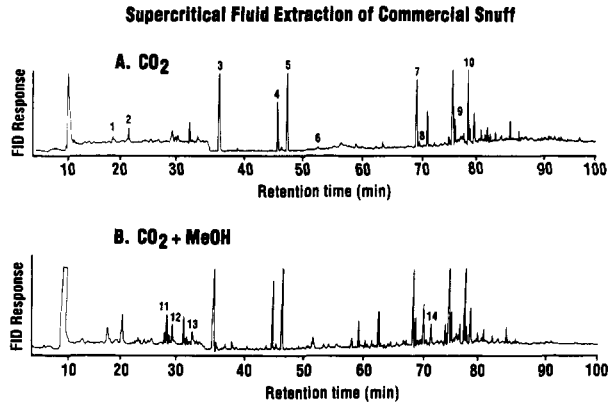

Figure 2. GC-FID trace of supercritical fluid extract of 100 mg of moist commercial snuff. Extraction conditions: pressure 8000 psi, temperature 60 °C, time 20 min. GC conditions: DB-5, 30 m × 0.25 mm i.d., FID at 250 °C, injection temperature 200 °C, He carrier gas, flow rate 1 mL/min, split ratio 1:10; oven temperature program, initial temperature 70 °C for 1 min, 2 °C/min, final temperature 250 °C for 14 min (for identity of peaks, see Table I).

Table I. Identification of Components in Commercial Snuff

peak no.	component	high-pressure extraction (8K, 60 °C, 20 min)[a]	CO_2 + 1 mL of MeOH (8K, 60 °C, 20 min)[a]
1	benzaldehyde	x	x
2	β-myrcene	x	x
3	methyl salicylate	x	x
4	dodecanoic acid	x	x
5	nicotine	x	x
6	myosmine	x	x
7	phenylmethyl benzoate	x	x
8	anthracene	x	x
9	2-(hydroxyphenyl) methyl benzoate	x	x
10[b]	neophytadiene	x	x
11	benzyl alcohol	-	x
12	phenethyl alcohol	-	x
13	benzothiazole	-	x
14	cotinine	-	x

[a] 8K = 8000 psi. [b] Mass spectrometry match only, 99% match.

RESULTS AND DISCUSSION

The GC-FID chromatogram of the extract of a 100-mg sample of commercial snuff, extracted with CO_2 at 8000 psi and at 60 °C for 20 min, is shown in Figure 2A. It is evident that supercritical CO_2 is able to extract a variety of organic components from this matrix. Several of these compounds have been identified (Table I). CO_2 as a supercritical fluid enables extraction of all classes of compounds including nonpolar unsaturated compounds (like myrcene, anthracene, and neophythadiene), alkaloids (nicotine, myosmine, and cotinine), esters (methyl salicylate, phenylmethyl benzoate), alcohols (benzyl alcohol, phenethyl alcohol, 1-decanol), and polar compounds such as benzaldehyde, benzothiazole and *n*-dodecanoic acid.

The differences seen in extracts obtained at low pressure (3000 psi) vs high pressure (8000 psi) appear to be quantitative rather than qualitative.

Effect of Entrainer. Adding a polar entrainer such as methanol to the matrix enhances the extraction of all components in general and some polar components in particular (Figure 2B). Compounds such as benzyl alcohol, phenethyl alcohol, benzothiazole, and cotinine could not be extracted without addition of methanol. How methanol enhances the extraction is not known. It probably acts as a solvent modifier, rendering the solvent mixture more polar, or simply as a displacing agent by wetting the matrix (Table I).

Quantitative Determination of Nicotine. The analytical reproducibility of the method was assessed by determining the quantity of nicotine present in tobacco (Table II). Various sample sizes ranging from 50 to 200 mg of commercial snuff were extracted, and nicotine was quantified by comparing the areas of its peaks from a standard solution with those obtained from the samples on GC-FID. For comparison with results from other methods, nicotine was quantified in the reference snuff sample (KY, 1S3). The moisture content, determined in reference and commercial snuff samples by the Dean–Stark procedure, was 49.8% and 50.6%, respectively.

On a dry-weight basis, the amount of nicotine determined with the SCFE method was compared to that determined in the 24-h liquid–liquid extraction performed on the same brand of commercial snuff by Djordjevic et al. (1989). This showed values obtained by SCFE to be about 5% lower than those reported with the conventional extraction (Djordjevic et al., 1989). However, SCFE is faster and leads to a cleaner extract. In addition, methyl salicylate (a major tobacco additive), cotinine and myosmine (trace amounts of tobacco alkaloids), and dodecanoic acid have been quantified at three different weight

Table II. Nicotine Content in Commercial and Reference Snuff Samples

sample	wt, mg	nicotine, mg/g of dry wt
commercial snuff	50.4	18.6
	150.3	17.5
	90.5	19.2
	110.2	17.9
	195.3	19.7
	80.6	18.4
reference snuff	100.2	23.2
	55.7	24.5
	85.5	22.8
av nicotine content in commercial snuff, mg/g of dry wt		18.55
RSD, %		4.1
av nicotine content in reference snuff, mg/g of dry wt		23.5

Table III. Quantitative Determination of Some Compounds in Commercial Snuff

wt of moist snuff, mg	methyl salicylate,[a] mg/g	dodecanoic acid,[a] mg/g	myosmine,[b] μg/g	cotinine,[b] mg/g
100.0	22.2	0.85	3.9	0.060
75.0	21.7	0.79	3.5	0.046
50.0	21.2	0.76	3.0	0.058

[a] All values are the average of three determinations at each weight level and are reported as milligrams per gram. [b] Supercritical fluid extraction in the presence of 0.5 mL of MeOH added to the tobacco directly in the extraction chamber.

levels in commercial snuff samples (Table III). For quantitation of myosmine and cotinine, 0.5 mL of MeOH was added to moist snuff directly into the extraction chamber.

CONCLUSION

SCFE with CO_2 alone or in conjunction with small amounts of a polar modifier enabled extraction of a wide variety of compounds ranging from extremely nonpolar to polar tobacco constituents. The methodology is fast (total sample handling time 50–55 min vs 24–30 h in conventional methods), requires small sample sizes (50–200 mg vs 50–500 g), and is highly reproducible and economical; in addition, CO_2 is nontoxic. It is expected that SCFE with CO_2 might become in the near future a viable alternative to classical methods of sample preparation.

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Registry No. Benzaldehyde, 100-52-7; β-myrcene, 123-35-3; methyl salicylate, 119-36-8; dodecanoic acid, 143-07-7; nicotine, 54-11-5; myosmine, 532-12-7; phenylmethyl benzoate, 120-51-4; anthracene, 120-12-7; 2-(hydroxyphenyl)methyl benzoate, 30923-59-2; neophytadiene, 504-96-1; benzyl alcohol, 100-51-6; phenethyl alcohol, 60-12-8; benzothiazole, 95-16-9; cotinine, 486-56-6; carbon dioxide, 124-38-9; methanol, 67-56-1.