

Determination of Volatile Acids in Tobacco, Tea, and Coffee Using Derivatization–Purge and Trap Gas Chromatography–Selected Ion Monitoring Mass Spectrometry

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Abstract

Short-chain organic acids from agricultural products are simultaneously extracted and derivatized using methanolic hydrochloric acid. Acetic, isobutyric, butyric, isovaleric, valeric, hexanoic, 3-methylvaleric, heptanoic, and nonanoic acids are quantitated using purge and trap gas chromatography with selected ion monitoring mode mass spectrometry. Flue-cured, burley, and oriental tobacco varieties are compared based on their volatile organic acid content. Commercial coffee, tea, and three cigarette brands are analyzed, and their differences are evaluated to demonstrate the utility of this technique for natural product analysis.

Introduction

Short-chain organic acids (C_1 – C_8) contribute significantly to the aroma and taste of consumer products (1). There have been numerous methodologies developed for the analysis of these components. However, analysis of these components by gas chromatography (GC) is challenging because of their polarity and low response to flame ionization detection (2). These acids are most often derivatized in order to enhance sensitivity, improve peak shape, and increase volatility (3). Two of the major drawbacks of these technologies are laborious sample preparation and poor recoveries due to the volatile nature of the derivatives (4).

Several derivatization techniques have been developed for the analysis of volatile organic acids (5–12). Silylation reactions have been used extensively and generally perform well for the analysis of “free” organic acids (5). These derivatives are extremely moisture-sensitive; precautions must be taken to ensure that hydrolysis does not occur. Methyl esterification has been widely used for the analysis of fatty acids, but short-chain acids lead to poor recoveries due to excessive sample volatility (6). Static headspace determination has been used to overcome

this limitation with moderate success (7). Numerous other procedures for the preparation of less volatile derivatives have been used for the analysis of low-molecular-weight acids (8–12).

Although the available methodologies provide accurate and precise data, most are not applicable to complex matrices such as agricultural products. Carboxylic acids in many natural products exist as “free” acids, salts, esters, and amides (13). To evaluate total organic acids in these matrices, the derivatization reagent must be capable of converting carboxylic acid functionality to the derivative, regardless of the form. Acid-catalyzed esterification fulfills these requirements and has been used successfully for the quantitation of higher molecular weight acids in plants and food products (14).

In this report, we treated tobacco, coffee, and tea samples with methanolic hydrochloric acid followed by analysis using purge and trap GC with selected ion monitoring mass spectrometry (SIMMS). This technique provides quantitative results for C_2 – C_8 organic acids with minimal sample preparation or loss of analytes.

Experimental

Instrumentation

A Tekmar 3000 (Cincinnati, OH) purge and trap concentrator interfaced with a Hewlett-Packard (HP) 5890 GC (Palo Alto, CA) and an HP 5970B mass selective detector was used for data collection and analysis. Splitless injections (78.5-mm inlet liner, 6.5 mm \times 1.5-mm i.d.) (Supelco, Bellefonte, PA) were made at 250°C. Separation was accomplished using a 30-m PTA-5 (Supelco) column (0.23-mm i.d., 0.20- μ m film thickness). The initial oven temperature was 50°C for 2 min and then increased to 250°C at a rate of 10°C/min. The GC–MS transfer line was maintained at 280°C.

Purge and trap concentrator

The purge and trap apparatus was equipped with a BTEX-TRAP trap (Supelco). Conditions for the Tekmar 3000 are listed in Table I.

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Calibration standards

In amounts of 0.04, 0.20, 0.50, 1.00, 2.00, 5.00, or 10.00 μg , carboxylic acid standard solution (acetic, isobutyric, butyric, isovaleric, valeric, hexanoic, 3-methylvaleric, heptanoic, and octanoic acids in methanol) (ChemService, Westchester, PA), 2-ethylhexanoic acid (surrogate) (0.50 μg), and methanolic HCl (400 μL) (Supelco) were heated at 55°C overnight (derivatization step). The samples were cooled in an ice bath, and 1M sodium bicarbonate solution (1600 μL) (Fisher Scientific) and 2,6-dichlorotoluene (internal standard, 0.50 μg in methanol) were added. The samples were placed in the purge and trap apparatus and analyzed as described. The MS was operated in SIM mode using molecular fragmentation ions of m/z 74, 87, and 125. Calibration curves were prepared (correlation coefficients of 0.999 or greater), and all exhibited linearity over the range evaluated.

Tobacco samples

Tobacco samples were dried at 70°C in a convection oven and ground to a powder. Tobacco (50 mg) and 2-ethylhexanoic acid (surrogate) (0.50 μg) were treated with methanolic hydrochloric acid (2000 μL) as described. A 400- μL aliquot of this material was treated with 1600 μL of 1M sodium bicarbonate solution and 2,6-dichlorotoluene (internal standard, 0.50 μg in methanol) and analyzed by SIMMS. Quantitation was performed by linear regression analysis using the standard HP ChemStation programming.

Table I. Tekmar 3000 Purge and Trap Parameters

Purge temperature	30°C
Prepurge time	2 min
Preheat time	5 min
Purge time	5 min
Helium purge flow rate	40 mL/min
Purge trap	BTEXTRAP
Trap pressure	20 psi
Desorption temperature	250°C
Transfer line	120°C
Desorption time	3 min

Table II. Volatile Organic Acids in Tobacco

Carboxylic acid (Retention time [min])	Flue-cured ($\mu\text{g/g}$ [%RSD])*	Burley ($\mu\text{g/g}$ [%RSD])	Oriental ($\mu\text{g/g}$ [%RSD])
Acetic (2.33)	773 (17.5)	554 (14.6)	575 (12.2)
Isobutyric (4.11)	14.68 (22.1)	13.39 (13.2)	108 (11.3)
Butyric (4.68)	6.95 (12.9)	4.69 (14.3)	19.85 (11.6)
Isovaleric (5.63)	38.69 (24.1)	37.75 (10.5)	290 (8.8)
Valeric (6.54)	5.86 (16.0)	8.5 (6.8)	10.11 (10.0)
3-Methylvaleric (7.69)	2.28 (28.0)	3.81 (26.8)	1787 (24.2)
Hexanoic (8.48)	†	18.14 (27.2)	14.33 (6.1)
Heptanoic (10.36)	1.68 (12.3)	1.90 (28.0)	3.25 (29.7)
Octanoic (10.71)	6.87 (22.8)	5.90 (8.6)	6.64 (30.6)

* RSD = Relative standard deviation.
† Quantitation was not possible due to interference.

Coffee and tea samples

Commercially available coffee and tea samples were dried at 70°C in a convection oven. Samples (20 mg) containing 2-ethylhexanoic acid (internal standard) (2.50 μg) were derivatized with methanolic HCl, and a 400- μL aliquot was treated with 1600 μL of 1M sodium bicarbonate solution and analyzed as described.

Results and Discussion

Derivatization

Derivatization of tobacco, coffee, and tea using methanolic hydrochloric acid provided an excellent method for extraction and conversion of organic acids to their more volatile methyl ester counterparts. This technique converts all carboxylic acid functional groups to the corresponding methyl esters, regardless of their form (i.e., "free acids," esters, amides, or salts).

Methyl esterification has not been used traditionally for the routine evaluation of volatile organic acids because sample preparation has often led to a loss of analyte. In this procedure, samples were reacted with methanolic hydrochloric acid by heating in sealed vials. The samples were cooled and opened, and the excess hydrochloric acid was destroyed with sodium bicarbonate. Samples were then analyzed directly with no further manipulation. This led to a decreased propensity for loss of analyte.

Purge and trap

The unique feature of this methodology is the use of the BTEXTRAP purge trap. This trap, which was initially developed for environmental analysis, exhibits a low retention for methanol (14). Methyl esters in aqueous methanol are easily separated from the matrix and absorb onto the trap with no solvent interferences. This is crucial because methanol would interfere with quantitation of the lower boiling methyl esters and, if retained on the purge trap, might occupy most of the active sites, leading to an overall decrease in sensitivity. The use of this purge and trap technique eliminated the need for solvent extraction and subsequent evaporation of solvent, which decreases recovery of volatile acid methyl esters.

Quantitative analysis

Tobacco samples were derivatized using methanolic hydrochloric acid and evaluated by purge and trap GC-SIMMS. Analytical data for 3-methylvaleric acid in flue-cured tobacco grades indicated values similar to data obtained using hexane extraction of the derivatives (15). Flue-cured and burley tobacco contain similar volatile acid content; however, oriental tobacco is distinctly different (Table II). Volatile organic acids have been known for some time to be more abundant in oriental tobacco (16). Oriental tobacco contains 400 times more 3-methylvaleric acid than flue-cured or burley tobaccos. The amount and presence of high levels of 3-methylvaleric acid in consumer

Table III. Volatile Organic Acids in Coffee, Tea, and Cigarette Products

Carboxylic acid (Retention time [min])	Coffee ($\mu\text{g/g}$ [%RSD])*	Tea ($\mu\text{g/g}$ [%RSD])	Cigarette A ($\mu\text{g/g}$ [%RSD])	Cigarette B ($\mu\text{g/g}$ [%RSD])	Cigarette C ($\mu\text{g/g}$ [%RSD])
Acetic (2.33)	577 (8.7)	887 (9.6)	546 (17)	655 (9.2)	674 (7.6)
Isobutyric (4.11)	5.11 (15)	5.73 (2.6)	19.6 (19)	33.6 (7.9)	30.73 (11)
Butyric (4.68)	7.96 (14)	6.26 (3.9)	5.57 (21)	7.83 (5.6)	6.72 (16)
Isovaleric (5.63)	113 (13)	1.90 (5.4)	68.2 (18)	101 (6.8)	88.0 (13)
Valeric (6.54)	0.68 (14)	6.48 (6.1)	6.28 (20)	6.41 (3.8)	5.52 (13)
3-Methylvaleric (7.69)	7.07 (34)	†	260 (30)	379 (17)	470 (18)
Hexanoic (8.48)	1.53 (22)	18.4 (8.4)	11.2 (47)	13.1 (39)	15.2 (7.7)
Heptanoic (10.36)	†	3.69 (27)	1.57 (22)	1.82 (26)	1.72 (5.9)
Octanoic (10.71)	6.75 (7.6)	7.82 (6.4)	5.20 (25)	7.69 (26)	6.54 (30)

* RSD = relative standard deviation.
† Not detected.

products could be used as an indicator of oriental tobacco. However, caution should be used with such assumptions because 3-methylvaleric acid is often used as a flavor additive material (1).

To demonstrate the utility of this technology in a variety of agricultural products, coffee, tea, and commercial cigarette samples were evaluated (Table III). The volatile acids present in all three products were similar and varied only in concentrations. Although citric, malic, and oxalic acids constitute the major acids in tobacco, the less abundant volatile organic acids contribute significantly to aroma and taste (1). Acetic acid was present in all samples at significant levels. The predominant volatile acid in coffee (other than acetic acid) was isovaleric acid, whereas hexanoic acid predominated in tea, and 3-methylvaleric acid was the major volatile acid in cigarette tobacco. Tea had slightly lower concentrations of volatile organic acids than tobacco or coffee. Volatile acid content profiles are one of the many differences in these products that contribute to their unique aroma profiles.

In summary, derivatization of agricultural products with methanolic hydrochloric acid followed by analysis using purge and trap GC-MS provided an excellent alternative to existing techniques for the analysis of organic acid content. This technology is rapid and requires limited sample preparation.

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