Analysis of Flue-cured Tobacco Extracts by Comprehensive Two-dimensional Gas Chromatography-Time-of-flight Mass Spectrometry

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1. Introduction

- Chemical composition of tobacco, extremely complex, more than 4000 estimated
- The physical and chemical properties of tobacco leaf, influenced by many factor, such as genetic, soil type and nutrients, climatic conditions, plant disease, stalk position, harvesting, and curing techniques.
- The change in any of these factors can markedly alter the chemical composition of leaf and thus affects smoking quality

1. Introduction

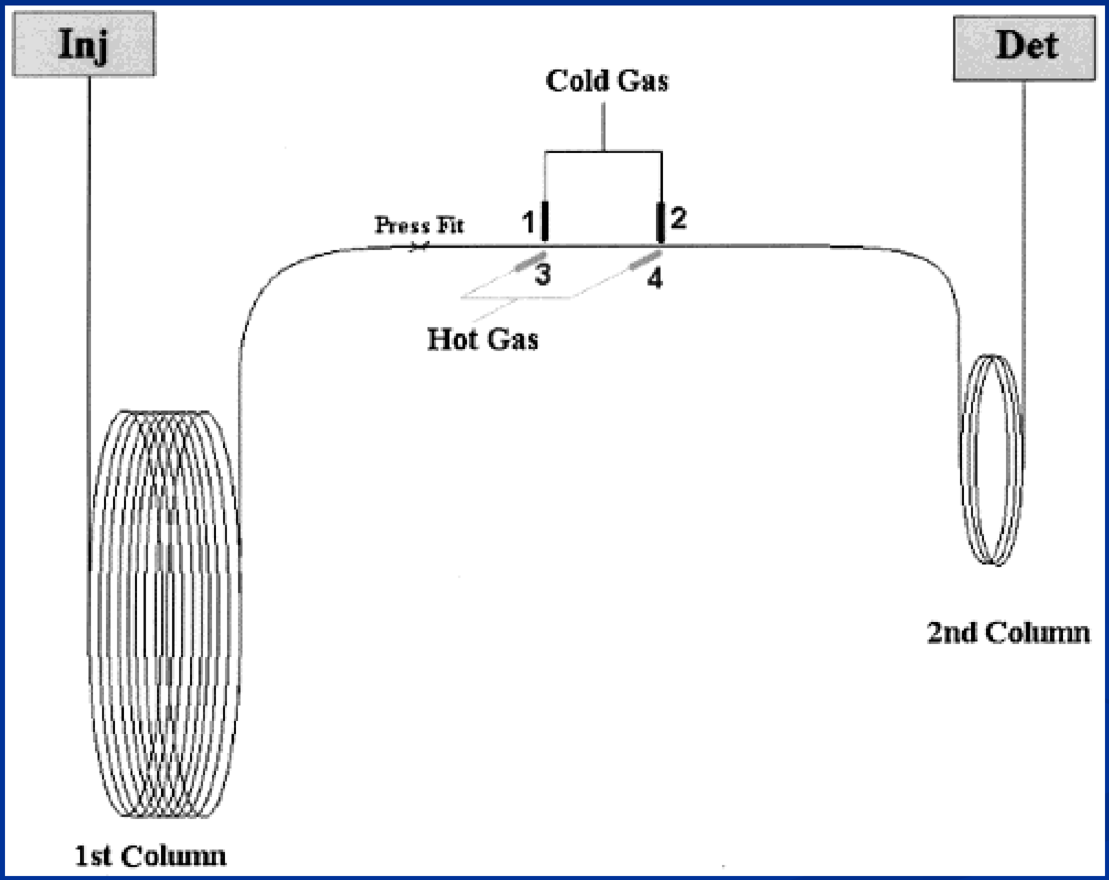
- Instrumental analysis methods, employed to analyze composition of tobacco leaf, including GC, GC/MS, HPLC, CE, NMR, IR, et al
- GC and HPLC have been demonstrated as the most effective analytical methods
- Tobacco are so complex that the separation power of one dimensional GC or HPLC, even with MS, may not be sufficient to solve emerging analytical challenges

1. Introduction

- GC×GC/ TOF-MS has been demonstrated to be a powerful analytical tool for the determination of the compounds in complex mixtures

- High resolution of GC×GC-TOFMS method has been developed for separation and identification of chemical composition in flue-cured tobacco extracts.

The Principle of Comprehensive two-dimensional gas chromatography



2. Experimental

2.1 Conditions of GC-MS and GC-TOFMS

GC/FID/MS	**QP 2010, Shimadzu**
Capillary column	**DB-Petro (50m*0.2mm *0.1μm)**
Program temperature	50ºC 2ºC/min 260ºC 10ºC/min 280ºC
Carrier gas	helium
Injection volume	3 μL
Split ratio	60:1
Mass Range	33～450 amu
GC/TOF-MS	**Agilent 6890N, Pegasus 4D GC×GC-TOFMS**
Capillary column	**DB-5 50m*0.2mm*0.33μm**
Program temperature	50ºC 2ºC/min 270ºC 10ºC/min 270ºC(25min)
Carrier gas	helium
Injection volume	3 μL
Split ratio	60:1
Mass Range	33～450 amu

2. Experimental

2.2 GC×GC Apparatus and Column Sets

- **GC×GC System:**
  - ✓ Agilent 6890 GC (Agilent Technologies,Wilmington,DE);
  - ✓ KT-2001 Retrofit prototype Cold-jet modulator (Zoex Corp,Lincoln,NE);
- **Column system:**
  - ✓ the first dimensional column DB-Petro (50 m *0.2 mm *0.5μm), the second dimensional column DB-1701(2 m *0.1 mm *0.1 μm),
  - ✓ Column Connection, by means of a press-fit connector

2. Experimental

2.3 Time-of-Flight-Mass spectrometer

Instrument	**Pegasus III TOF-MS(Leco Corp., St Josephs, MI, USA)**
Conditions	
EI	**70eV**
Transfer line temperature	270^{o}C
Ion source temperature	200^{o}C
Detector voltage	**1550V**
Mass Scan	**33-450 m/z with 50 spectra/s**

2. Experimental

2.4 Preparation of tobacco extract sample

- **Tobacco sample :**

M_2OL(zimbabwe) , C_2F(Yunnan, China), C_2F(Henan, China), C_2F(Fujian, China)

- **Sample preparations :**

Simultaneous distillation and extraction(Fig.1)

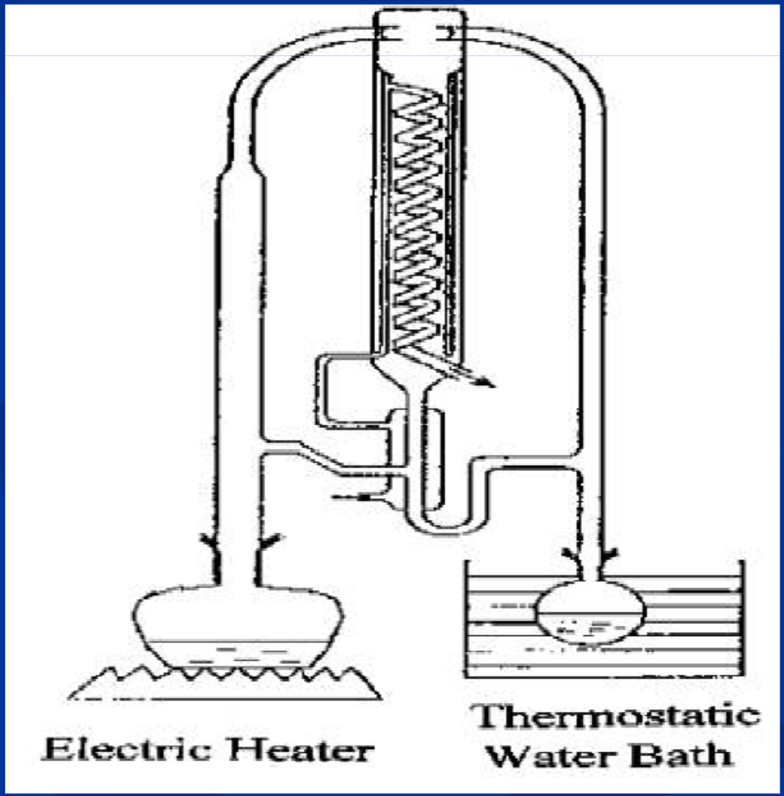


Fig.1 Simultaneous Distillation and Extraction

3. Results and Discussions

3.1 Selection of GC×GC Operation condition

Table 1

Set	1st column	2nd column
Length (m)	50	2.0
Diameter (mm)	0.2	0.1
Stationary phase	DB-Petro [a]	DB-1701 [e]
Film thickness (μm)	0.5	0.1
Temperature program	50℃ →2℃/min→270℃(20min)	50℃ →2℃/min→270℃(20min)

Carrier gas: helium, column head pressure: 600 kPa

a DB-Petro (J&W Scientific, Folsom, CA, USA), a 100% Dimethylpolysiloxane column

b DB-17ht (J&W), a 50% Phenyl-methylpolysiloxane column

c CEC-WAX (Chrom Expert Company, USA) a Polythylene glycol

d DB-WAX(J&W) a Polythylene glycol

e DB-1701(J&W), a (14%-Cyanopropyl-phenyl)-methylpolysiloxane

3. Results and Discussions

3.2 1D-GC/FID and 1D-GC/MS analyses

- **FID: 120 components detected**
- **MS: 46 components detected, shown in Table 2**
- **Common shortage: peeks overlap, partially resolved peaks, and shoulders**

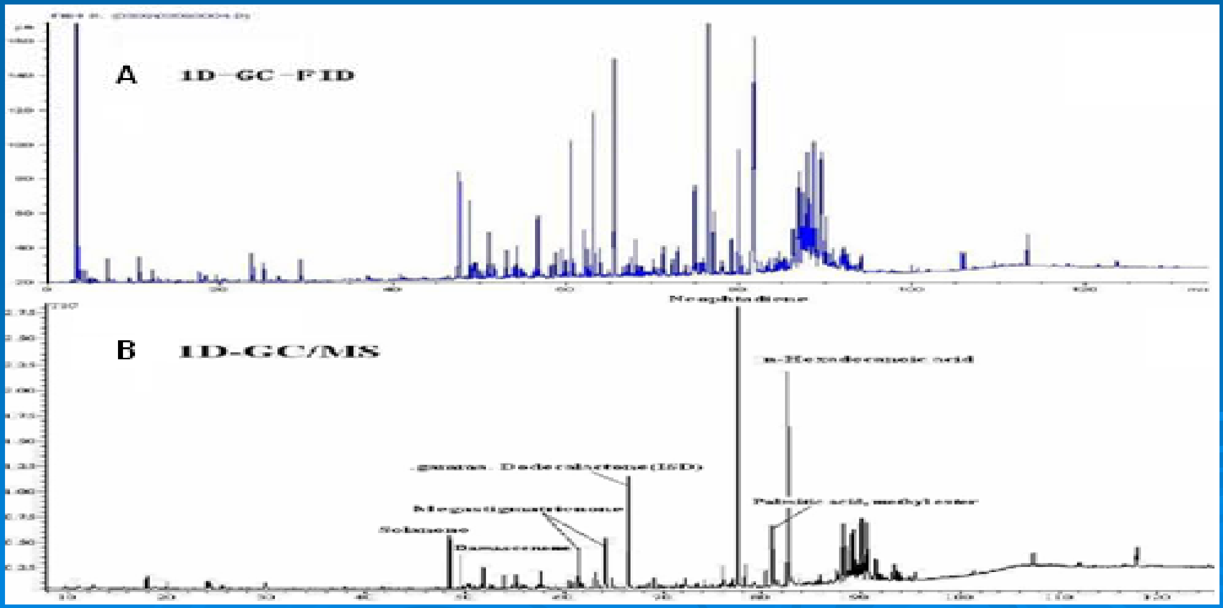


Fig.2 TIC of tobacco extracts by 1D-GC/FID (A) and 2D-GC/MS(B)

Table2 Components detected by 1D-GC/MS in tobacco extraction

RT/min	SI	Name	RT/min	SI	Name
7.17	96	(S)-(+)-1,2-Propanediol	63.46	89	Megastigmatrienone
9.61	88	3(2H)-Furanone, dihydro-2-methyl-	63.80	74	Benzene, (4,5,5-trimethyl-1,3-cyclopentadien-1-yl)-
10.85	97	Furfural	64.18	92	Megastigmatrienone
12.44	85	2-Furanmethanol	64.88	91	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-
17.89	86	Cyclopentane, 1,2,3,4,5-pentamethyl-	66.57	93	.gamma. Dodecalactone
19.96	91	Cyclohexane, 1-methyl-2-propyl-	72.21	93	Tetradecanoic acid
24.01	95	Benzyl Alcohol	74.13	87	Solavetivone
24.30	93	Benzeneacetaldehyde	77.07	90	2-Pentadecanone, 6,10,14-trimethyl-
25.47	91	2-Acetylpyrrole	77.52		**Neophtadiene**
29.85	94	Phenylethyl Alcohol	79.20	93	Benzeneacetic acid, 2-phenylethyl ester
48.46	92	Solanone	79.31	83	Linolenic acid, methyl ester
49.66	96	β-Damascenone	81.07	94	Palmiticc acid, methyl ester
50.33	83	Benzene, 1-methyl-4-[(1-methylethylidene)cyclopropyl]-	82.65	93	n-Hexadecanoic acid
50.53	86	Naphthalene, 1,2-dihydro-1,4,6-trimethyl-	83.69	89	3-(4,8,12-Trimethyltridecyl) furan
51.60	73	4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	88.93	93	Linoleic acid, methyl ester
51.73	85	β-Damascone	89.12	85	Linolenic acid, ethyl ester
51.92	87	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	90.20	93	Phytol
55.74	83	1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	90.44	93	Linoleic acid
57.64	89	Dihydroactinidiolide	90.62	93	Stearic acid, methyl ester
60.50	88	Megastigmatrienone	90.85	83	Oleic acid
61.55	93	Megastigmatrienone	107.22	92	Pentacosane
61.78	76	2-Butanone, 1-(2,3,6-trimethylphenyl)-	107.53	96	1,2-Benzenedicarboxylic acid, diisooctyl ester
63.09	79	3-Hydroxy-.beta.-damascone	117.87	92	Heptacosane

3. Results and Discussions

3.3 GC/TOFMS analyses

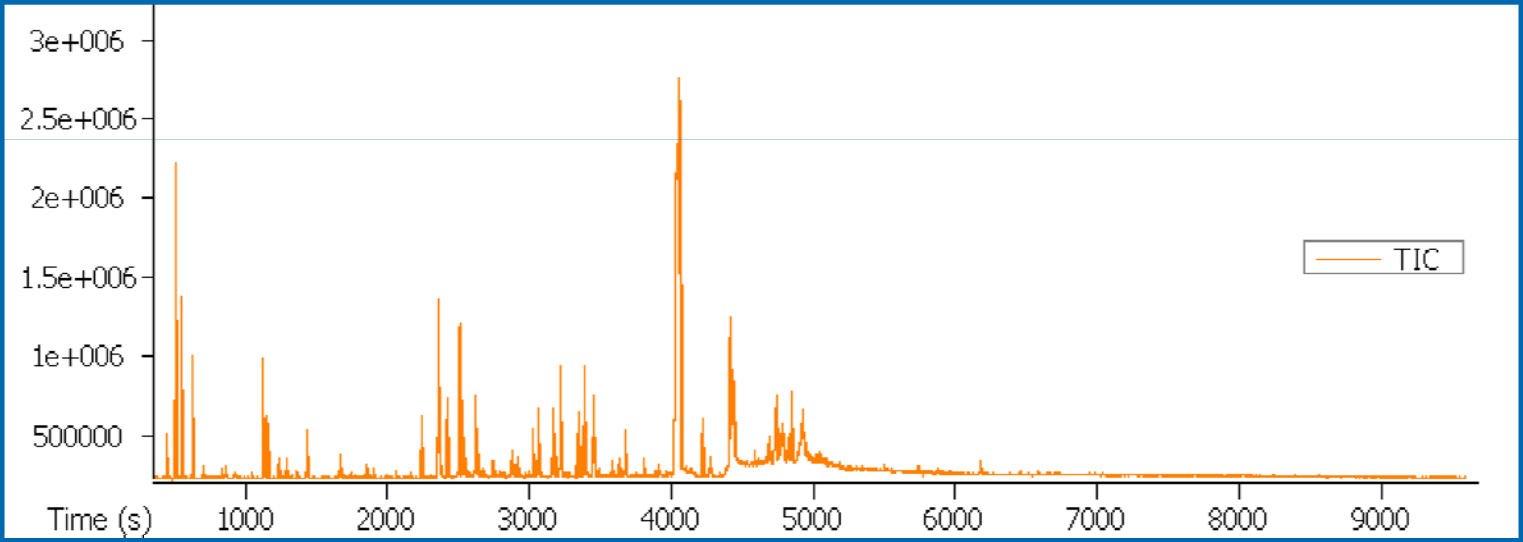


Fig. 3 TIC of tobacco extract by GC/TOFMS

- 79 components detected (Similarity>800)
- Peak Overlap, **partially separation peaks, shoulders, and shift** of base line

3. Results and Discussions

3.4 GC×GC analyses

Tobacco extracts sample was analyzed by GC×GC/FID and GC×GC/MS , respectively. The result shown as Figure 4 indicated:

- GC×GC/ FID: 2100 components identified
- GC×GC/ TOFMS: 1093 components identified (Similarity>800), which is over 20 times than that by 1DGC-MS method
- Good resolution, no peak overlap
- Groups separation can be achieved by adjusting the frequency of modulation period

3. Results and Discussion

3.4 GC×GC analyses

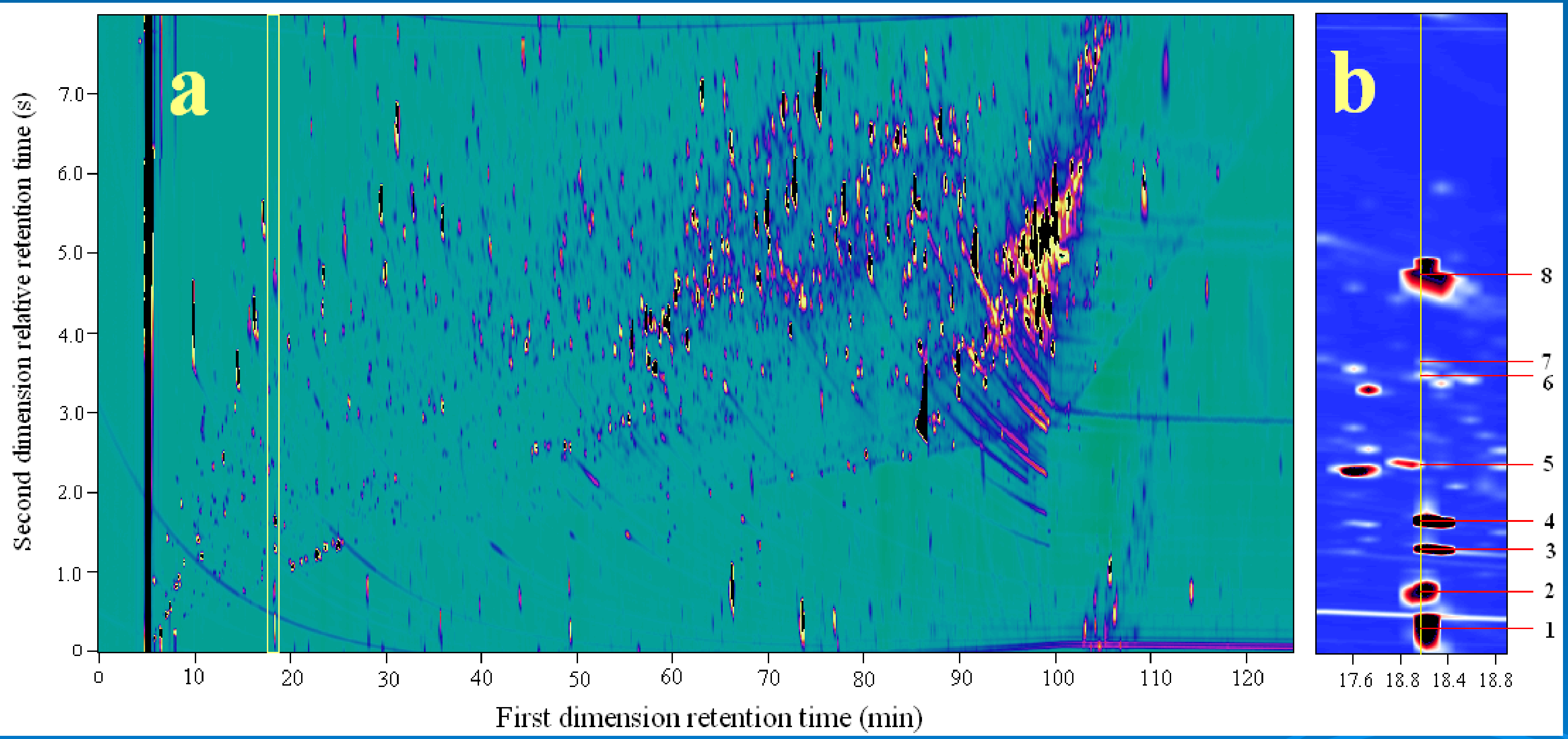


Figure 4. GC×GC separation of a typical tobacco condensate (a) and enlargement (b) Compounds

3. Results and Discussion

3.5 Identification of components in tobacco

- **Four tobacco extracts (Zimbabwe, Yunnan, Henan, and Fujian) were analyzed by GC×GC/ TOFMS method. The analytical result of Tobacco extract (Zimbabwe) was shown as table 3**

Table 3. Compounds identified by GC×GC/TOFMS (similarity>800)

Group	Num		Num
Hydrocarbons	**244**	**Oxygen-containing compounds**	**786**
Nitrogen-containing compounds	**53**	Ketones	245
pyridines	17	Aldehydes	82
pyrazines	5	Fruans	23
quinolines	6	Phenols	22
pyrazoles	3	Alcohols	167
indoles	2	Esters	134
pyrroles	2	Acids	62
nitriles	5	Others	51
others	13	**Sulfur-containing compounds**	**10**
Total		**1093**	

3. Results and Discussion

3.5 Identification of components in tobacco

- **244 hydrocarbons, 53 nitrogen-containing compounds, 786 oxygen-containing compounds, and 10 sulfur-containing compounds were Identified from Zimbabwe tobacco extract**
- **Oxygen-containing compounds is the most abundant group , including ketones, aldehydes, furans, phenols, alcohols, esters and acids**
- **Nitrogen-containing compounds include pyridines, pyrazines, quinolines, pyrazoles, indoles, nitrile derivatives**

3. Results and Discussion

3.6 Comparison of components in different tobacco

- Four tobacco extracts(Zimbabwe, Yunnan, Henan, and Fujian) were investigated
- Tobaccos from different growing district
  - Difference of Chemical component group
  - Difference of Chemical component content
  - Difference of the content in the same group components

4. Conclusions

- GC×GC in combination with TOFMS detection has been demonstrated as a powerful tool for the investigation of chemical composition in tobacco extracts
- A total of 1093 compounds were tentatively identified in flue-cured tobacco extracts, including 244 hydrocarbons, 53 nitrogen-containing compounds, 786 oxygen-containing compounds and 10 sulfur-containing compounds
- GC×GC method could distinguish tobaccos from different growing districts by analyzing the tobacco extracts.

Thank for your attention!