Asian Journal of Chemistry Vol. 22, No. 2 (2010), 1192-1196

Study on Determination of Polyphenols in Tobacco by Gas Chromatography-Mass Spectrometry

HONG-LIN SHI†, YI-RAN XU†, MING-MING MIAO, GUANG-YU YANG† and QIU-FEN HU*†
Department of Chemistry, Yunnan Institute of the Nationalities, Kunming 650031, P.R. China
E-mail: huqiufena@yahoo.com.cn

A gas chromatography-mass spectrometry method for the determination of polyphenols (chlorogenic acid, rutin and scopoletin) in tobacco samples was studied. The polyphenols were extracted from the samples by ultrasonic extraction and a 99:1 mixture of N,O-*bis*(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane was used for derivatization. With GC-MS detection in single-ion-monitoring mode, the main polyphenols (chlorogenic acid, rutin and scopoletin) were analyzed quantitatively. The limit of detections (S/N = 3) were below 50 ng/mL, the relative standard deviations (RSD) were below 3.2 % (n = 7) and the recoveries were in the range of 92-106 %.

Key Words: Gas-MS, Polyphenols, Derivatization, Tobacco.

INTRODUCTION

Polyphenols are important components in tobacco. There is a close relationship between polyphenols and the quality of tobacco. The polyphenols in tobacco greatly affect the odour and taste of smoking[1,2]. Therefore, the determination of polyphenols in tobacco and cigarettes is important. For the determination of polyphenols, the main techniques are spectrophotometry, gas chromatography (GC) and high-performance liquid chromatography (HPLC)[3-6].

The gas chromatographic method has the advantages of high separation capacity and extremely sensitive and selective when combined with mass spectrometry. Nevertheless, because of their high boiling points and their tendency to decompose at high temperatures, polyphenols should be derivatized before analysis to reduce polarity and boiling point. Silylation is the method of derivatization of polyphenols most often used before GC analysis. Li *et al.*[7] reported faster silylation when acetone was used as solvent (reaction medium) to accelerate the reaction. When rutin, scopoletin and chlorogenic were analyzed using this method, the results showed that the silylation of phenolic analytes can occur at room temperature and the reaction time can be reduced to 30 s. It was assumed that the rate of reaction is increased by the increased stability of the intermediate oxonium ions in polar solvents. However, the research on acetone-assisted derivatization of tobacco polyphenols has not been reported yet.

†Yunnan Academy of Tobacco Sciences, Kunming 650106, P.R. China.

In this work, a new GC-MS method was developed for rapid analysis of polyphenols in tobacco samples. Acetone-assisted silylation was followed by GC-MS analysis. A 99:1 mixture of N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) was used as the derivatizing reagent. The derivatization reaction time for the polyphenols could be reduced to within 5 min. This method has been successfully applied to the determination of polyphenols in tobacco with good results.

EXPERIMENTAL

Rutin, scopoletin and chlorogenic acid were purchased from the Fluka Corporation (Switzerland). Stock standard solutions of the three compounds were prepared by dissolving 25.0 mg solute in 100 mL methanol. The solutions were stored under refrigeration at 4 ºC in the dark. Five working solutions were prepared by mixing the stock solutions and diluting with methanol. The derivatizing reagent BSTFA plus TMCS (99:1) was purchased from Supelco (USA). Methanol and acetone were HPLC-grade obtained from Fisher Corporation (USA).

Extraction of polyphenols: Dry tobacco powder (60-80 mesh, *ca.* 0.2 g) was weighed in a vessel and 10 mL of *n*-hexane was added. The mixture was then placed in an ultrasonic water bath for 15 min. After centrifugation for 4.0 min at 4500 rpm, the upper solution was discarded (The objective of this step is to eliminate triglycerides[8] and the step was repeated twice for complete elimination). For the extraction of the phenolic, a 50 mL of methanol was added into the vessel. After 0.5 h ultrasonic extraction in an ultrasonic cleaner, the extract was filtered. Finally, the filtrate was diluted to 50 mL. The solutions were subjected to the derivatization procedures.

Derivatization: Derivatization was performed on 0.1 mL solution. Because the silylation reagent reacts with methanol, this solvent was evaporated by use of a stream of nitrogen before derivatization. Acetone (0.3 mL) and BSTFA-TMCS (99:1, 200 L) were then added at room temperature. For comparison, the conventional thermal method with diethyl ether as the solvent was also used[3]. The main conditions derivatization time and derivatization temperature were investigated.

GC-MS Analysis: GC-MS was performed on Agilent 6890 GC and 5973 mass spectrometer (Agilent Corporation, USA). Full-scan mass spectra (mass range 80-700 m/z) were acquired by electron-impact ionization at an electron energy of 70 eV. For quantitative analysis the MS was used in single-ion-monitoring (SIM) mode. Samples (2.0 µL) were injected by autosampler in splitless mode and the compounds were separated on a HP-5 MS column (30 m × 0.25 mm id × 0.25 µm film). Helium (99.999 % purity) was used as carrier gas at a flow rate of 1.0 mL min^{-1}. The injector temperature was 275 ºC. The oven temperature was held at 60 ºC for 1.0 min after injection, then programmed at 30 ºC min^{-1} to 130 ºC, which was held for 2.0 min, then programmed at 20 ºC min^{-1} to 205 ºC, which was held for 1.0 min and finally programmed at 30 ºC min^{-1} to 275 ºC, which was held for 15 min. The transfer line temperature was 280 ºC.

RESULTS AND DISCUSSION

Selection of derivatization method for the polyphenols: The acetone-assisted method was investigated at room temperature as suggested in the literature[7]. The most important condition affecting the reaction is derivatization time; 5, 30, 60, 90 and 120 min were therefore studied. The result shows that 5.0 min is sufficient for the polyphenols determined. The polarity of acetone may be the reason for its acceleration of the derivatization. This result is similar with that reported by Li *et al.*[7] who assumed that formation of an intermediate is favoured during the derivatization and that stabilization of the intermediate oxonium ion depends on the polarity of the solvent. Compared with the time required for conventional thermal derivatization (50 min)[3], the acetone-assisted method requires much less time (5 min only). The retention time and mass spectra of the derivatives obtained from the two methods were compared. The results showed that the products of acetone assisted derivatization were almost the same as those from conventional thermal derivatization. Acetone-assisted derivatization clearly performs better and was, therefore, used for the subsequent study.

Analyses of standards: Five working solutions were prepared by mixing the stock solutions and diluting with methanol. They were then derivatized by the acetone-assisted method, as described above. The solution of the derivative (2.0 μL) was injected for analysis. The three polyphenols can be separated successfully. Retention times and mass spectra were both used to identify the polyphenols. Retention times, with their relative standard deviations (RSD, n = 7) and the characteristic ions for each compound are listed in Table-1. The regression equations and their correlation coefficients are listed in Table-2. To investigate the repeatability of the method, seven measurements were repeated. The RSD of peak area was between 2.2 % (n = 7); these results are also listed in Table-2.

TABLE-1
RETENTION TIMES AND CHARACTERISTIC IONS OF THE POLYPHENOLS

Compound	Retention time (mean ± σ, n = 5)	Characteristic ions (abundance, %)
Rutin	24.46 ± 0.08	575 (36), 647 (100)
Chlorogenic acid	22.31 ± 0.08	255 (90), 345 (100)
Scopoletin	13.82 ± 0.68	234 (100), 249 (29), 264 (46)

σ is the relative standard deviation (RSD, %)

Analysis of tobacco samples: Polyphenols were extracted from tobacco samples by ultrasonic extraction as stated above. The time and temperature for ultrasonic extraction were 0.5 h and room temperature. Fig. 1 shows an example of a thin layer chromatogram of the tobacco sample. The SIM plots obtained for chlorogenic acid, rutin and scopoletin are also shown in Fig. 2. From these SIM plots the amounts of the polyphenols extracted were calculated by use of the regression equations. The precision and recovery of the method was measured. The results are listed

TABLE-2
CALIBRATION PLOTS FOR THE THREE POLYPHENOLS COMPOUND

Compound	Calibration range (μg/mL)	Regression equation	LOD (ng ML^{-1})	RSD % (n = 7)
Rutin	0.5–50	$A = -384 + 1.28 \times 10^5 C$, r = 0.992	50	1.8
Chlorogenic acid	0.6–80	$A = 587 + 1.54 \times 10^5 C$, r = 0.990	40	2.2
Scopoletin	0.8-80	$A = 626 + 1.92 \times 10^5 C$, r = 0.990	35	2.0

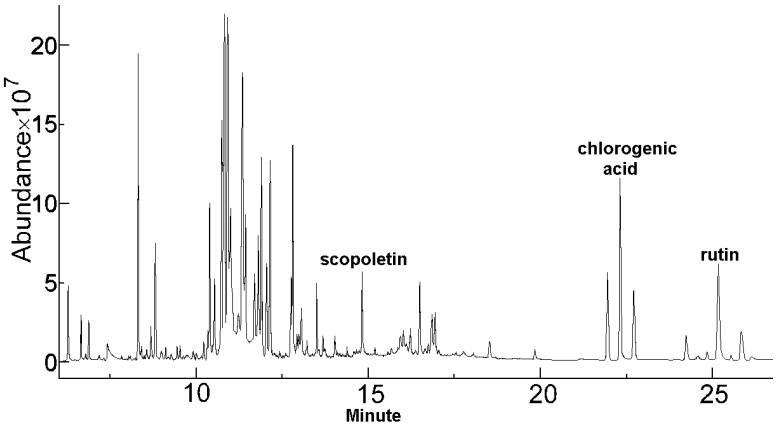

Fig. 1. Total-ion chromatogram of a tobacco sample

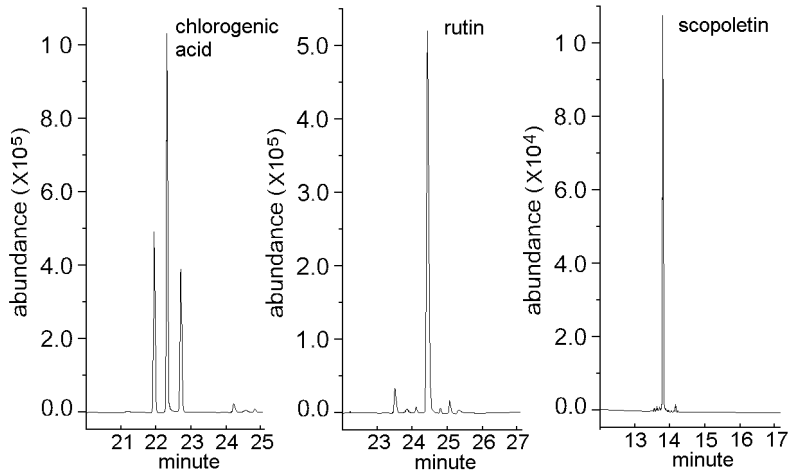

Fig. 2. SIM chromatograms of tobacco sample

in Table-3. By comparing the results obtained with the reflux extraction and routine derivatization method[3], it was found that the efficiency of the two methods was similar. Because of its simplicity, the use of the ultrasonic method is suggested.

TABLE-3
DETERMINATION RESULTS (%) OF THE SAMPLES

Components	Tobacco samples (%)				RSD % (n = 5)	Recovery % (n = 5)
	Yuxi C_2F	Yuxi B_2F	Dali C_3F	Dali X_2F		
Rutin	0.95	1.21	0.76	0.64	3.2	96-106
Chlorogenic acid	1.54	1.33	1.17	0.94	2.8	94-105
Scopoletin	0.225	0.312	0.174	0.148	3.0	92-103

CONCLUSIONS

In this paper, rapid acetone-assisted derivatization of polyphenols was investigated for analysis of polyphenols in tobacco. It was found that with acetone as solvent in the derivatization the reaction time for the polyphenols could be reduced to within 5 min. Three polyphenols (chlorogenic acid, rutin and scopoletin) can be analyzed quantitatively. The developed method was applied to tobacco samples. The results show that this method can therefore be used for rapid analysis of polyphenols in the tobacco industry.

REFERENCES

1. J. L. Kallianos. *Recent Adv. Tobacco Sci*., **20**, 61 (1976).
2. M.E. Snook, *Coresta*, **1**, 17 (1994).
3. H.L. Zhang and C.Y. Ge. Tobacco Analysis and Inspection, Henan Science and Technology Press, Zhengzhou, China, pp. 243-45 (1994).
4. Z. Li, L. Wang, G.Y. Yang, H.L. Shi, C.Q. Jiang and W. Liu, *J. Chromatogr. Sci*., **41**, 1 (2003).
5. A. Zafra, M.J.B. Juarez, R. Blanc, A. Navalon, J. Gonzalez and J.L. Vilchez, *Talanta*, **70**, 213 (2006).
6. A. Kakasy, Z. Fuzfai, L. Kursinszki, P.I. Molnar and E. Lemberkovics, *Chromatographia*, **63**, S17 (2006).
7. D.H. Li, J. Park and J.R. Oh, *Anal. Chem*., **73**, 3089 (2001).
8. M. Saitta, S. Lo Curto, F. Salvo, G. Di Bella and G. Dugo, *Anal. Chim. Acta*, **466**, 335 (2002).

(*Received*: 2 February 2009; *Accepted*: 22 October 2009) AJC-7970