Quantitative Analysis of Cigarette Smoke Condensate Monophenols by Reverse-Phase High-Performance Liquid Chromatography *

by

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SUMMARY

A rapid and quantitative method for the determination of cigarette smoke condensate monophenols using high-performance liquid chromatography (HPLC) was developed. Total particulate matter was collected on cigarette filter tips and Cambridge filter pads. Phenolic compounds were extracted with a 1 N sodium hydroxide solution.

Five different brands of filter-tipped cigarettes from the French market were analysed. Their phenol, guaiacol, methylphenols and dimethylphenols levels were determined in comparison with an internal standard, o-chlorophenol.

With the same method we compared the phenolic retention efficiency of different cigarette filter tips.

ZUSAMMENFASSUNG

Es wird ein Verfahren beschrieben, das die schnelle und quantitative Bestimmung von Monophenolen im Kondensat von Zigarettenrauch mittels Hochleistungsflüssigkeitschromatographie (HPLC) ermöglicht. Die Gesamtpartikelphase wird im Zigarettenfilter und in Cambridge-Filtern aufgefangen. Die phenolischen Ver-

bindungen werden mit einer 1 N Natriumhydroxidlösung extrahiert.

Fünf verschiedene französische Filterzigarettensorten wurden untersucht. Der Gehalt an Phenol, Guajakol, an Methylphenolen und Dimethylphenolen wurde unter Verwendung eines inneren Standards (o-Chlorphenol) bestimmt.

Unter Einsatz derselben Methode wurde das Filtrationsvermögen verschiedener Zigarettenfilter gegenüber den genannten Phenolen miteinander verglichen.

RÉSUMÉ

Nous proposons une méthode rapide et quantitative par chromatographie liquide à haute performance (HPLC), pour analyser les monophénols du condensat de la fumée de cigarette. La phase particulaire de la fumée est piégée sur bout-filtre de cigarette et sur filtre Cambridge. Les composés phénoliques sont extraits par une solution de soude 1 N.

Pour cinq types de cigarettes du marché français, les quantités de phénol, gaïacol, méthylphénols et diméthylphénols sont déterminées par rapport à l'o-chlorophénol selon la méthode de l'étalonnage interne.

Par la même méthode, nous avons comparé l'efficacité de rétention vis-à-vis de ces phénols de différents filtres de cigarette.

* Received: 30th January 1984 — accepted: 1st August 1984.

INTRODUCTION

Numerous quantitative methods have been published for determining phenolic compounds in cigarette smoke (1–8). Several leaf constituents (cellulose, lignin, polyphenolic pigments) were identified as pyrolytic precursors of smoke phenolic compounds (9–13).

Colorimetric methods involving the reactions between phenols and aryldiazonium salt or 4-aminophenazone give only an overall value of smoke phenols. Generally, this value underestimates the actual amount of smoke phenols. Chromatographic methods allow the selective and rapid analysis of the most abundant monophenols from tobacco smoke (1, 2, 4–9). Gas chromatography has been widely used. To our knowledge, only *Kuwata* et al. (14, 15) have used high-performance liquid chromatography (HPLC) to analyse tobacco smoke monophenols as azo dyes.

We propose an analytical method for determining the monophenols present in tobacco smoke condensate. Reverse-phase high-performance liquid chromatography is used for this method without derivatization.

EXPERIMENTAL

Apparatus and Reagents

- Liquid chromatographic separations were performed on a Waters Associates liquid chromatograph with Model 6000 A pumps. The detector was a Schoeffel M 450 variable-wavelength UV detector. Samples were injected via a Model U6K injection system.
- A 250 mm × 4 mm inside diameter LiChrosorb RP-18 (7 μm) (Merck) column was used at room temperature.
- Solvents and chemical compounds were analytical grade. Phenol standards were obtained from commercially available sources. Purity was greater than 98 % except for 2,4-dimethylphenol which was a mixture of 85 % 2,4-dimethylphenol and 8 % 2,5-dimethylphenol; it was used without further purification.
- Cigarettes, from homogeneous batches, were smoked on a commercial Model 301 Filtrona smoking machine according to French standard NF V 37-001 using Cambridge filter pads. The Cambridge and cigarette filters were subsequently collected for analytical procedures.

Analytical Procedures

First we determined the best separation conditions for reverse-phase chromatography of the major monophenols from tobacco smoke: phenol, guaiacol, methylphenols, dimethylphenols. The eluting solvents studied were: water/methanol, water/acetonitrile and water /

Figure 1.

Separation of monophenolic compounds on a LiChrosorb RP-18 column. Column: LiChrosorb RP-18 (7 μm; 25 cm × 4 mm inside diameter); mobile phase: water / acetonitrile / acetic acid (700/300/5 (v/v)); flow rate: 1.0 ml / min at room temperature; UV detector: 275 nm wavelength).

acetonitrile / acetic acid in different ratios. Comparison of the capacity factor ($k' = (V_R - V_m)/V_m$) and resolution ($R_s = 2 \Delta V_R / (W_1 + W_2)$) between chromatograms shows that we obtain a good separation with an eluting solvent which has the following ratio: 700-300-5 (v/v) water / acetonitrile / acetic acid, and 1 ml / min as flow rate (Fig. 1). The ultraviolet absorbance detector was adjusted to 275 nm. It is the maximum absorbance mean of the various phenols analysed.

With this procedure and an 0.01 AUFS (absorption units full scale) sensitivity, the minimum detectable amounts were: 10 ng of phenol, 15 ng of methylphenols, 100 ng of 3,4-dimethylphenol. The relationship between the injected amount of each phenol and the corresponding peak height was linear at 0.1 AUFS sensitivity. Graphs for each analysed phenol were plotted at this detection sensitivity. Calibration curves were calculated by the internal standard method with *o*-chlorophenol as internal standard (4, 6). The results are shown in Table 1 and Figure 2. Figure 3 shows a chromatogram from a real sample.

Table 1.
Linear equation parameters.

$$q_x = m q_e \frac{h_x}{h_e} + b$$

q_e : amount of internal standard
 h_e : peak height measurement of internal standard
 q_x : amount of phenol analysed
 h_x : peak height measurement of phenol analysed
 m : slope of the line
 b : y intercept

	m	b	r
phenol	0.547	- 17.0	0.999
gualacol	0.564	+ 8.3	0.991
3-methylphenol	0.922	- 3.6	0.998
4-methylphenol	0.926	- 3.0	0.999
2-methylphenol	0.874	- 3.8	0.997
3,4-dimethylphenol	1.506	- 3.8	0.999

$$\text{correlation coefficient } r = m \frac{\sigma(q_e h_x / h_e)}{\sigma q_x}$$

Figure 2.
Calibration curves for phenols.

Figure 3.
HPLC chromatograms of cigarette smoke condensate mono-phenols (cigarette E). HPLC conditions and peak identity are identical with Figure 1 conditions.

Sample Preparation

Cigarettes, selected by weight and draw resistance, were smoked through a Cambridge filter on an eight-channel smoking machine. Five cigarettes were smoked successively on each channel. The resulting total particulate matter was collected on the cigarette filter tips and on the Cambridge filter. A sample batch comprised two Cambridge filters or ten of the corresponding cigarette filter tips.

Each batch was extracted with 50 ml of 1 N sodium hydroxide for two hours with intermittent stirring. The solution was filtered through a sintered glass funnel (No. 3) and then poured into a 100 ml calibrated flask. The residue and the flask were washed with 4×10 ml portions of 1 N NaOH, then all basic extracts were combined and diluted to a final volume of 100 ml. A 20 ml aliquot was transferred to a separatory funnel where the basic and neutral compounds were extracted from the alkaline solution with *n*-hexane (3×2 ml). Phenols were regenerated from their salt by acidification (6 N H_2SO_4) and extracted into ether (5×5 ml). The ether fraction was extracted with saturated sodium hydrogen carbonate solution to eliminate organic acids. It was then concentrated to approximately 1 ml at 45 °C in a water bath at atmospheric pressure. HPLC eluting solvent (2 ml) was added and the residual ether was evaporated at room temperature and 100 mm Hg pressure. The phenolic fraction was poured into a 5 ml volumetric flask. Internal standard was added and the solution diluted to 5.0 ml with the eluting solvent. The various extractions were quantitative for monophenols. This fact was confirmed by HPLC analysis. We did not observe the presence of studied phenols in the final extractions or residual ether.

Table 2.
Major phenols ($\mu\text{g}/\text{cigarette}$) and phenolic retention efficiency R (%) of cigarette filters.

Type of cigarette *	Filter type	Colorimetric method		HPLC method										Total
		$\mu\text{g/cig.}$	R (%)	phenol		guaiacol		3- and 4-methylphenols		2-methylphenol		3,4-dimethylphenol		
				$\mu\text{g/cig.}$	R (%)	$\mu\text{g/cig.}$	R (%)	$\mu\text{g/cig.}$	R (%)	$\mu\text{g/cig.}$	R (%)	$\mu\text{g/cig.}$	R (%)	
Cigarette A (dark tobacco (Caporal))	paper	172 $\pm$ 3	56 $\pm$ 2	101 $\pm$ 5	63 $\pm$ 6	41 $\pm$ 3	61 $\pm$ 10	61 $\pm$ 2	62 $\pm$ 4	25 $\pm$ 1	64 $\pm$ 4	166 $\pm$ 7	60 $\pm$ 4	394 $\pm$ 18
Cigarette B (dark tobacco (Caporal))	paper	193 $\pm$ 4	60 $\pm$ 2	109 $\pm$ 5	60 $\pm$ 5	40 $\pm$ 3	57 $\pm$ 9	57 $\pm$ 2	58 $\pm$ 4	22 $\pm$ 1	59 $\pm$ 4	185 $\pm$ 8	56 $\pm$ 4	413 $\pm$ 19
Cigarette C (dark tobacco (Caporal doux))	paper	152 $\pm$ 3	58 $\pm$ 2	87 $\pm$ 4	63 $\pm$ 6	43 $\pm$ 3	63 $\pm$ 10	52 $\pm$ 2	60 $\pm$ 4	22 $\pm$ 1	64 $\pm$ 4	137 $\pm$ 6	56 $\pm$ 4	341 $\pm$ 16
Cigarette D (blended tobacco)	cellulose acetate	194 $\pm$ 4	78 $\pm$ 3	102 $\pm$ 5	81 $\pm$ 7	45 $\pm$ 4	73 $\pm$ 11	48 $\pm$ 2	73 $\pm$ 5	21 $\pm$ 1	81 $\pm$ 5	147 $\pm$ 6	61 $\pm$ 4	363 $\pm$ 18
Cigarette E (Virginia tobacco)	cellulose acetate	207 $\pm$ 4	75 $\pm$ 3	114 $\pm$ 6	75 $\pm$ 7	37 $\pm$ 3	65 $\pm$ 10	47 $\pm$ 2	68 $\pm$ 5	22 $\pm$ 1	73 $\pm$ 4	165 $\pm$ 7	56 $\pm$ 4	385 $\pm$ 19

* cigarettes B and C:
length 78 mm, diameter 8.65 mm, cigarette ventilation 3%, filter ventilation 0%.

cigarettes A, D and E:
length 84 mm, diameter 7.90 mm, cigarette ventilation 17%, filter ventilation 0% except for cigarette D (2.1%).

Table 3.
Retention efficiency R (%) of various cigarette filters. *

Filter	Phenol		Guaiacol		3- and 4-Methylphenols		2-Methylphenol		3,4-Dimethylphenol	
	type of cigarette		type of cigarette		type of cigarette		type of cigarette		type of cigarette	
	dark tobacco (Caporal)	blended tobacco	dark tobacco (Caporal)	blended tobacco	dark tobacco (Caporal)	blended tobacco	dark tobacco (Caporal)	blended tobacco	dark tobacco (Caporal)	blended tobacco
Paper	64 ± 6		62 ± 10		62 ± 4		62 ± 4		60 ± 4	
F. P. A. **	70 ± 6	74 ± 6	63 ± 10	67 ± 10	64 ± 4	69 ± 4	64 ± 4	71 ± 5	57 ± 4	62 ± 4
S. P. A. ***	72 ± 6	76 ± 6	69 ± 11	74 ± 11	68 ± 4	71 ± 5	71 ± 5	77 ± 5	58 ± 4	62 ± 4
Cellulose acetate	81 ± 7	81 ± 7	68 ± 11	74 ± 11	75 ± 5	74 ± 5	78 ± 5	81 ± 5	61 ± 4	61 ± 4

* All of the filter cigarettes were 84 mm long, 7.90 mm in diameter and with 17 % cigarette ventilation and 0 % filter ventilation.

** Cellulose and cellulose acetate fibre blend without definite structure.

*** Paper filter with cellulose acetate fibres. The paper is formed to S structure.

RESULTS AND DISCUSSION

Five different brands of filter-tipped cigarettes from the French market were analysed by this method and compared by colorimetric determination of the total steam-volatile phenols. The results are given in Table 2. No considerable variation was observed from cigarette to cigarette in the amounts of the corresponding individual phenols. The pattern of phenol distribution was generally the same for all cigarettes studied.

Resolution of *ortho*-substituted dimethylphenols was insufficient but they were present at low levels. We did not observe the presence of 3,5-dimethylphenol. 3,4-Dimethylphenol was present as often noted in cigarette smoke but its level was unusually high (5–8, 16). The difference between colorimetric and chromatographic results is explained by the fact that *para*-substituted phenols do not react with 4-aminophenazone (3).

Using the same method we have compared the efficiency of different filters in relation to phenolic retention. These results are given in Table 3. We observed that the efficiency of removal of the phenolic fraction increases with the presence of cellulose acetate. The retention by cellulose acetate filters differed for individual phenols, in contrast to paper filters, with which there was no selectivity.

CONCLUSION

This paper shows that high-performance liquid chromatography is a good analytical tool for the determination of monophenols in cigarette smoke condensate. In comparison with colorimetric or gas chromatographic methods it is simple, rapid and selective. Steam distillation and derivatization to azo dyes or volatile compounds are not necessary. Individual monophenols are identified by their reten-

tion times or elution volumes with a low detection limit. This method shows good reproducibility and its automation is possible at least for chromatographic measurement.

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