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Determination of organic acids in cigarette smoke by high-performance liquid chromatography and capillary electrophoresis

D. Lagoutte*, G. Lombard, S. Nisseron, M.P. Papet, Y. Saint-Jalm

Research Department, SEITA, 4 Rue André Dessaux, 45401 Fleury-les-Aubrais Cedex, France

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Abstract

The most abundant low-molecular-mass organic acids in cigarette smoke are glycolic, lactic, formic and acetic acids. In this study, these substances were detected and determined by high-performance liquid chromatography (HPLC) and by capillary electrophoresis (CE). HPLC analysis used precolumn derivatization with the *p*-bromophenacyl bromide. The two methods were compared. The levels of each organic acid in a typical "European blend" cigarette smoke measured by HPLC and CE were comparable. The corresponding run-to-run relative standard deviations (R.S.D.s) ranged from 6 to 12.7% for HPLC and from 2.8 to 12.4% for CE. The smoking-to-smoking reproducibility (R.S.D.) was between 4.2 and 11.0% for HPLC and between 1.2 and 14.0% for CE. The limit of detection, calculated at a signal-to-noise ratio of 3 for each acid, was about 10^{-6} mol/l for the two methods, corresponding to 5 pmol of analyte injected for HPLC and 0.5 pmol for CE. CE was shown to be a good alternative to HPLC, requiring almost no sample preparation other than dilution, and giving a short analysis time (less than 15 min).

1. Introduction

The mainstream smoke aerosol phase produced by burning tobacco is a complex matrix composed predominantly of water, nicotine and organic molecules. Several organic acids have been identified in cigarette smoke: low-molecular-mass organic acids [1–5], mainly acetic, formic, lactic and glycolic [6]; aromatic acids [7,8]; and high-molecular-mass organic acids [9–11]. Analytical approaches adopted for the measurement of these compounds have been based on gas chromatographic (GC) methods with derivatization steps [6–13] or high-performance liquid chromatography (HPLC) [14,15].

GC methods used the conversion of organic acids into methyl [7,9,10], butyl [6] and pentafluorobenzyl [12,13] esters, or trimethylsilyl derivatives [2,6,8,9]. In all instances, complex extraction steps were necessary, because these methods were not specific for organic acids and the reagents used could interact with many compounds containing functional groups with an active hydrogen. Moreover, GC methods were difficult to use in a routine manner.

We have found no report on the use of high-performance ion-exclusion chromatography for the analysis of tobacco smoke and only two

* Corresponding author.

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references to anion-exchange chromatography. This is probably because smoke produced by burning tobacco is largely insoluble in water and most samples for ion chromatography are aqueous or are solubilized in an aqueous medium. As with GC methods, the HPLC methods also used complex extraction steps followed by separation on an anion-exchange column. Owing to the poor resolution of the analysis, only acetic and formic acids could be detected and determined [14].

Current HPLC or GC methods thus lack specificity and ruggedness for routine work. The aim of this work was to develop a routine method for the determination of the four most abundant low-molecular-mass organic acids (glycolic, lactic, formic, acetic) in cigarette smoke. We focused our attention on reversed-phase HPLC, including a derivatization step with *p*-bromophenacyl bromide, and capillary electrophoresis (CE). CE is a method with great potential for the high-resolution separation of various substances [16]. The detection and determination of organic acids by CE has been carried out in many raw materials such as wines [17], sugar refinery juices [18] and food samples [19]. To our knowledge, no report has been published concerning the CE separation of organic acids in cigarette smoke.

2. Experimental

2.1. Instrumentation

HPLC

The HPLC apparatus used in this study consisted of an LDC/Milton Roy Constametric I and II solvent-delivery system and an LDC/Milton Roy SM 4000 UV detector. The separation was carried out with a Nucleosil $5C_{18}$ column (12.5 cm × 3 mm I.D.) from Macherey–Nagel equipped with the corresponding precolumn (8 × 4 mm I.D.). Samples and standards were injected using a Rheodyne 5-μl loop. Data acquisition and treatment were accomplished by using a Minichrom data acquisition system (VG Data Systems).

CE

The CE instrument used was a Quanta 4000 (Waters Chromatography Division of Millipore) with a Maxima 820 data station (version 3.30). Conventional fused-silica capillaries (100 cm × 375 μm O.D. × 75 μm I.D.) were used. Detection was carried out by measuring the absorbance on the column at a position 10 cm from the end of the capillary tube. All pH values were measured with a Schott Geräte Model 6820 pH meter calibrated immediately prior to use.

2.2. Reagents

Deionized water (produced with a Millipore Milli-Q water-purification system) was used to prepare all solutions. Acetonitrile was obtained from Merck (LiChrosolv grade), methanol from Carlo Erba (for HPLC grade) and acetone from Merck (for analysis grade). All solvents were used as received. Sodium hydrogencarbonate was obtained from Fluka (Microselect grade). Sodium hydroxide (1 *M*) was purchased from Merck. The reagents used for HPLC were *p*-bromophenacyl bromide (Merck) and two crown ethers, dicyclohexane-18-crown-6 and 1,4,7,10,13,16-hexaoxacyclooctadecane (Fluka). The internal standards were 2,5-dimethylacetophenone (ICN) for HPLC and methylsuccinic acid (Fluka) for CE. The electroosmotic flow modifier (OFM Anion-BT) was obtained from Millipore. It is composed mainly of tetradecyltrimethylammonium bromide (TTAB). The UV-active component used for CE, with the same charge as the analytes, was potassium hydrogenphthalate (Aldrich). The standard samples used for calibration were sodium L-lactate (Fluka, purum), potassium acetate (Prolabo, Normapur), potassium formate (Fluka, Microselect), glycolic acid (Merck, for analysis) and sodium succinate (Merck, for synthesis).

2.3. Mechanical smoking of cigarettes and trapping of smoke

Mechanical smoking of cigarettes was carried out under normal conditions [20]. A twenty-channel Filtrona 300 smoking machine was used.

The particulate phase of the smoke was trapped by filtration through a normalized Cambridge fibre-glass filter (Borgwaldt, Hamburg, Germany). Twenty-five cigarettes were smoked for each determination.

2.4. Procedures

HPLC

Immediately after smoking, five Cambridge filters (each corresponding to the smoke of five cigarettes) were placed in 25 ml of an acetone solution containing the internal standard (2,5-dimethylacetophenone, 0.5 μl/ml). After mixing, the filters were allowed to stand overnight in the acetone solution, which is the necessary duration to perform the extraction of the acid fraction of the smoke. The day after, the filters were crushed and the extract was filtered. The derivatization reaction was then performed following Durst et al.'s procedure [21]: 500 μl of the extract were added to 100 mg of sodium hydrogencarbonate, 100 μl of a solution of *p*-bromophenacyl bromide (80 mg/ml) in acetonitrile and 100 μl of a solution of dicyclohexane-18-crown-6 (8 mg/ml) and 1,4,7,10,13,16-hexaoxacyclooctadecane (8 mg/ml) in acetonitrile. The reaction was allowed to proceed at 80°C for 1 h, then the preparation was injected into the liquid chromatograph without further purification. The mobile phase consisted of a mixture of 39% acetonitrile–methanol (52:48) and 61% water between 0 and 20 min, then a linear gradient between 61 and 0% water was applied for 3 min. The flow-rate was 1 ml/min. All HPLC solvents were filtered and degassed prior to use. The absorption of the eluate was measured at 255 nm (direct UV detection). All experiments were performed at room temperature (22°C).

CE

Immediately after smoking, five Cambridge filters (each corresponding to the smoking of five cigarettes) were placed in 25 ml of acetone containing the internal standard (methylsuccinic acid, 50 μl of a 0.52 g/l aqueous solution). After overnight extraction, the filters were crushed and the extract was filtered. Sample solutions were placed in the CE apparatus after diluting each acetone solution with four volumes of deionized water. The background electrolyte was 5 m*M* potassium hydrogenphthalate + 1 m*M* OFM Anion-BT (TTAB) flow modifier. Its pH was adjusted to 5.6 with 10 m*M* NaOH. The indicated concentration of OFM Anion-BT refers to the concentration of the commercial solution; 5% (v/v) is equivalent to 1 m*M* active substance. Samples were introduced hydrostatically by elevation of the sample vials to 10 cm for 30 s. The migration voltage and the current were kept constant at 18 kV and 5.8 μA, respectively. Analyte zone were detected by indirect UV absorbance at 254 nm. The total analysis time was 15 min. The capillary was purged with 1 *M* NaOH for 10 min, followed by a 5-min purge with deionized water and a 5-min purge with the separation buffer, prior to the initial run. It was also purged with the separation buffer for 5 min between each run. All experiments were performed at 24°C (internal temperature of the CE apparatus).

3. Results and discussion

3.1. Identification of low-molecular-mass organic acids in cigarette smoke by HPLC and CE

The samples of smoke treated as described above were analysed by HPLC and CE and compared with a mixture of synthetic products. The corresponding chromatogram and electropherogram obtained from a typical "European blend" cigarette smoke are shown in Figs. 1 and 2, and those corresponding to the synthetic products are shown in Figs. 3 and 4. Peak confirmation was achieved by comparison of migration times and co-injections of authentic standards with the smoke samples. Four organic acids were detected by HPLC, glycolic, lactic, formic and acetic acids, and five were detected by CE, formic, succinic, glycolic, acetic and lactic acids.

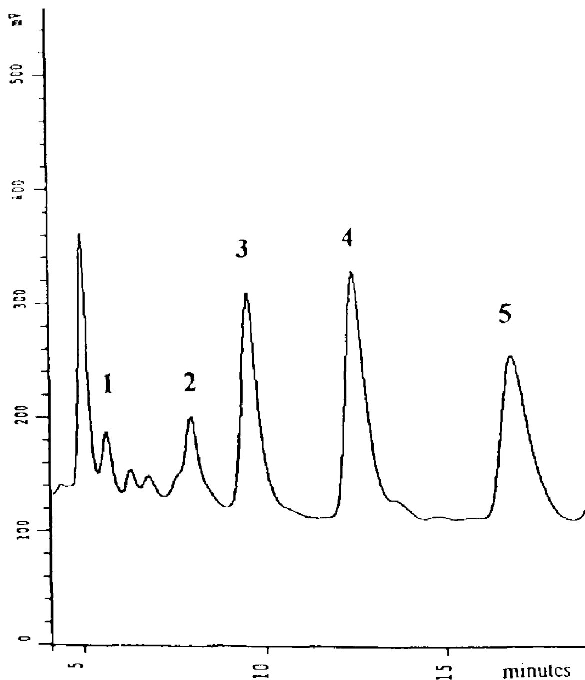

Fig. 1. Chromatogram of a European blend cigarette smoke. Peaks: 1 = glycolic acid; 2 = lactic acid; 3 = formic acid; 4 = acetic acid; 5 = internal standard.

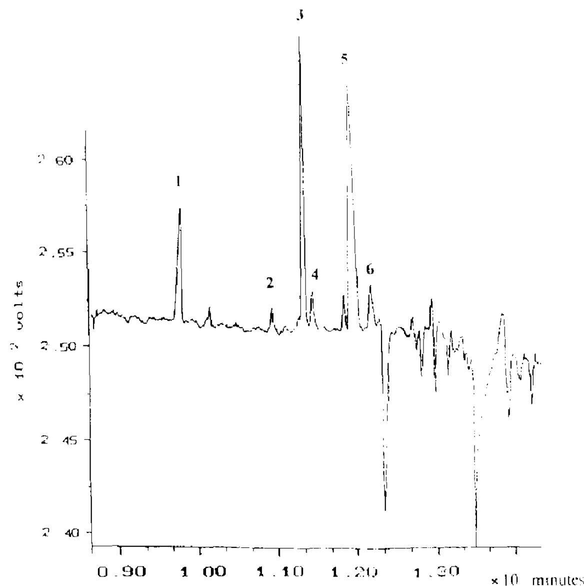

Fig. 2. Electropherogram of a European blend cigarette smoke. Peaks: 1 = formic acid; 2 = succinic acid; 3 = internal standard; 4 = glycolic acid; 5 = acetic acid; 6 = lactic acid.

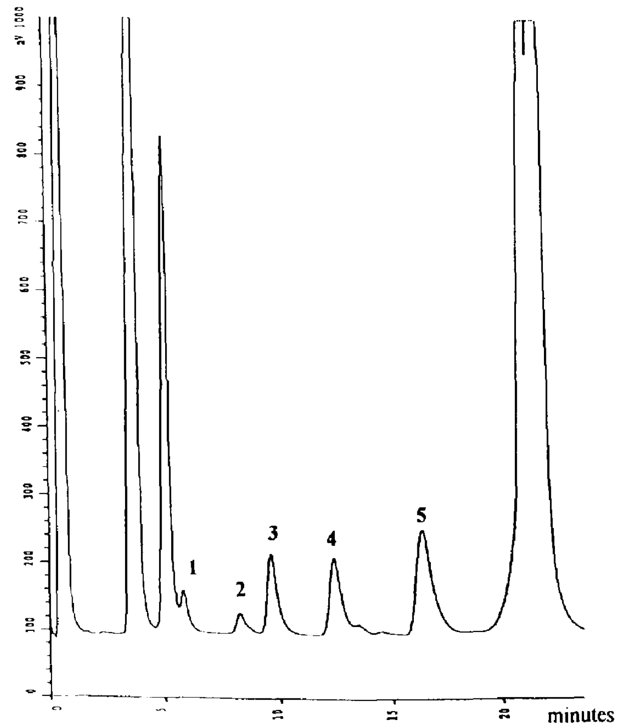

Fig. 3. Chromatogram of a standard mixture of four synthetic organic acids in acetone–water (9:1). Peaks: 1 = glycolic acid (52.6 μg/ml); 2 = lactic acid (57 μg/ml); 3 = formic acid (110 μg/ml); 4 = acetic acid (196 μg/ml); 5 = internal standard (0.5 μl/ml).

3.2. Quantitative study of the three main organic acids (lactic, formic, acetic) in a "European blend" cigarette smoke

Kinetics of the derivatization reaction (HPLC)

The kinetics of the reaction were studied directly on the smoke acetone solution. HPLC analyses of the reaction mixture were carried out at regular time intervals. The reaction was completed in 1 h (Fig. 5). A slower reaction rate was noted for acetic acid. The products formed were stable in the reaction mixture for at least 24 h.

Reproducibility and detection limit

A sample of the acetone smoke condensate was injected nine times into the HPLC apparatus and eight times into the CE apparatus. The reproducibility (R.S.D.) of the retention times was found to range from 1.4 to 1.7% for HPLC

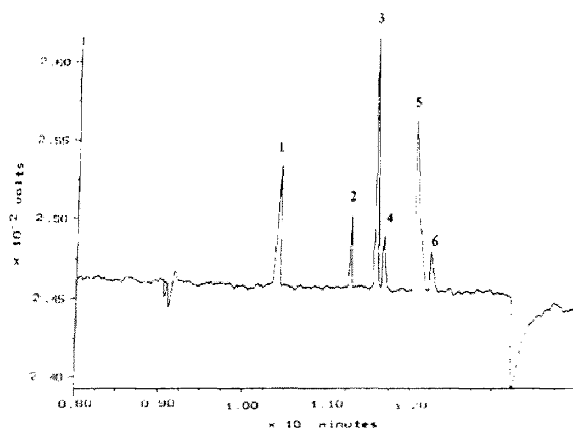

Fig. 4. Electropherogram of a standard mixture of five synthetic organic acids in acetone–water (9:1). Peaks: 1 = formic acid (27.3 μg/ml); 2 = succinic acid (26 μg/ml); 3 = internal standard (2 μl/ml); 4 = glycolic acid (28.3 μg/ml); 5 = acetic acid (97.5 μg/ml); 6 = lactic acid (30 μg/ml). Before injection into the CE apparatus, the standard solution was diluted with four volumes of deionized water.

and from 0.4 to 0.7% for CE (Table 1). The concentration reproducibility (R.S.D.) was 6–12.7% for HPLC and 2.8–10.5% for CE (Table 2). R.S.D.s obtained for retention time (Table 3) and concentration (Table 4) by repetition of the smoking were of the same magnitude: between 4.2 and 11.0% for HPLC, and between 1.2 and 14.0% for CE for concentration.

The detection limit, calculated at a signal-to-noise ratio 3 for each acid, was about 10^{-6} mol/l for both methods, corresponding to 5 pmol of analyte injected for HPLC and 0.5 pmol for CE.

Calibration

Standard samples were obtained by dissolving the acids, or their salts, in deionized water. Least-squares calibration graphs were constructed for lactic, formic and acetic acids (three concentration points). The HPLC calibration appeared to be linear in the concentration ranges 14–57 μg/ml for lactic acid ($R^2 = 1.00$), 27–110 μg/ml for formic acid ($R^2 = 1.00$) and 49–196 μg/ml for acetic acid ($R^2 = 1.00$) and CE calibration in the concentration ranges 7–30 μg/ml for lactic acid ($R^2 = 1.00$), 14–55 μg/ml for formic acid ($R^2 = 0.998$) and 25–100 μg/ml for acetic acid ($R^2 = 1.00$).

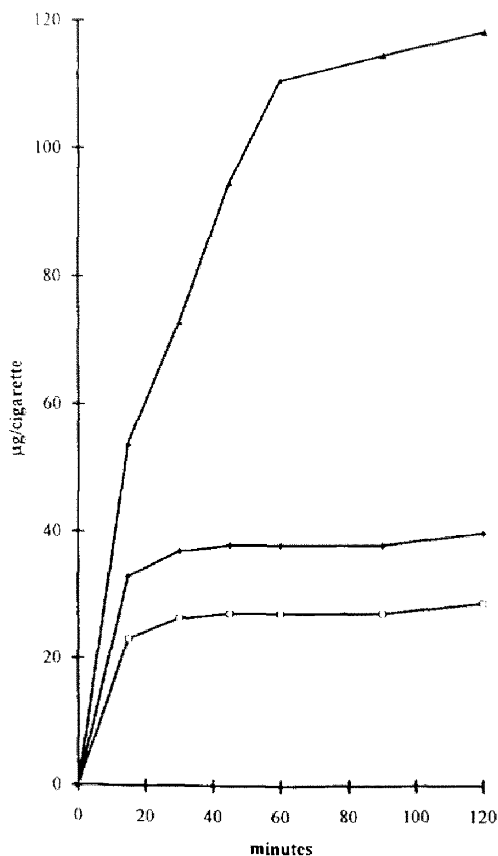

Fig. 5. Kinetics of the derivatization reaction with p-bromophenacyl bromide. Amount (μg per cigarette) of (▲) acetic acid, (□) lactic acid and (◆) formic acid derivatized versus the heating time (min).

Results of quantitative analysis of a "European blend" cigarette by both techniques

By repeating the smoking of 25 cigarettes, we carried out two series of measurements by both

Table 1
Run-to-run retention time (t_r) reproducibility of the HPLC and CE analyses

Compound	HPLC		CE	
	Mean t_r (min)	R.S.D. (%) ($n = 9$)	Mean t_r (min)	R.S.D. (%) ($n = 8$)
Lactic acid	7.9	1.7	12.21	0.7
Formic acid	9.5	1.5	9.66	0.5
Acetic acid	12.4	1.4	12.02	0.4

Table 2
Run-to-run concentration (c) reproducibility of the HPLC and CE analyses (μg per cigarette, obtained from the peak area by using the calibration graphs determined for each acid)

Compound	HPLC		CE	
	Mean c (μg/cig.)	R.S.D. (%) ($n=9$)	Mean c (μg/cig.)	R.S.D. (%) ($n=8$)
Lactic acid	32.9	6.0	22.8	10.5
Formic acid	41.7	12.7	41.6	2.8
Acetic acid	111.4	8.2	112.3	3.8

Table 3
Smoking-to-smoking retention time (t_r) reproducibility of the HPLC and CE analyses

Compound	HPLC		CE	
	Mean t_r (min)	R.S.D. (%) ($n=5$)	Mean t_r (min)	R.S.D. (%) ($n=3$)
Lactic acid	7.9	1.5	12.22	0.9
Formic acid	9.4	2.0	9.64	0.5
Acetic acid	12.2	1.5	12.00	0.3

Table 4
Smoking-to-smoking concentration (c) reproducibility of the HPLC and CE analyses (μg per cigarette, obtained from the peak area by using the calibration graphs determined for each acid)

Compound	HPLC		CE	
	Mean c (μg/cig.)	R.S.D. (%) ($n=4$)	Mean c (μg/cig.)	R.S.D. (%) ($n=3$)
Lactic acid	28.4	11.0	24.0	14.0
Formic acid	42.2	4.2	40.2	6.5
Acetic acid	120.5	8.5	113.9	1.2

HPLC and CE and the results are given in Table 5. The average results for both series are comparable. The calculation of the experimental F values between the two methods gives $F=1.77$ for lactic acid, 1.58 for formic acid and 5.08 for acetic acid. The Snedecor table gives the critical F at 95% confidence level ($F=18.51$ for acetic acid and 200 for lactic and formic acids). In all three instances, the calculated F value is less than the corresponding F value in the Snedecor table [22], so that one can conclude that the probability of the results obtained by the two methods for the three acids being the same is 95%.

Table 5
Comparison of HPLC and CE results (μg per cigarette) obtained for two smokings of 25 European blend cigarettes

Smoking	Lactic acid		Formic acid		Acetic acid	
	HPLC	CE	HPLC	CE	HPLC	CE
No. 1	24.3	23.4	41.1	42.1	118.6	110.7
No. 2	27.3	24.1	38.0	41.1	118.7	115.4

4. Conclusions

The two methods proposed for the determination of organic acids were sufficiently selective and sensitive to be applied directly to complex cigarette smoke mixtures. Both techniques provided rapid analyses, yielding quantitative information. They have distinct practical advantages over previous methods (GC or ion-exchange HPLC): they do not need complex extraction or prepurification steps, they are easy to use in a routine manner and they can detect and determine simultaneously the four most abundant low-molecular-mass organic acids in a cigarette smoke (glycolic, lactic, formic, acetic). Only two acids (acetic and formic) were determined using the ion-exchange HPLC method.

The agreement between the reversed-phase HPLC and CE methods was checked and it was concluded that the results provided by the two methods were the same. However, CE offers several features that make it more attractive than HPLC: (a) simplicity, as CE offers a rapid and simple means of identifying and analysing multicomponents mixtures such as cigarette smoke acid fraction without a derivatization step and sample preparation, other than dilution, and it is possible to inject dilute acetone smoke solutions

directly into the CE apparatus; (b) speed, as an analysis by HPLC takes 23 min (plus 1 h for derivatization) whereas CE takes only 15 min; (c) low costs and low solvent consumption (millilitres per day for CE versus centilitres per day for HPLC); and (d) the use of non-hazardous solvents (deionized water).

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