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Short communication

Determination of organic acids in tobacco by capillary isotachophoresis

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Abstract

Nine organic acids and phosphate have been separated and quantified in tobacco by capillary isotachophoresis. Two operating systems for the separation were found: hydrochloric acid (10 mmol l^{-1}) adjusted with β-alanine to pH 2.9 plus 0.1% poly(vinylpyrrolidone) was used as the leading electrolyte and 10 mmol l^{-1} nicotinic acid as the terminating electrolyte and hydrochloric acid (10 mmol l^{-1}) including 5.5 mmol l^{-1} 1,3-bis[tris(hydroxymethyl)methylamino]propane plus 0.1% poly(vinylpyrrolidone) was used as the leading electrolyte and 5 mmol l^{-1} 2-morpholinoethanesulfonic acid as the terminating electrolyte. Linearity was observed from 0.008 to 0.100 mmol l^{-1} with a coefficient of determination (r^2) of 0.999. The separation of anions was achieved in less than 16 min. The minimal sample pretreatment and relatively low running cost make isotachophoresis a good alternative to existing methods.

Keywords: Isotachophoresis; Tobacco; Organic acids; Phosphates

1. Introduction

A number of organic acids in tobacco plants have been identified, such as acetic, citric, formic, lactic, malic, malonic, oxalic, pyroglutamic and succinic acids and the major acids are acetic, citric, malic and oxalic acids. Organic acids are important contributors to leaf quality. The taste and aroma of tobacco products are closely linked with contents of some organic acids [1]. Therefore, organic acids responsible for these characteristics are determined not only in tobacco products but also in various phases of the production process.

Tobacco also contains inorganic ions, e.g., phosphate, nitrate and perchlorate [2]. These ions are accumulated by tobacco plants in the tobacco leaves from soil amended with fertilizers.

Gas chromatography with trimethylsilylation can be used for the determination of organic acids in tobacco, which provides excellent resolution and a high detection sensitivity [3–6]. However, this method requires a complicated and time-consuming extraction and derivatization procedure.

Ion-exclusion chromatography with either a refractive index (RI) detector or a UV detector is commonly employed for the determination of organic acids in tobacco [7,8]. Sample clean-up procedures are required in both RI and UV detection. For detection of organic acids, conductivity detection would provide greater selectivity and sensitivity than RI or UV

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detection. Recently, Qiu and Jin [9] reported an ion chromatographic (IC) method with suppressed conductivity detection for separating eight organic acids and phosphates from tobacco. The optimal conditions were found at a column temperature of 42 °C and a mobile phase of 1.8 mmol l^{-1} heptafluorobutyric acid solution.

Capillary isotachophoresis (cITP) is a convenient alternative for the separation and determination of inorganic and organic anions. However, despite the large number of publications which have appeared in the literature on the determination of organic acids in different materials, no isotachophoretic method for the assay of these acids in tobacco has yet been reported. The present study describes the isotachophoretic procedure for the determination of nine organic acids in tobacco. The samples, after efficient extraction, were directly analyzed by cITP. cITP is a simple, sufficiently sensitive and inexpensive method and therefore suited for routine analysis.

2. Materials and methods

2.1. Apparatus

Isotachophoretic separations were performed using a ZKI 02 isotachophoretic analyzer (Villa Labeco, Spišská Nová Ves, Slovak Republic) operated in the single-capillary mode. The analyzer was equipped with a sample valve of 30 μl fixed volume, an analytical capillary (160 mm×0.3 mm I.D.) made of fluorinated ethylene–propylene copolymer and a conductivity detector. The isotachopherograms were evaluated by a personal computer software package supplied with the analyzer.

2.2. Chemicals

The chemicals used were of analytical-reagent grade. Hydrochloric acid, sulfuric acid, sodium acetate, trihydrate, sodium formate, sodium oxalate, ammonium perchlorate, sodium phosphate, monobasic dihydrate and poly(vinylpyrrolidone) were obtained from Lachema (Brno, Czech Republic), β-alanine, 2-morpholinoethanesulfonic acid, citric acid, lithium lactate, malonic acid, malic acid, nicotinic acid, pyroglutamic acid and succinic acid from Merck and 1,3-bis[tris(hydroxymethyl)methylamino]propane from Sigma–Aldrich. Deionized and double distilled water with a resistivity of 18 MΩ cm was used to prepare all solutions.

2.3. Isotachophoretic conditions

System A

The leading electrolyte was 10 mmol l^{-1} hydrochloric acid including 0.1% poly(vinylpyrrolidone) adjusted with β-alanine to pH 2.9. The terminating electrolyte was 10 mmol l^{-1} nicotinic acid. The driving current was initially 80 μA. During detection, the current was reduced to 40 μA.

System B

Hydrochloric acid (10 mmol l^{-1}) including 5.5 mmol l^{-1} 1,3-bis[tris(hydroxymethyl)methylamino]-propane plus 0.1% poly(vinylpyrrolidone) was used as the leading electrolyte and 5 mmol l^{-1} 2-morpholinoethanesulfonic acid as the terminating electrolyte. The driving current was initially 60 μA. During detection, it was reduced to 40 μA.

2.4. Sample preparation

During development of the present method, the acidic, water and alkaline extraction procedures were tested and compared. It was found that the phosphoric, oxalic and malic acid levels were lower in both the alkaline and water extracts. Nevertheless, both the water and alkaline extracts had an inadequate stability (results not shown).

Tobacco samples were prepared by acidic extraction, as previously described Qiu and Jin [9]. Tobacco (2 g) was extracted with a sulfuric acid solution (100 ml, 5 mmol l^{-1}) by vigorous shaking for 3 h. The extract was filtered through a 0.45 μm membrane (Millipore) and diluted 25-fold with double distilled water. A 30-μl volume of solution was injected.

3. Results and discussion

The ITP operating systems were chosen with the

aim of differentiating the acids according to their p*K* values (system A) [10] and charges (system B) [11]. The isotachopherogram for the separation of the anionic constituents shown in Fig. 1 was obtained using system A. Eleven anions can be separated with adequate resolution, however, sulfate migrated in the zone of the leading ion (chloride). Since the chloride ion is not an interesting anion in tobacco there is no need to separate sulfate from chloride. The leading electrolyte pH had a significant effect on the resolution. For example, at pH>3.1 citrate, malate and pyroglutamate will migrate in the mixed zone. Therefore, the pH of the leading electrolyte has to be adjusted carefully. A full resolution of the anionic constituents was achieved with operating system B (Fig. 1).

For the linearity, precision and recovery, both the A and B systems were tested. Linear regression analysis by the least-squares method was performed for the test mixture (100%; 0.1 mmol l^{-1} each of acetic, citric, formic, lactic, malic, malonic, oxalic, perchloric, phosphoric, pyroglutamic and succinic acids prepared in 0.2 mmol l^{-1} H_2SO_4), using stepwise dilution with 0.2 mmol l^{-1} H_2SO_4 down to 8% (n=7). The calibration graphs of zone length vs. acid concentration for all these acids were linear at least in a range of 0.008–0.100 mmol l^{-1} with high coefficients of determination >0.999.

The relative standard deviation (RSD) value of the zone length estimation was less than 1% (n=7) for all these acids at the 0.05 mmol l^{-1} level. High reliability was thus demonstrated.

For the limit of detection (LOD), we used the value $(y+3S)/b$, where the calculated intercept of the calibration line can be used as an estimate of y, S is the standard deviation in the y direction of the calibration line and b is the slope of the calibration line [12]. LODs for analytes were 0.002–0.006 mmol l^{-1}. The possibility of further decreasing of LOD was examined. In general, it is necessary to decrease the driving current when an analyte concentration in sample solution is low. The shortest detectable zone length was 1.0 s at 10 μA; according to the calibration equation this zone length equals to 1 μmol l^{-1}. The comparison of the LODs and RSDs obtained by the proposed methods with those obtained by IC is as follows. The LOD for citric, malic, malonic and succinic acid was almost equal to that obtained by IC [9]. The LOD for the other acids was lower than that obtained by IC. The RSDs for CITP were almost equal to those obtained by IC.

Sample analysis

The samples of tobacco treated as described above

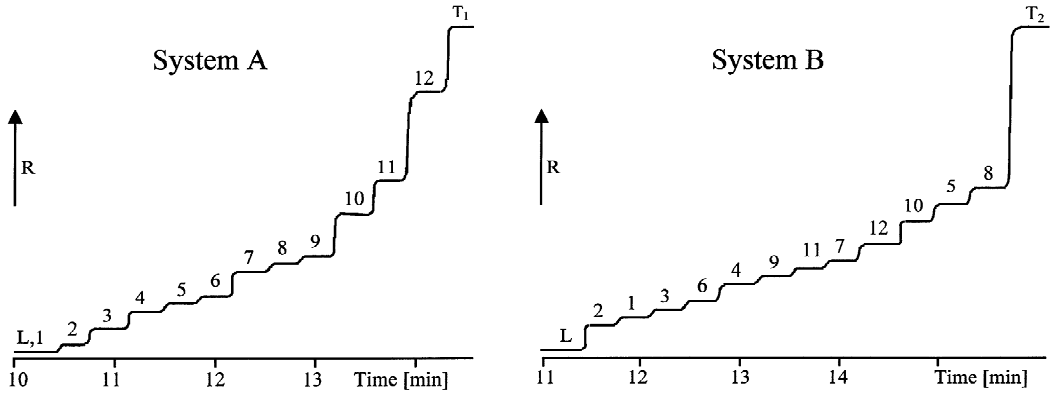

Fig. 1. The isotachophoretic separation of sulfate (1), perchlorate (2), oxalate (3), malonate (4), phosphate (5), formate (6), citrate (7), pyroglutamate (8), malate (9), lactate (10), succinate (11) and acetate (12) (0.1 mmol l^{-1} of each) in operating system A [the leading electrolyte (L), 10 mmol l^{-1} hydrochloric acid, 0.1% poly(vinylpyrrolidone), β-alanine, 2.9; the terminating electrolyte (T_1), 10 mmol l^{-1} nicotinic acid; the driving current 40 μA] and operating system B {the leading electrolyte (L), 10 mmol l^{-1} hydrochloric acid, 0.1% poly(vinylpyrrolidone, 5.5 mmol l^{-1} 1,3-bis[tris(hydroxymethyl)methylamino]propane; the terminating electrolyte (T_2), 5 mmol l^{-1} 2-morpholinoethanesulfonic acid; the driving current 40 μA}. R: Increasing resistance.

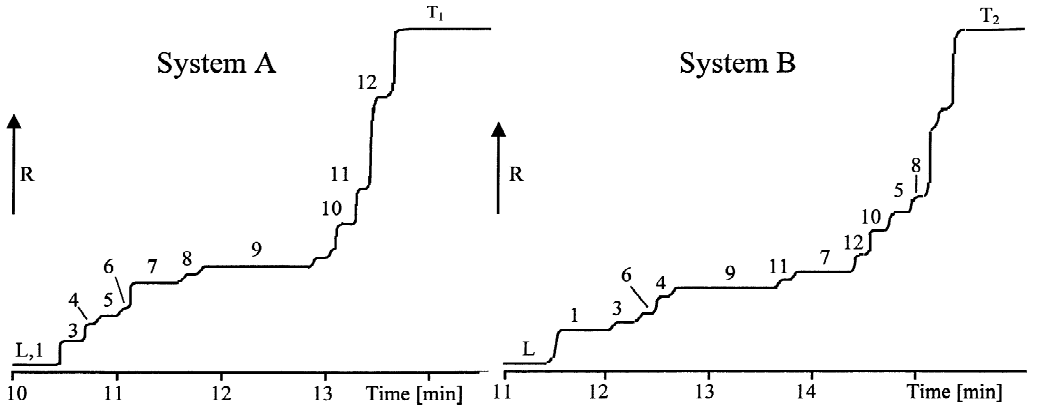

Fig. 2. Isotachopherograms of tobacco extract in system A and system B. Conditions and symbols as in Fig. 1.

were analyzed by cITP in both the A and B systems. Fig. 2 shows the typical isotachopherograms obtained from tobacco extract. The zones in the isotachopherograms of the tobacco extract were identified by comparing their relative step heights with those of the authentic standard, by spiking the extract with a single standard compound in the subsequent run and also by checking the results obtained from two operating conditions. Oxalate, malonate, phosphate, formate, citrate, pyroglutamate, malate, lactate, succinate and acetate were identified, however, perchlorate was not detected in tobacco extract.

The precision of the method was calculated by extraction and cITP assay of independent samples ($n=5$) from the same tobacco. The precision was represented by RSD values of 0.8–1.2 and 0.9–1.2% for systems A and B (Table 1), respectively.

For the recovery experiment, an acidic solution containing suitable amounts of authentic standards (10 ml solution containing 5 mmol l^{-1} H_2SO_4, 25 mmol l^{-1}each of acetic, citric, malic and oxalic acids and 2.5 mmol l^{-1} each of formic, lactic, malonic, perchloric, phosphoric, pyroglutamic and succinic acids) was added to a tobacco sample (2 g) of known contents, vortexed and allowed to stand for 2 h. The sample was than made up to volume 100 ml with 5 mmol l^{-1} H_2SO_4, extracted for 3 h, filtered, diluted 25-fold with double distilled water and injected. The recoveries of the constituents were 96–101% as shown in Table 1.

Table 1
Limit of detection, precision and recovery

Solute	System A			System B		
	LOD (mmol l^{-1})	RSD (%)	Recovery±RSD (%)	LOD (mmol l^{-1})	RSD (%)	Recovery±RSD (%)
Perchloric acid	0.006	ND	96±3	0.005	ND	96±4
Oxalic acid	0.004	1.2	99±2	0.003	1.1	100±3
Malonic acid	0.004	0.8	99±2	0.003	0.9	99±3
Phosphoric acid	0.002	0.9	100±3	0.002	0.9	99±2
Formic acid	0.004	0.8	99±2	0.004	1.0	99±3
Citric acid	0.004	1.0	98±2	0.004	1.1	97±3
Pyroglutamic acid	0.003	0.9	97±4	0.003	1.0	98±2
Malic acid	0.003	0.8	99±2	0.002	0.9	99±2
Lactic acid	0.004	1.1	99±2	0.003	1.2	99±2
Succinic acid	0.004	0.9	101±3	0.003	1.1	100±4
Acetic acid	0.004	1.1	100±2	0.003	1.0	100±3

ND=Not detected.

Table 2
Amounts of acids in tobacco determined with cITP system A and system B

Solute	Amount (%, $n=3$)	
	System A	System B
Perchloric acid	ND	ND
Oxalic acid	1.05±0.07	1.02±0.04
Malonic acid	0.31±0.01	0.32±0.02
Phosphoric acid	0.63±0.01	0.65±0.02
Formic acid	0.42±0.02	0.40±0.02
Citric acid	2.52±0.05	2.48±0.07
Pyroglutamic acid	0.60±0.02	0.59±0.01
Malic acid	5.46±0.10	5.55±0.12
Lactic acid	0.84±0.05	0.89±0.04
Succinic acid	0.53±0.02	0.53±0.02
Acetic acid	1.30±0.04	1.27±.0.03

ND=Not detected.

The amounts of 10 acids in the five tobacco prepared as described in Section 2.4 were measured to investigate relationship between determination by cITP systems A and B. The obtained relationships (cITP system A versus cITP system B) with a slope nearly 1 (0.992–1.005) and a nearly zero intercept (from −0.29 to +0.26) validate the techniques. The results calculated for the individual tobacco are given in Table 2.

4. Conclusions

Organic acids can be successfully determined in tobacco samples using present cITP methods with conductivity detection. It was found that the content of perchlorate was lower than the detection limit. The other constituents can determined by both the A and B systems. The two systems proposed for the determination of organic acids were sufficiently selective and sensitive. The agreement between the A and B systems was checked and it was concluded that the results provided by the two systems were the same. The different effects defining the effective mobilities of the components in different operating systems can facilitate identification and makes determination of the same constituents in two systems partly comparable to determinations performed by independent methods. cITP offers some advantages over chromatographic methods: (i) non-ionic compounds (e.g., sugars, propylene glycol), which are frequently compounds of tobacco, do not interfere with the analysis of the ionic compounds, (ii) low running cost, decreased cost of capillaries.

References

[1] A.G. Kallianos, Recent Adv. Tob. Sci. (1976) 61.
[2] J.J. Ellington, N.L. Wolfe, A.W. Garrison, J.J. Evans, J.K. Avants, Q. Teng, Environ. Sci. Technol. 35 (2001) 3213.
[3] T.J. Clark, J.E. Bunch, J. Chromatogr. Sci. 35 (1997) 206.
[4] T.J. Clark, J.E. Bunch, J. Chromatogr. Sci. 35 (1997) 209.
[5] L.K. Ng, M. Hupe, M. Vanier, D. Moccia, J. Agric. Food Chem. 49 (2001) 1132.
[6] S.S. Yang, C.B. Huang, I. Smetena, J. Chromatogr. A 942 (2002) 33.
[7] J. Qiu, J. Chromatogr. A 859 (1999) 153.
[8] A.K. Sharma, S.A. Clauss, G.M. Mong, K.L. Wahl, J.A. Campbell, J. Chromatogr. A 805 (1998) 101.
[9] J. Qiu, X. Jin, J. Chromatogr. A 950 (2002) 81.
[10] F.M. Everaerts, Th.P.E.M. Verheggen, J.L. Beckers, Isotachophoresis: Theory, Instrumentation and Applications, Elsevier, Amsterdam, Oxford, New York, 1976.
[11] D. Kaniansky, V. Madajova, I. Zelensky, S. Stankoviansky, J. Chromatogr. 194 (1980) 11.
[12] J.C. Miller, J.N. Miller, Statistics for Analytical Chemistry, Ellis Horwood, Chichester, 1984.