

Short communication

Ion chromatography methods for the simultaneous determination of mineral anions in plant sap

P. Masson*, G. Hilbert, D. Plenet

Station d'Agronomie, Centre de Recherches INRA de Bordeaux, B.P. 81, 33883 Villenave d'Ornon Cedex, France

Received 12 February 1996; revised 8 May 1996; accepted 14 May 1996

Abstract

The use of two ion chromatographic systems, with and without a chemical conductivity suppressor, is described for the determination of Cl^- , NO_3^- , PO_4^{3-} and SO_4^{2-} in plant sap. Samples were prepared just by dilution. Separations have suffered from interference due to the presence of other solutes. Usually, organic acid ions such as malate coelute with sulphate ions on the non-suppressed system. An appropriate eluent, formed with boric and tartaric acids at pH 4.0, was used to overcome this problem. Organic acids were eliminated in the void volume but phosphate appeared as a negative peak and cannot be quantitated. Suppressed ion chromatography permits the measurement to be performed with more efficiency, but coelutions also occurred with carbonate–hydrogencarbonate eluent for Cl^- and SO_4^{2-} . The best separation of ions was achieved on the Dionex AS 11 column with a sodium hydroxide gradient elution and a 5 min running time. The selectivity and sensitivity of the methods are discussed.

Keywords: Chloride; Nitrate; Phosphate; Sulfate; Anions

1. Introduction

Diagnostic tools for the determination of plant nutritional needs are required by agronomists in the fertiliser management of crops. With this goal, various techniques, based on sap analysis, have been developed to estimate the levels of mineral ion (e.g. NO_3^- and PO_4^{3-}) uptake by plants. Fast and accurate measurement of inorganic nutrients would be extremely helpful in agronomic studies. Therefore, we propose to study the simultaneous determination of chloride, nitrate, phosphate and sulphate ions in plant sap samples by ion chromatography.

To date, there is very little literature on this subject; a single report on plant sap analysis by ion

chromatography is available. This study only investigated the determination of nitrate ions and it lacks details on the method used [1]. However, rapid tests are widely used for the direct analysis of NO_3^- or PO_4^{3-} in sap samples [1–4]. These methods have limitations in terms of detection limits (5 mg/l) and suffer from interference by a variety of ions such as Fe^{3+} , K^+ and Cl^- (Merck data). Conventional colorimetric, titrimetric, turbidimetric and gravimetric methods are time-consuming or difficult to perform [5,6]. Concurrently, anions in other plant extracts have been analysed by several investigators using ion chromatography [7–10]. The samples, generally plant powders, were extracted with purified water or salt solutions. However, papers describing the elution behaviour of common inorganic anions in organic-rich samples are unusual [11,12].

*Corresponding author.

The separation of inorganic anions is easily carried out by ion chromatography. The advantages over alternative techniques are in the speed of analysis, greater sensitivity of detection and the variety of species that may be determined in a single run. Moreover, minimal sample preparation is required. For this study, anions were measured directly in diluted sap samples with two chromatographic systems, either with or without a background conductivity suppressor. For each analytical method, the separation efficiencies were investigated.

2. Experimental

2.1. Instrumentation

Two high-performance liquid chromatographic (HPLC) systems were used. Each system contained a conductivity detector and an anion-exchange analytical column and precolumn. Table 1 summarises the details of the operating parameters.

The non-suppressed ion chromatography system included a Beckman (Fullerton, CA, USA) programmable solvent pump module 126, a sample injection valve 210A and a Waters (Milford, MA, USA) conductivity detector 431 (10 mV output) coupled with an analogic interface Beckman module 406.

The suppressed system was a Dionex (Sunnyvale,

CA, USA) Model DX 500 IC equipped with a GP 40 gradient pump, an ED 40 electrochemical detector and an AS 40 automated sampler. The separation was carried out on two different columns. The detector was preceded by an ASRS 1 anion self-regenerating suppressor, to suppress the background conductivity of the eluents.

2.2. Eluents

The eluents were chosen to give the best overall selectivity for a standard mixture of anions within a reasonable elution time. All reagents were of analytical grade. Water that had been purified (18 M Ω) using a Millipore Milli-Q water purification system (Bedford, MA, USA) was used for all solutions.

For the Waters column, two eluents were used. The borate–gluconate had the following composition; sodium gluconate (1.48 mM), boric acid (5.82 mM), sodium tetraborate decahydrate (1.30 mM), acetonitrile 12% (v/v) and glycerol 0.25% (v/v). The borate–tartrate eluent, used to eliminate interference during separation, was made with tartaric acid (3 mM), boric acid (200 mM) and acetonitrile 10% (v/v) and was adjusted to pH 4.0 with lithium hydroxide. Eluents were prepared daily, filtered at 0.22 μ m and degassed.

For the Dionex AS 9-SC column, the mobile phase was a solution containing sodium hydrogen-

Table 1
Operating parameters for the liquid chromatography systems

Suppressed ion chromatography	Isocratic elution	Gradient elution
Sample loop volume	25 μ l	25 μ l
Separator column	Dionex AS 9-SC (250 \times 4 mm I.D.)	Dionex AS 11 (250 \times 4 mm I.D.)
Guard column	Dionex AG 9-SC (50 \times 4 mm I.D.)	Dionex AG 11 (50 \times 4 mm I.D.)
Eluent	2.0 mM Na ₃ CO ₃ + 0.75 mM NaHCO ₃	5 mM NaOH/200 mM NaOH
Eluent flow-rate	2 ml/l	2 ml/l
Pump pressure	1100 p.s.i.	1300 p.s.i.
Background conductivity	15 μ S	2 μ S
Non-suppressed ion chromatography	Alkaline pH elution	Low pH elution
Sample loop volume	20 μ l	20 μ l
Separator column	Waters IC-PACK Anion HR	Waters IC-PACK Anion HR
Guard column	Waters IC-PACK Anion Guard Pak	Waters IC-PACK Anion Guard Pak
Eluent	borate–gluconate, pH 8.5	borate–tartrate, pH 4.0
Eluent flow-rate	1 ml/l	1 ml/l
Pump pressure	1000 p.s.i.	1000 p.s.i.
Background conductivity	274 μ S	350 μ S

Table 2
Gradient profile for dionex AS11 column

Time (s)	Flow-rate (ml/min)	A (%)	B (%)	C (%)	Curve	Comments
<i>Column equilibration</i>						
Initial	2.0	4.0	40.0	56.0	5.0	
4.60	2.0	4.0	40.0	56.0	5.0	sampling
7.00	2.0	4.0	40.0	56.0	5.0	injection
<i>Analysis</i>						
0.00	2.0	4.0	40.0	56.0	5.0	start reading
1.50	2.0	11.0	60.0	29.0	7.0	
2.50	2.0	17.5	65.0	17.5	3.0	
5.00	2.0	17.5	65.0	17.5	2.0	end reading

Eluent A: 200 mM NaOH. Eluent B: 5 mM NaOH. Eluent C: water.

carbonate (0.75 mM) and sodium carbonate (2.0 mM). Elution was achieved in an isocratic run. Sodium hydroxide gradient elution was used for the Dionex AS 11 column using three different solutions (purified water, 5 mM NaOH and 200 mM NaOH). Table 2 shows the gradient profile. Eluents were prepared daily, degassed and pressurised under helium (grade C).

2.3. Sample preparation

The test plant chosen for these experiments was maize. Sap was extracted from the petioles using a simple press, as described by Burns and Hutsby [2,5]. The resulting juices were diluted (1:20) with 18 M Ω Milli-Q water and directly injected into the HPLCs. The instruments were calibrated with blanks and three mixed standards, prepared by dissolving the corresponding sodium salts in Milli-Q water (10, 20 and 30 mg/l Cl⁻; 30, 60 and 90 mg/l NO₃⁻; 10, 20 and 30 mg/l PO₄³⁻ and 5, 10 and 15 mg/l SO₄²⁻). The linearity of the standards was excellent for each ion species ($r^2 \geq 0.998$) over these concentration ranges with peak area or peak height evaluation. A synthetic solution containing a mixture of pure organic and inorganic anions was used for solute peaks identification.

3. Results and discussion

Plant sap is a complex solution containing many organic compounds such as organic acids derived

from the Krebs cycle, sugars and proteins [13]. The presence of high levels of organic acids in the sample matrices affect the quality of separation and detection of the components of interest. Table 3 summarises the efficiency of each method tested in this study based on detection limits, reproducibility of measurements and resolution (R_s) of peaks when coelutions occurred. The limit of detection, L_D , was defined as three times the standard deviation of the background signal which was determined by measuring ten replicate samples of Milli-Q water. Reproducibility was assessed as the relative standard deviation from triplicate analyses of four anions in the sap.

3.1. Non-suppressed ion chromatography

Borate–gluconate eluent was previously shown to be effective for the separation of anions using non-suppressed ion chromatography [14]. A typical chromatogram for maize sap is given in Fig. 1. Results show that among plant macro-constituents, SO₄²⁻ is the most resistant to quantitative measurements. Divalent organic acid anions (e.g. malate and succinate) formed a large peak which completely overlapped the SO₄²⁻ peak ($R_s=0.2$). The same result was observed by Smolders et al. [10]. Coelution of these compounds probably occurred due to their nearly equal ionic radii. Moreover, malic acid is generally the major constituent of the total organic acid content in sap [13] and is present in excess compared to SO₄²⁻. Various method modifications were considered to overcome the problem. UV detection [10,11] or sample pre-treatment [12] can be used, but the simplest solution was to find an eluent with appropriate selectivity for this application. The other ions (Cl⁻, NO₃⁻ and PO₄³⁻) can be easily quantitated in the sap samples, with good sensitivity and reproducibility (Table 3).

Jackson and Boswer [14] recommended the use of a borate–tartrate mixture at low pH to obtain selective separation between sulphate and organic acid peaks. Under these conditions, all organic acids eluted in the void volume. The low eluent pH protonates these species, further decreasing their retention. In this way, no compounds interfered with the sulphate peak and the total separation was achieved in 8 min (Fig. 2). The results show that the phosphate peak gave a negative response with con-

Table 3
Efficiency of methods

<i>Non-suppressed ion chromatography system with borate–gluconate eluent</i>				
Analytical ions	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻
Detection limit (μg/l)	10	30	70	50
Reproducibility (%)	1.7	3	1.1	2.6
Similar solutes	–	–	–	malate, succinate
Resolution factor				0.2
<i>Non-suppressed ion chromatography system with borate–tartrate eluent</i>				
Analytical ions	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻
Detection limit (μg/l)	10	50	negative peak	30
Reproducibility (%)	1.2	2.1		1.6
Similar solutes	all organic acids eluted in the void volume			
<i>Suppressed ion chromatography system with isocratic elution</i>				
Analytical ions	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻
Detection limit (μg/l)	4	7	18	10
Reproducibility (%)	0.7	2	1.8	4.2
Similar solutes	unknown	–	–	tartrate
Resolution factor	0.6			0.7
<i>Suppressed ion chromatography system with gradient elution</i>				
Analytical ions	Cl ⁻	NO ₃ ⁻	PO ₄ ³⁻	SO ₄ ²⁻
Detection limit (μg/l)	5	12	35	20
Reproducibility (%)	1.4	3.4	1.3	0.8
Similar solutes	–	malate, succinate	–	fumarate, oxalate
Resolution factor		1.2		1.1

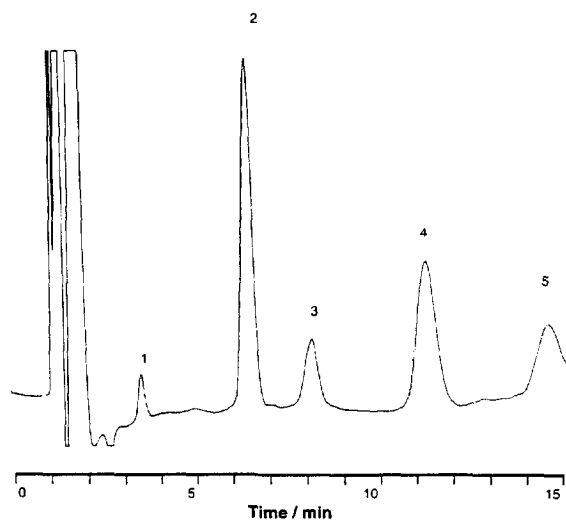


Fig. 1. Ion chromatogram of maize sap. Column=Waters IC PACK HR; eluent=5.82 mM borate–1.48 mM gluconate. Peaks: 1=Chloride; 2=nitrate; 3=phosphate; 4=sulfate+malate+succinate and 5=oxalate.

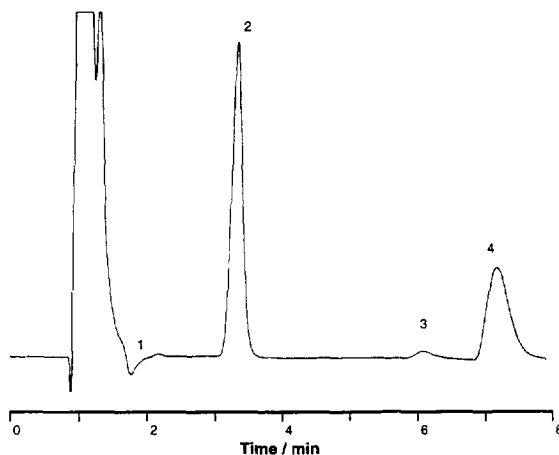


Fig. 2. Ion chromatogram of maize sap. Column=Waters IC PACK HR; eluent=200 mM borate–3.0 mM tartrate. Peaks: 1=Phosphate; 2=chloride; 3=sulfate and 4=nitrate.

ductivity detection and could not be quantitated. The eluent pH changes the retention times of the analytes simply by altering the degree of ionization of the species. Also, nitrate eluted later than sulphate. The same facts were observed by Jackson and Boswer [14]. The borate–tartrate eluent was applicable to the

analysis of inorganic anions in samples containing high levels of organic acids, but was restricted to analysis that did not require phosphate quantitation. The total measurement of all plant nutrients present in plant sap, with non-suppressed ion chromatography, therefore required two separate runs of the same sample, with two different eluents to complete analyses.

The relative standard deviations measured for the ion concentrations present in sap were less than 5% for this method (Table 3).

3.2. Suppressed ion chromatography

Suppressed ion chromatography permits the best sensitivity of measurement (Table 3). The autosuppressed conductivity device decreases the base noise compared to that seen in the non-suppressed system.

Carbonate–hydrogencarbonate was the most versatile eluent when used with suppressed conductivity detection for isocratic elution of anions in a single column [14]. The results obtained on the AS 9-SC column also resulted in poor resolution of the SO_4^{2-} peak. Slight overlapping peaks ($R_s=0.7$) for sulphate and tartrate appeared on the chromatogram of the sample of maize sap (Fig. 3). Similarly, an unidentified species was weakly retained and coeluted with Cl^- ($R_s=0.6$). Nevertheless, sulphate and chloride ions were present in large excess compared to the interfering solutes and data was calculated by measuring peak heights. Even with excessive peak overlap, the peak height calculation is more effective than that of the peak area.

The Dionex AS 11 column was specifically used for the determination of organic acids. Gradient elution with NaOH maximised the resolving power of ion chromatography. Investigations were conducted to obtain a sufficiently good separation of Cl^- , NO_3^- , PO_4^{3-} and SO_4^{2-} from the organic acids. Ultimately, we developed a selective elution where the time required for complete separation of the compounds was approximately only 5 min (Table 2). Fig. 4 depicts a typical chromatogram obtained. All anions, organic and inorganic, showed clear individual separation ($R_s \geq 1$), fast retention time, good sensitivity and reproducibility of measurement. The same results were observed on two other sap samples, strawberry plant and wheat (data not shown).

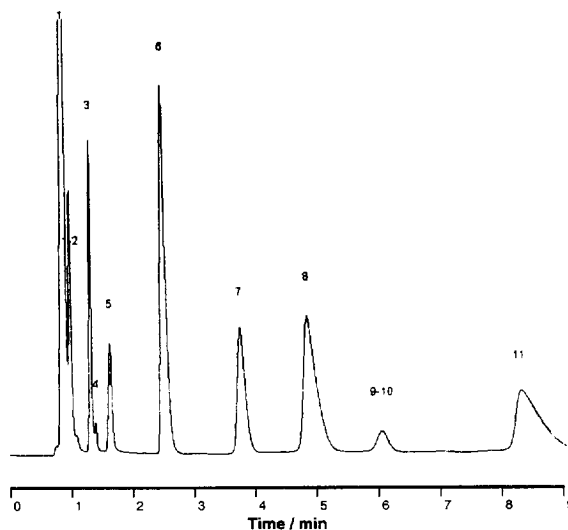


Fig. 3. Ion chromatogram of maize sap. Column=Dionex AS 9-SC; eluent=2.0 mM carbonate–0.75 mM hydrogenocarbonate. Peaks: 1–2=Organic acid ions (pyruvate, acetate, lactate...); 3=chloride; 4=unknown; 5=unknown; 6=nitrate; 7=phosphate; 8=malate+succinate; 9=tartrate; 10=sulfate and 11=oxalate.

The reproducibility was good for the Dionex methods (standard deviation less than 5%). These results indicate that the present system is also applicable to the simultaneous determination of carboxylic acids and inorganic anions in samples having matrices as complex as plant sap.

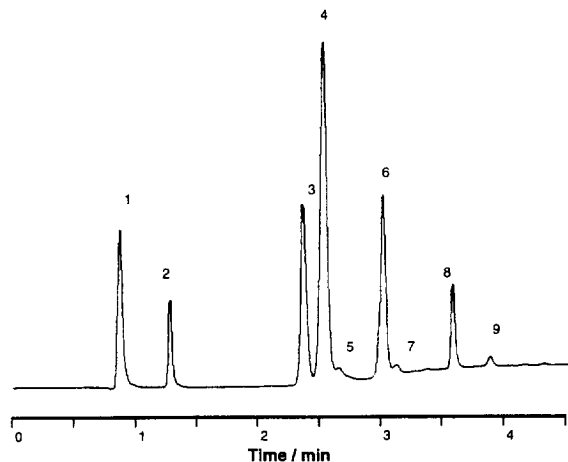


Fig. 4. Ion chromatogram of maize sap. Column=Dionex AS11; eluent=NaOH with gradient elution. Peaks: 1=pyruvate; 2=chloride; 3=nitrate; 4=succinate+malate; 5=tartrate; 6=sulfate; 7=oxalate; 8=phosphate and 9=citrate.

4. Conclusion

For soluble samples with high organic compound content, such as plant sap, ion chromatography has often given poor resolution of the SO_4^{2-} peak. Coelutions between mineral species and organic acids occurred when using neutral-to-alkaline eluents.

Borate–gluconate eluent, usually used in non-suppressed ion chromatography, was unsuitable for separating sulphate and malate ions. The low pH borate–tartrate eluent decreased the retention of organic acids, making it ideal for the analysis of sap samples. However, this eluent was of limited interest as a general purpose eluent because phosphate eluted as a negative peak when using conductivity detection and could not be quantitated.

Slight coelutions also occurred for sulphate and chloride ions in suppressed ion chromatography when using carbonate–hydrogencarbonate eluent. However, the resolution was high enough to analyse chromatograms by peak height evaluation. The best overall separation for sap samples was achieved on the Dionex AS 11 column, which is specifically designed for the analysis of organic acids. Improved efficiency can be easily achieved with a gradient elution. Our investigations led to the development of a rapid program which can be run in only 5 min.

With these conditions, ion chromatography was certainly the most reliable method for the rapid and sensitive quantification of anions in plant saps.

References

- [1] N.L. Schaefer, *Commun. Soil Sci. Plant Anal.*, 17 (1986) 937.
- [2] I.G. Burns and W. Hutsby, *Commun. Soil Sci. Plant Anal.*, 15 (1984) 1463.
- [3] R.R. Coltman, *J. Plant Nut.*, 10 (1987) 1699.
- [4] E. Justes, *Proc. 2nd ESA congress, Warwick University*, 1992, p. 262.
- [5] I.G. Burns and W. Hutsby, *Commun. Soil Sci. Plant Anal.*, 17 (1986) 839.
- [6] S. Ferrario, I. Agius and A. Morisot, *J. Plant Nut.*, 15 (1992) 85.
- [7] W.T. Frankenberger, H.C. Mehra and D.T. Gjerde, *J. Chromatogr.*, 504 (1990) 211.
- [8] A.A. Hafez, S.S. Goyal and D.W. Rains, *J. Chromatogr.*, 546 (1991) 387.
- [9] J. Pentchuk, U. Haldna and K. Ilmoja, *J. Chromatogr.*, 364 (1986) 189.
- [10] E. Smolders, M. Van Deal and R. Merckx, *J. Chromatogr.*, 514 (1990) 371.
- [11] H. Itoh and Y. Shinbori, *Bull. Chem. Soc. Jpn.*, 60 (1987) 1327.
- [12] W. Shotyk, *J. Chromatogr.*, 640 (1993) 309.
- [13] M.J. Canny and M.E. McCully, *Aust. J. Plant Physiol.*, 15 (1988) 557.
- [14] P.E. Jackson and T. Boswer, *J. Chromatogr.*, 602 (1992) 33.