CHROM. 22 509

Note

Simultaneous determination of extractable sulphate and malate in plant extracts using ion chromatography

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Since its introduction in the mid-1970s, ion chromatography (IC) has become widely used for the determination of all types of anions or cations. Its importance in soil science and plant analysis has increased considerably in recent years[1-6].

Of all plant macroconstituents, SO_4^{2-} has been the most resistent to quantitative determinations[7]. Turbidimetric, gravimetric, titrimetric and spectrophotometric methods[8] are time consuming, difficult to perform or require expensive equipment[9]. With an ion chromatographic technique, however, SO_4^{2-} can be rapidly quantified at an acceptable level of accuracy with a rather simple procedure. An additional advantage of this method in plant and soil research is that many other anionic compounds, such as Cl^-, NO_3^-, $H_2PO_4^-$ and some organic acids can also be determined in the same run.

The determination of SO_4^{2-} in plant materials has been described previously[2], but the resolution of the SO_4^{2-} and organic acid (malate) peaks is poor, probably owing to their nearly equal ionic radii. Nevertheless, methods have been developed to overcome this problem making use of a programmed run with a specific column. In this work we explored the possibility of a simple isocratic system with tandem detectors (a conductivity cell and UV absorbance detector). Double monitoring has been used before by others to widen the range of applications of ion chromatography[10]. In this instance nitrite could be quantified in a large background of chloride by combined monitoring.

EXPERIMENTAL

Ion chromatography

The system used was a Dionex (Sunnyvale, CA, U.S.A.) Model QIC analyser. Details of the operating parameters are given in Table I. The instrument consists of a high-performance liquid chromatograph with an analytical column and precolumn, both containing an anion-exchange resin in the HCO_3^- form. Other components are a single-piston pump with pulse damper and an injection valve with a 50-μl sample loop. The first detector was a Dionex conductivity detector preceded by a cation-

TABLE I

OPERATING PARAMETERS FOR THE QIC ANALYSER AND THE UV ABSORBANCE DE-
TECTOR

Instrument	Parameter	Value
QIC analyser	Sample loop volume	50 μl
	Guard column	HPIC-AG4A (50 × 3 mm I.D.)
	Separator column	HPIC-AS4A (250 × 3 mm I.D.)
	Eluent	1.8 mM Na$_2$CO$_3$ + 1.7 mM NaHCO$_3$
	Eluent flow-rate	2.0 ml/min
	Pump pressure	ca. 70 bar (1000 p.s.i.)
	Suppressor	Anion fibre suppressor 1–2 P/N
	Regenerant for suppressor	12.5 mM H$_2$SO$_4$
	Regenerant flow-rate	3.0 ml/min
	Background conductivity	21 μS
	Conductivity full-scale	100 μS
UV absorbance detector	Wavelength	190 nm
	Filter	0.5 s
	Minimum absorbance	0.001

exchange membrane in the H$^+$ form to suppress the background conductivity of the
eluent. The second detector was a variable-wavelength UV monitor (Waters Assoc.
Model 484) connected through 30 cm × 0.3 mm I.D. tubing in series with the
conductivity cell. The reference cell in the UV monitor was left empty. Absorbance was
measured at the highest sensitivity at 190 nm. Sufficient back-pressure was achieved by
connecting a 160 cm × 0.3 m I.D. tubing after the outlet of the UV monitor. Data
acquisition and peak-area calculation were performed using Shimadzu C-R6A and
C-R3A integrators connected to the first and second detectors, respectively.

Extraction of plants and preparation of standards

A commercially available spinach (*Spinacia oleracea* L.) sample was dried for
3 days at 70°C and ground to pass through a 0.4-mm screen (Tecator Cyclotec 1092
sample mill). A 150-mg amount of the ground sample was extracted with 1.8 mM
Na$_2$CO$_3$–1.7 mM NaHCO$_3$ solution. The mixture was heated for 30 min in
a water-bath at 70°C and the extract was centrifuged for 15 min at 20 000 g before
injecting the supernatant into the ion chromatographic system. Standards (between
0.02 and 0.1 mM for SO$_4^{2-}$ and between 0.05 and 0.25 mM for malate) were prepared
by dissolving appropriate weights of analytical-reagent grade potassium sulphate
(UCB) and DL-malate (Riedel-de Haën) in deionized water that had been filtered and
purified through a Millipore Milli-Q purification system. Aliquots (*ca*. 2 ml) of the
extract and standards were used to flush and fill the sample loop using a plastic syringe
equipped with a Swinnex filter holder with Schleiger & Schüll membrane filters (0.45
μm, diameter 13 mm). Colloidal particles which are retained on the membrane filter act
as an ion exchanger and reduce the recovery of the anions. Alkaline instead of aqueous
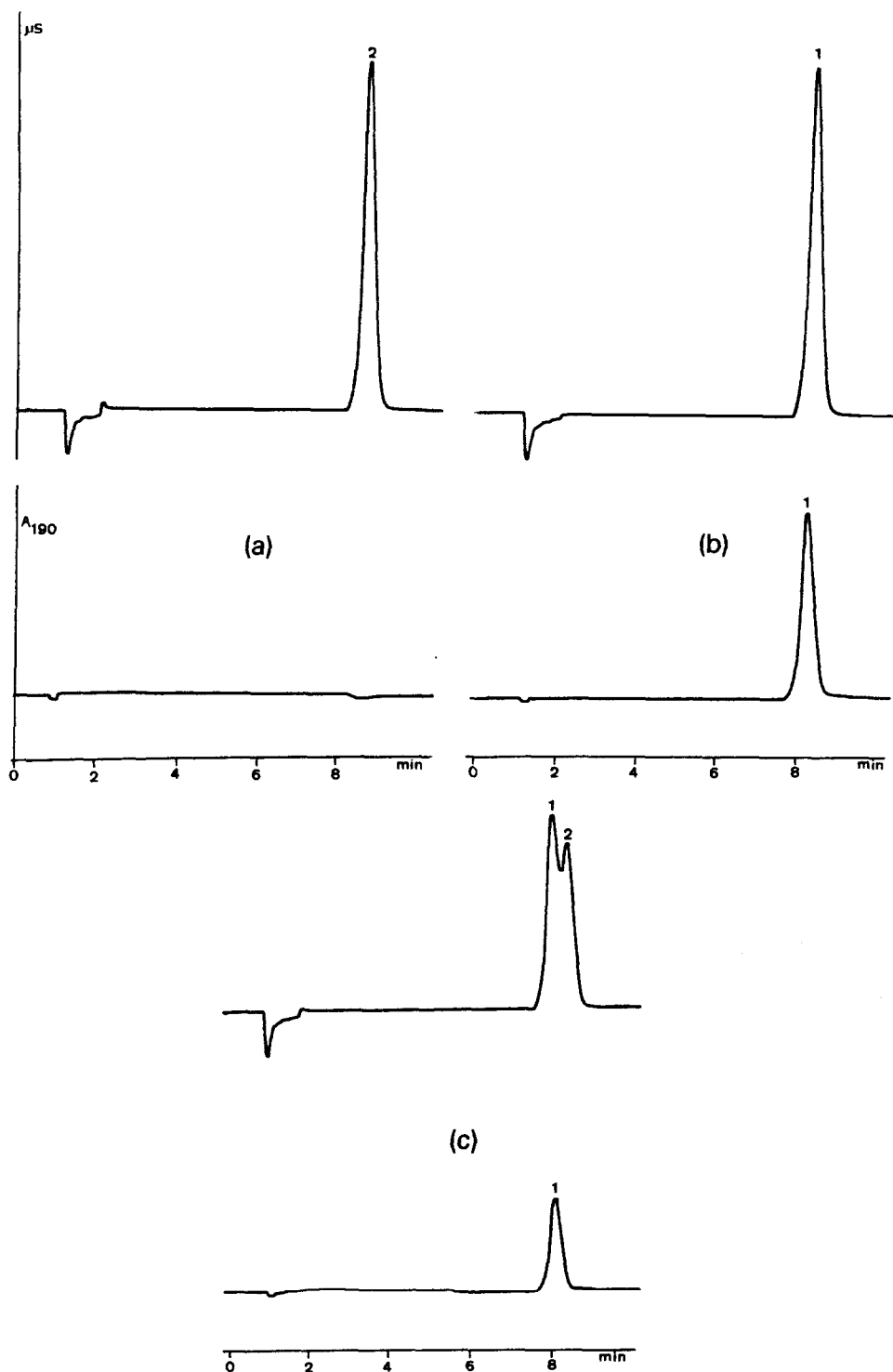extraction has been found[11] to reduce this effect.

Fig. 1. Typical chromatograms of aqueous standard solutions of (a) 0.1 mM SO_4^{2-}, (b) 0.25 mM malate and (c) 0.05 mM SO_4^{2-} + 0.125 mM malate monitored with the conductivity cell (upper chromatograms) or the UV absorbance detector (lower chromatograms). Operating conditions are given in Table I. Peaks: 1 = malate; 2 = SO_4^{2-}.

TABLE II

DETERMINATION OF SO_4^{2-} AND MALATE CONCENTRATIONS IN PURE AQUEOUS MIXTURES BY ION CHROMATOGRAPHY WITH DOUBLE MONITORING

Results are expressed as means (mM) $\pm$ standard deviation (S.D.) of duplicate injections.

Sample composition	[SO_4^{2-}] (mM) (x $\pm$ S.D.)	[malic acid] (mM) (x $\pm$ S.D.)
0.05 mM SO_4^{2-} + 0.1 mM malate	0.048 $\pm$ 0.001	0.104 $\pm$ 0.003
0.1 mM SO_4^{2-} + 0.25 mM malate	0.102 $\pm$ 0.001	0.250 $\pm$ 0.001

RESULTS AND DISCUSSION

Some typical chromatograms obtained after injecting solutions of SO_4^{2-} or malate and of their mixtures are shown in Fig. 1. When introduced as a single compound the absorbance at 190 nm of SO_4^{2-} does not exceed the noise of the baseline. Therefore, the double peak on the conductivity detector of SO_4^{2-} and malate in Fig. 1c is reduced to a single peak of malate in the UV absorbance detection mode. In all runs a small peak (not shown) was registered by the second detector at a retention time of 23 min. Whether this was an impurity in the malate or a component of malate formed during separation was not investigated further.

When both constituents were present the SO_4^{2-} concentration could be calculated as follows: the concentration of malate was obtained from the area of the single detected peak in the UV absorbance mode; the corresponding area on the conductivity detector was calculated using a calibration graph for pure malate standards and was subtracted from the total area of the unresolved peaks from this detector. The remaining area was used to calculate the SO_4^{2-} concentration.

TABLE III

DETERMINATION OF SO_4^{2-} AND MALATE CONCENTRATIONS IN AQUEOUS EXTRACTS OF SPINACH USING STANDARD ADDITIONS BY ION CHROMATOGRAPHY WITH DOUBLE MONITORING

Results are expressed as means (mM) $\pm$ standard deviation (S.D.) of duplicate injections. The expected values of the total concentrations are given in parentheses and were calculated with the mean SO_4^{2-} and malate concentrations as the correct value.

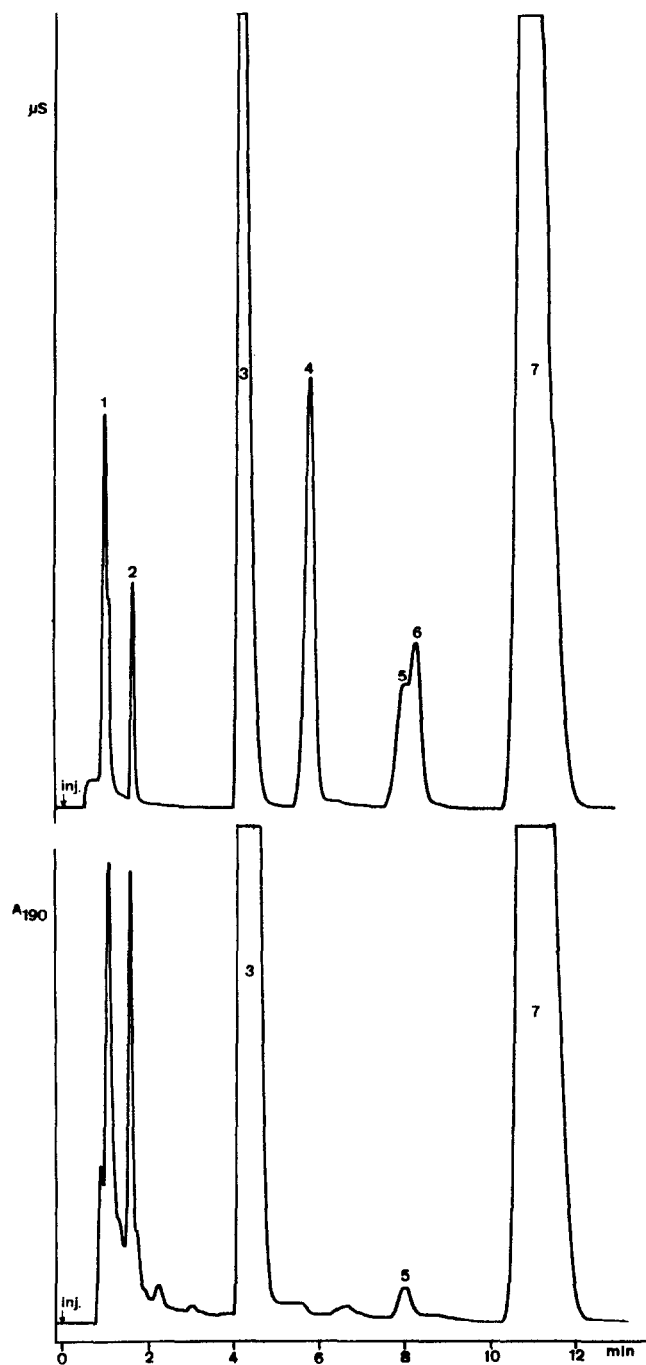| Sample composition	[SO_4^{2-}] (mM) (x $\pm$ S.D.)	[Malic acid] (mM) (x $\pm$ S.D.)
Spinach extract	0.094 $\pm$ 0.005	0.164 $\pm$ 0.015
Spinach extract, diluted 1:2	0.045 $\pm$ 0.004 (0.047)	0.079 $\pm$ 0.001 (0.082)
Spinach extract + SO_4^{2-} and malate solution	0.081 $\pm$ 0.001 (0.078)	0.210 $\pm$ 0.001 (0.22)
Spinach extract, diluted 1:2 + SO_4^{2-} and malate solution	0.057 $\pm$ 0.001 (0.056)	0.128 $\pm$ 0.002 (0.132)

Fig. 2. Typical ion chromatogram of an aqueous spinach extract monitored with the conductivity cell (upper chromatogram) or the UV absorbance detector (lower chromatogram). Operating conditions are given in Table I. Peaks: 1 = unknown; 2 = Cl^-; 3 = NO_3^-; 4 = $H_2PO_4^-$; 5 = malate; 6 = SO_4^{2-}; 7 = oxalate.

To test the assumption that the conductivity peak caused by a mixture of SO_4^{2-} and malate can be obtained by a linear summation of the separate contributions, well standardized mixtures of both components were analysed. On the above basis the concentrations were calculated with the results given in Table II, and are considered to be satisfactory.

To test the appropriateness of the method for plant extracts dilution and standard addition procedures were applied to a spinach extract and the results are given in Table III. Typical chromatograms of the pure extract obtained with both detectors are shown in Fig. 2. The identification of the first peak remains unclear. The results in Table III indicate that SO_4^{2-} can be quantified by the described system with double monitoring within an accepted level of accuracy ($\leqslant 5\%$) except for the diluted sample (The second sample in Table III). This indicates that the detection limit is within this range. However, this limit seems to depend on the malate concentration, as indicated by the smaller relative standard deviation for the last sample ($<2\%$).

CONCLUSIONS

In the isocratic ion chromatographic system described, SO_4^{2-} and malate peaks show poor resolution. However, using the double monitoring system (conductivity cell and UV absorbance at 190 nm), both can be quantified at an accepted level of accuracy with a simple procedure. In spinach extracts (150 mg in 100 ml of 1.8 mM Na_2CO_3–1.7 mM $NaHCO_3$) SO_4^{2-} and malate were determined with relative standard deviations of 5 and 9%, respectively, for sequential injections. As could be inferred from standard addition procedures, the recoveries of both constituents were satisfactory.

ACKNOWLEDGEMENTS

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