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Note

Anion chromatography on a conventional reversed-phase column using two ultraviolet detectors

JAN ANDRASKO
The National Laboratory of Forensic Sciences, S-581 01 Linköping (Sweden)
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Ion chromatography, introduced in 1975, has become a widely accepted method for quantitative determination of anions in aqueous samples[1]. In addition to the work done with fixed-site ion-exchange materials, alternative methods for anion analysis using conventional high-performance liquid chromatographic (HPLC) equipment have appeared in the literature[2–9]. High-efficiency reversed phases and the equipment are readily available in routine analytical laboratories.

In the majority of publications conductometric detectors were used, but standard UV-absorbance detectors were also employed for direct[2–5,7,8] and indirect[6,10,11] detection of anions.

Recently, Wheals[9] described a very ambitious approach to anion screening using a combination of several columns and four detectors (UV absorbance, conductometric, electrochemical and refractive index). Such a system is very efficient, and high selectivity is achieved in the detection of a variety of anions.

In the present work an attempt is made to use simple HPLC equipment (a single reversed-phase column and two UV detectors connected in series) for anion screening. Both UV-absorbing and non-absorbing anions may be separated and detected with similar sensitivity in a single system. This equipment is suitable for those laboratories that do not always require the highest sensitivity for some specific ion.

EXPERIMENTAL

The chromatographic system consisted of a Varian Model 5000 liquid chromatograph equipped with a variable-wavelength UV detector (Model 100). In series with this detector, a Waters Assoc. fixed-wavelength detector (Model 440) operating at 254 nm was connected. The time delay between the responses of the two detectors for the same analyte was *ca.* 2 sec at a flow-rate of 1.5 ml/min.

The HPLC column was a 5 μm Nucleosil-C_{18} (200 × 4.6 mm I.D.) from Scandinaviska GeneTec. The temperature was ambient. The injections were made via a valve injector (Rheodyne type 7125) with a 10-μl loop. The eluent was prepared by dissolving cetyl trimethylammonium *p*-toluenesulphonate (CpTS), purchased from Sigma, and *p*-toluenesulphonic acid (Kebo-Grave) in water–acetonitrile, and the pH of the solution was adjusted to 5.5–6.0 with ammonium hydroxide. The concentration of CpTS varied from 0.1 to 0.16%, that of *p*-toluenesulphonic acid from

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0 to 0.003 *M*. The acetonitrile–water ratio was within the range 35:65 to 40:60. For anion screening, two eluent compositions were found to be suitable: 0.13% CpTS plus 0.003 *M p*-toluenesulphonic acid in acetonitrile–water (35:65), and 0.11% CpTS plus 0.002 *M p*-toluenesulphonic acid in acetonitrile–water (39:61). The former eluent has higher UV absorbance and needs good backing-off facilities of the UV detectors.

The flow-rate was held at 1.5 ml/min in all experiments. The pH of the samples injected was close to that of the eluent.

RESULTS AND DISCUSSION

Several inorganic anions absorb strongly at low wavelengths[12], and UV monitoring is an excellent method of detection. The anions that do not show strong UV absorbance may be detected by indirect photometry[10] when a UV-absorbing counter-ion is incorporated in the eluent. If small ions are to be separated by a reversed-phase system, an ion-pairing compound must be added. Direct UV detection of UV-absorbing ions with high sensitivity is achieved using cetyl trimethylammonium chloride[2,5,8] or bromide[9], whereas chloride must be replaced by a suitable UV-absorbing ion for detection of non-absorbing ions[6]. We chose *p*-toluenesulphonate for three reasons:

(1) It is commercially available and relatively cheap.

(2) It exhibits a UV spectrum with a local minimum at wavelengths where many inorganic anions absorb more strongly.

(3) It is unlikely to be an anion of interest for detection by this method.

The UV spectrum for CpTS is depicted in Fig. 1. The UV absorbance of this compound is due to the *p*-toluenesulphonate ion. It shows high absorbance below *ca.* 225 nm and exhibits a minimum close to 236 nm and a maximum at *ca.* 259 nm.

In our experimental arrangement, one UV detector is fixed at 254 nm. At this wavelength the majority of simple inorganic anions do not absorb significantly, they give negative responses and are detected by indirect photometry. The wavelength of the second UV detector is set close to the minimum in the UV spectrum of CpTS (*ca.* 236 nm). UV-transparent anions, such as Cl^-, ClO^-, ClO_3^-, SO_4^{2-}, $H_2PO_4^-$ and CN^-, will give negative responses at this wavelength; other anions, such as NO_2^-, NO_3^-, I^-, IO_3^-, SCN^- and $S_2O_3^{2-}$, will give positive peaks.

The composition of the eluent system as described in the Experimental section may be varied to some extent. Thus, separation of common univalent anions may be achieved using CpTS without incorporation of an additional counter-ion. Fig. 2 shows the separation of some small ions using this eluent. The observed signals for NO_2^- and NO_3^- change the sign between the two wavelengths of detection. In the forensic laboratory the separation and detection of NO_3^- and ClO_3^- is of importance in the analysis of explosives and explosive residues. The specificity of such an analysis is improved by this simple arrangement.

If two variable-wavelength detectors are, used wavelengths longer than 254 nm might be employed for detection, which would result in improved specificity for other anion specimens. As an illustration, Fig. 2 includes an additional analysis of the same mixture of anions with detection wavelengths 260 and 270 nm, respectively (two separate analyses). At 270 nm *p*-toluenesulphonate still exhibits some UV absorbance, and all the anions in Fig. 2 are detected by indirect photometry.

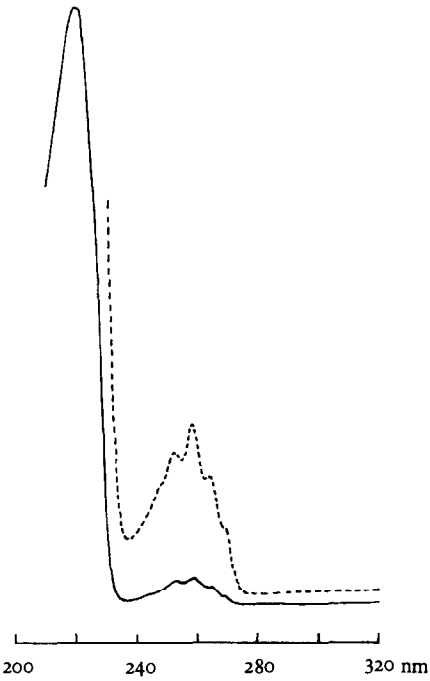

Fig. 1. The UV spectrum of cetyl trimethylammonium *p*-toluenesulphonate in water. The dashed line shows a vertically expanded part of the spectrum.

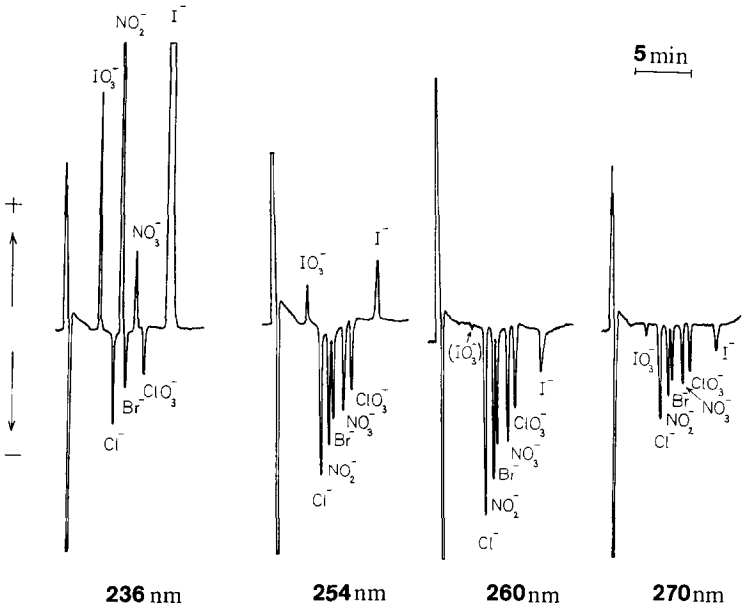

Fig. 2. The separation of several univalent inorganic anions (1.4 μg of each). The eluent was 0.13% CpTS in acetonitrile–water (40:60). The flow-rate was 1.5 ml/min, and the temperature ambient. The time scale in this figure and in all subsequent chromatograms corresponds to 5 min. The detection at 236 and 254 nm was performed simultaneously using two detectors connected in series. (The other two chromatograms were obtained by separate analyses.) Sensitivity: 0.05 a.u.f.s. at 254 nm, otherwise 0.02 a.u.f.s.

To elute bivalent and more strongly retained univalent anions within a reasonable time and using a single analytical step, an additional counter-ion must be incorporated in the eluent. Owing to the competition between *p*-toluenesulphonate, the analysed anions and any additional counter-ion, the peaks observed for non-absorbing anions were sometimes distorted or completely missing. This occured particularly with HPO_4^{2-} as counter-ion, and the resulting signals for Cl^-, for example, were partly positive and partly negative.

To avoid these problems, *p*-toluenesulphonate was included in the eluent as an additional counter-ion. Even though the UV absorbance of the eluent increased, satisfactory separations without distorted peaks (except the system peak) were observed. The separation of a mixture of eight inorganic anions is depicted in Fig. 3. It can be seen in this figure that the separation between Br^- and NO_2^- is not as good as that in Fig. 2. However, both anions may be easily detected even at 236 nm, where the NO_2^- signal is positive and the Br^- signal negative. Some other examples of separation and detection of univalent and bivalent anions are given in Figs. 4 and 5.

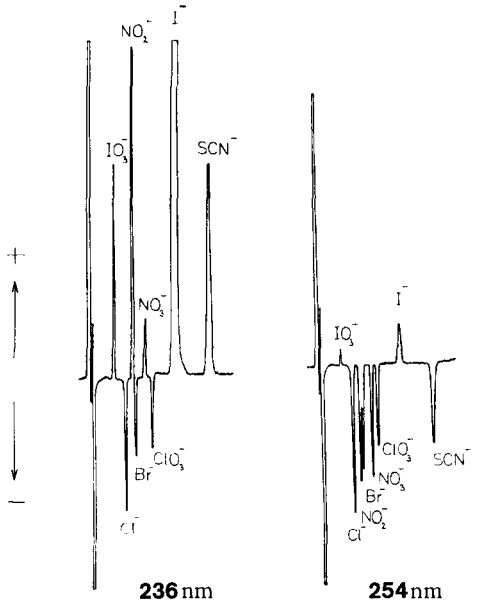

Fig. 3. The separation and UV detection of eight univalent anions (1 μg of each). Mobile phase, 0.11% CpTS plus 0.002 *M* *p*-toluenesulphonate in acetonitrile–water (39:61). Sensitivity: 0.05 a.u.f.s. at 254 nm and 0.02 a.u.f.s. at 236 nm.

Polyvalent counter-ions, such as citrate and tartrate, have the advantage of eluting bivalent ions more rapidly than univalent *p*-toluenesulphonate. Citrate was used by Wheals[9] as a counter-ion with very good results. Fig. 6 shows the separation of a mixture of anions with tartrate as an additional counter-ion. The separation is good but the negative signals in the chromatograms become weaker because tartrate (and also citrate) is not appreciably UV absorbing at wavelengths longer than *ca.* 230 nm.

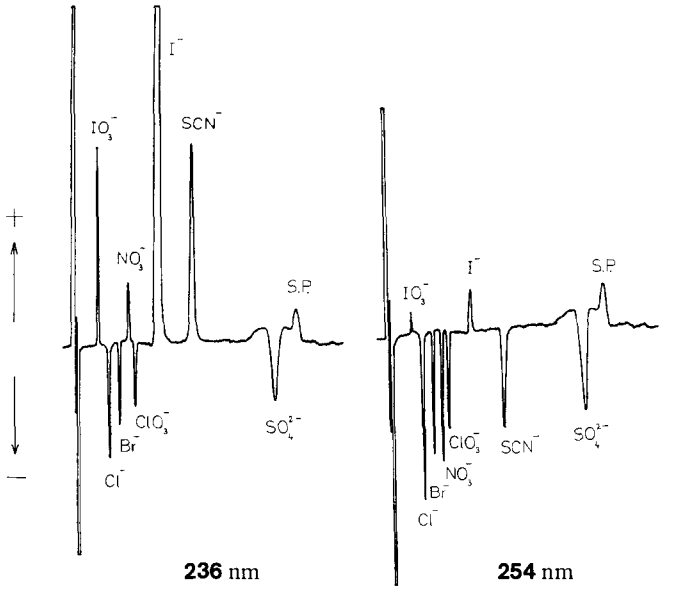

Fig. 4. HPLC chromatograms for a mixture of several anions (1 μg of each). The experimental conditions are the same as those in Fig. 3. S.P. = System peak.

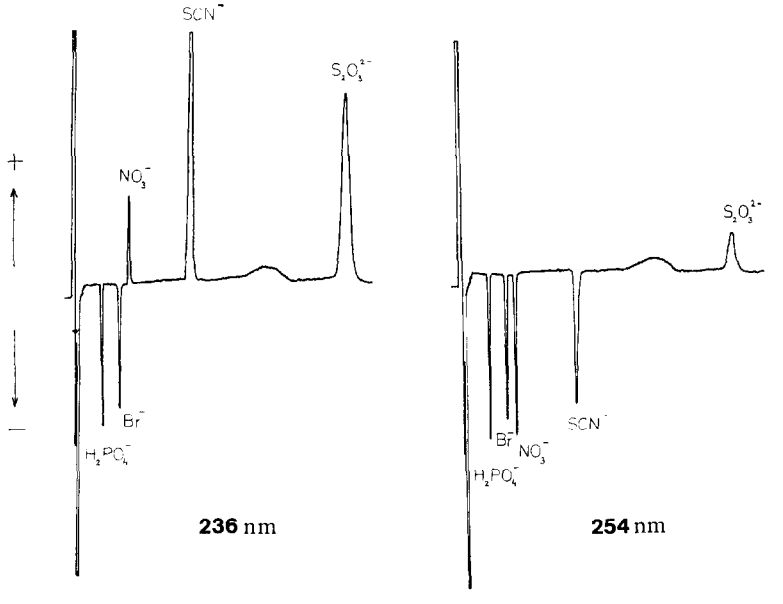

Fig. 5. The separation and UV detection of $H_2PO_4^-$, Br^-, NO_3^-, SCN^- and $S_2O_3^{2-}$ (2 μg of each). The experimental conditions are the same as those in Fig. 3.

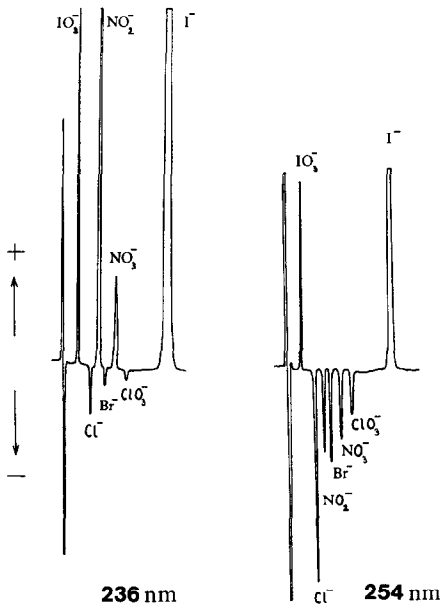

Fig. 6. HPLC chromatograms obtained for a mixture of seven anions. Mobile phase: 0.13% CpTS plus 1.5 m*M* potassium tartrate in acetonitrile–water (35:65).

The analytical system described here will separate and detect common inorganic anions with similar sensitivity. The detection limit is of the order 100 ng and is better for some strongly absorbing species such as I^-. The disadvantage of ion-pairing techniques is the long time necessary to equilibrate the C_{18} column. As we used a relatively high concentration of the ion-pairing compound in the eluent, equilibrium was reached in less than 2 h (at a flow-rate of 1.5 ml/min). Another disadvantage of ion-pairing systems and C_{18} columns is a slow decrease in retention times of analysed compounds. However, as in Wheals' paper[9] no change in the retention times for anions was noticeble during a working day.

Also the presence of a system peak is undesirable. In our system, the system peak appears shortly after the SO_4^{2-} peak, but in a positive direction.

We believe that in laboratories that do not carry out daily screening analyses of anions and cannot afford ion-exchange columns and specific detectors, the use of the ion-pairing technique on conventional columns with a combination of normally UV detectors should be a very convenient solution. Because the time taken to reach equilibrium in the system is relatively long, the analyses may be performed when a sufficient number of samples has accumulated.

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