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Reversed-phase ion-pair chromatography with indirect photometric detection of inorganic anions from residues of low explosives[a]

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ABSTRACT

The analysis of inorganic anions found in residues of low explosives present a difficult forensic problem. Current methods are neither quantitative nor sensitive. While the liquid chromatographic separation of these anions is relatively easy, their detection is difficult since they cannot be detected by conventional high-performance liquid chromatography detectors. We report a method that can detect inorganic anions, which are commonly found in combustion residues of low explosives, by reversed-phase ion-pair liquid chromatography, using indirect UV detection. The ion-pair reagent is benzyltributylammonium chloride. In addition, the phosphate buffer mobile phase contains hexane sulfonate. There is little sample preparation since no interferences were encountered while testing real samples from explosive residues. Detection limits are low and selectivity is high compared with existing techniques. Analysis time is short and the separation can be repeated immediately.

INTRODUCTION

Low explosive residues are difficult to analyze since they contain inorganic salts which originate from the oxidizers. For example, the most popular oxidizers are usually nitrate and chlorate salts with potassium or ammonium as the co-ion[1,2]. Since these explosives are frequently improvised (home made), and since there is no way to predict the ingredients and their amounts in the bomb, they present a complex analytical problem. Additional difficulties in the analysis involve (a) handling the traces which are left for analysis after an explosion, and (b) interferences that result from

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debris and other impurities that are collected together with the sample at the scene of the explosion[2].

The analysis of post explosion samples involves techniques such as extraction with water (for inorganic compounds) or acetone (for organic compounds), spot tests, Fourier-transform infrared spectroscopy, mass spectrometry and ion-exchange chromatography. Other methods are microscopic analysis and X-ray diffraction[3–5]. These methods, while useful, are frequently not specific or sensitive enough for the analysis of inorganic anions. There is a need to develop an analytical method that will be able to detect inorganic anions of explosives and explosive residues.

The method which is described here can separate and detect inorganic anions, which are commonly found in combustion residues of low explosives, by reversed-phase ion-pair liquid chromatography, using indirect UV detection. Inorganic anions are difficult to detect by conventional UV detectors because they are UV transparent. The problem is solved in the present work by using a mobile phase containing a UV-absorbing ion-pair reagent (IPR). Due to adsorption of the IPR on the stationary phase, the reversed-phase column becomes a dynamically coated anion exchanger. The solutes interact with the IPR and, as a result, zones of IPR deficiencies are formed in the column[6]. The inorganic anions are detected indirectly as a result of the detector response to this deficiency of IPR in the mobile phase. The continuous flow of the IPR ensures the stability of the column and of the separation. Indirect detection in high-performance liquid chromatography (HPLC) has been used previously in the forensic laboratory for the analysis of many anions; see for the example the work of Wheals[7].

Reversed-phase columns are commonly used in modern chromatography and are available at a reasonable cost. The instrumentation needed for this method consists of an HLPC system, which is very common in many forensic laboratories. Thus, this new method can be used in many laboratories immediately and without any major expenses which are associated with new instrumentation or columns.

EXPERIMENTAL

Materials

The mobile phase was prepared using deionized water which was filtered through a 0.45-μm Schleicher & Schull membrane filter membrane. Benzyltributylammonium chloride (BTA) and hexane sulfonate were obtained from Sigma Israel (Tel-Aviv, Israel). All chemicals were of analytical-reagent grade.

The column (50 mm × 4.5 mm I.D.) and the guard colum (25 mm × 4.5 mm I.D.) were LiChrospher RP-18 cartridges (Merck, Darmstadt, F.R.G.).

The mobile phase consisted of 4 m*M* BTA, 0.14 m*M* hexane sulfonate and 7 m*M* phosphate buffer (pH 5).

Instrumentation

The chromatographic system consisted of a SP 8000 HPLC system (Spectra-Physics) with a Rheodyne injector (10-μl loop). The detector was a Perkin-Elmer 85B UV–VIS variable-wavelength detector. The recorder and integrator were part of the HPLC system. The column was thermostated at 35 ± 0.1°C.

The spectrum of BTA was obtained with a Perkin-Elmer Lambda 5 UV–VIS spectrophotometer.

Sample preparation

Samples containing post-explosive residues were extracted with water and then filtered and concentrated over a water bath. The solids which remained in the flask were weighed (between 15–30 mg) and diluted to 5–10 ml. Samples were filtered and injected immediately.

RESULTS AND DISCUSSION

In this study we have examined the behavior of nitrite, nitrate, chlorate, sulfate (as sodium salts), thiocyanate and perchlorate (as potassium salts) ions as the inorganic anions. The chromatographic method is a modification of the system described by Barber and Carr[6]. The modifications were made to fit the needs of a forensic laboratory.

Characterization of the ion-pair reagent

The UV spectrum of BTA is shown in Fig. 1. BTA has three absorption maxima: at 269, 262 and 257 nm. We chose 262 nm as the working wavelength of detection since at this wavelength BTA absorbs the most and it should provide the most sensitive detection. Another wavelength, 222 nm, was investigated to detect nitrite and nitrate. The high native absorbance of these anions at this wavelength ensures high sensitivities.

To maintain short analysis times, we used a 5-cm column. This short column did not affect the selectivity but shortened the retention times of most anions and gave sharper peaks. Phosphate buffer was used in order to further ensure sharp chromatographic peaks. The buffer was kept at pH 5 due to the better buffering capacity at that pH. To better control the analysis time and the selectivity of the column, hexane sulfonate was added to the mobile phase. Fig. 2 shows a typical chromatogram of this system with a standard mixture of anions.

Before describing the actual separations, we will examine the effects of some of the experimental parameters. Many parameters influence the retention and selectivity

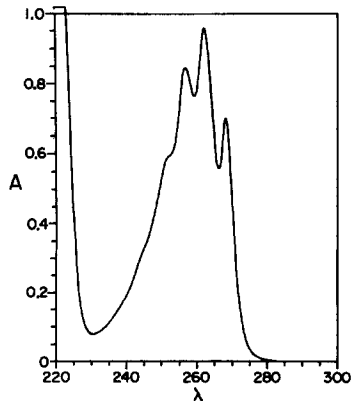


Fig. 1. The UV spectrum of BTA. The solvent is water. λ = Wavelength in nm.

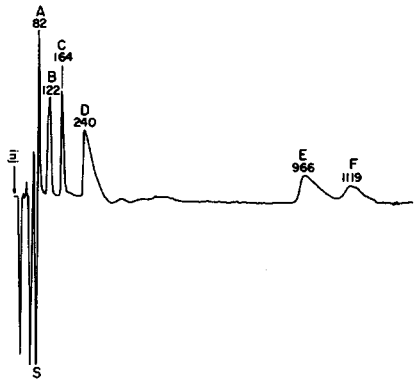


Fig. 2. Chromatogram showing the separation of all six anions studied. Chromatographic conditions are given in the heading of Table I. Peaks: S = system peak; A = nitrite; B = nitrate, C = chlorate; D = sulfate; E = thiocyanate; F = perchlorate. Numbers at peaks are retention times in s.

of the chromatographic system. To optimize the chromatographic system, these experimental parameters must be studied and understood.

Temperature effects

The effect of the temperature on the retention is shown in Fig. 3 which plots ln k' (k' = capacity factor) of five anions as a function of the reciprocal of the temperature (T). The nitrate, chlorate, perchlorate and thiocyanate behave in the 'normal fashion'; namely, the retention times of these solutes decrease as the temperature increases. The retention of sulfate, on the other hand, *increase* with increasing temperatures. As will be shown throughout this paper, the sulfate ion behaves in a different fashion than the rest of the anions. It is thought that in the present chromatographic system the adsorption isotherm of the sulfate ion is not linear.

The slopes of the perchlorate and thiocyanate lines are close in value (2140 as

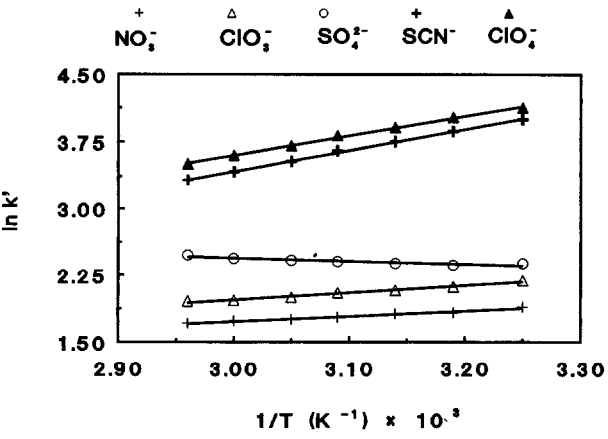


Fig. 3. Dependence of ln k' on $1/T$. The mobile phase contained 4 mM BTA, 5 mM phosphate buffer pH 4.6 and 0.25 mM hexane sulfonate. The flow-rate was 2 ml/min. Detection at 262 nm.

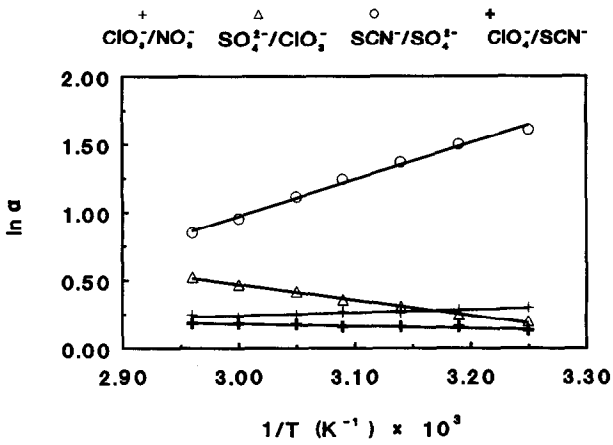


Fig. 4. Dependence of ln α on $1/T$. Chromatographic conditions as in Fig. 3.

compared to 2307). Thus, the selectivity of the system toward these two ions should be, to a large extent, temperature independent. Similarly, the slopes of the nitrate and chlorate lines are fairly similar (590 as compared to 800). The temperature dependence of the selectivity between these two ions should be small. Fig. 4, which gives the dependence of the selectivity, α, on the temperature, shows the temperature independence of α in the case of SCN^- and ClO_4^- and the small temperature dependence of α in the case of nitrate and chlorate. Because of the temperature dependence of the sulfate retention, the selectivity between the sulfate and the chlorate increases with increasing temperatures, while the selectivity between thiocyanate and sulfate decreases sharply as the temperature increases.

Effect of buffer concentration

Fig. 5 shows the dependence of the k' values of nitrite, nitrate, chlorate and

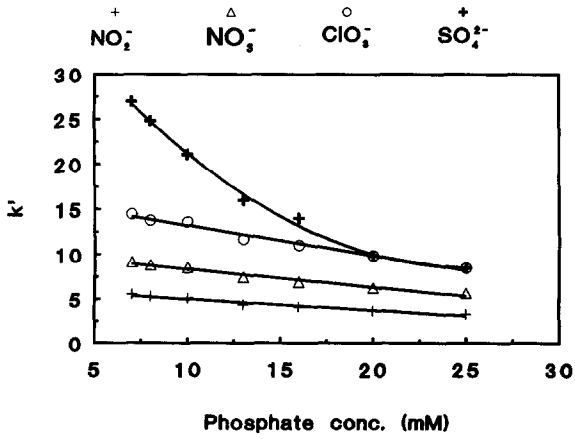


Fig. 5. Dependence of k' on the phosphate concentration. Chromatographic conditions as in Fig. 3, with the exception that the phosphate concentration varied.

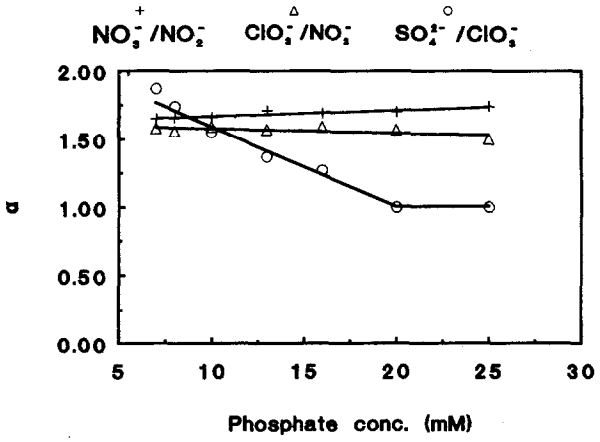


Fig. 6. Selectivity, α, as a function of phosphate concentration. Chromatographic conditions as in Fig. 3, with the exception that the phosphate concentration varied.

sulfate on the buffer concentration. As expected, the k' values of the solutes decrease as the phosphate concentration increases. The greatest change occurs in the case of the sulfate ions. This great change in the retention of sulfate ions with buffer concentration is in agreement with the non-linearity of its adsorption isotherm.

Fig. 6 shows the dependence of the selectivity on the buffer concentration. The selectivity between nitrate and nitrite increases a little with increasing buffer concentrations. The selectivity between chlorate and nitrate decrease a little with increasing phosphate concentrations. However, the selectivity between sulfate and chlorate decreases drastically with buffer concentrations. In fact, at high amounts of phosphate, the resolution between these two solutes disappears completely. Similar behavior of the retention of sulfate was observed by Barber and Carr[8]. Clearly, for the chromatographic system at hand, running at low buffer concentration is advantageous.

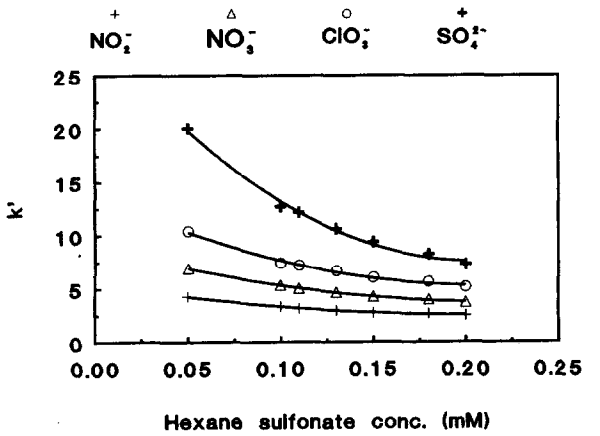


Fig. 7. Dependence of k' on the hexane sulfonate concentration. Chromatographic conditions as in Fig. 3, with the exception that the hexane sulfonate concentration varied.

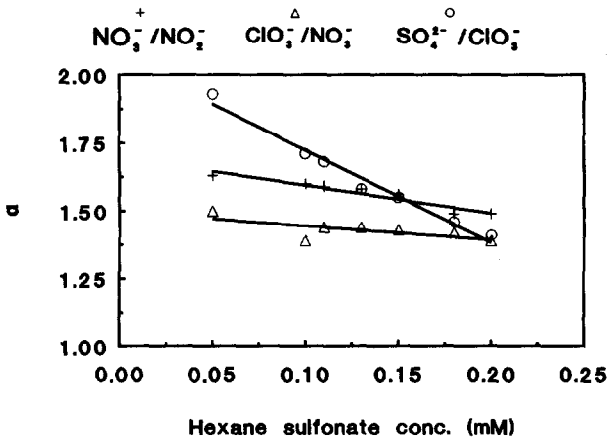


Fig. 8. Dependence of α on the hexane sulfonate concentration. Chromatographic conditions as in Fig. 3, with the exception that the hexane sulfonate concentration varied.

Effect of hexane sulfonate concentration.

The effect of increasing the concentration of the hexane sulfonate in the mobile phase is similar to the effect of changing the buffer concentration. As the hexane sulfonate concentration increases, the retention of the solutes decrease. Fig. 7 shows the k' values of four anions as a function of the sulfonate concentration. Again, the sulfate ions show the greatest dependence on the concentration of the sulfonate.

The selectivity dependence on hexane sulfonate is given in Fig. 8. In all cases, the selectivity decreases with increasing sulfonate in the mobile phase. However, in cases where sulfate ions are not involved, the decrease in the selectivity is small. Figs. 7 and 8 indicate that is is more advantageous to achieve the separation at low concentration of the sulfonate.

Based on the above studies, the chromatographic system which we chose for the separation of nitrite, nitrate, chlorate and sulfate ions, found in post-explosive residues, consisted of an RP-18 column, 4 m*M* BTA, 7 m*M* phosphate buffer at pH 5, 0.14 m*M* hexane sulfonate and temperature of 35°C.

Detection linearity and detection limits

The linearity and the detection limit of the present chromatographic system was examined by preparing calibration curves for all anions at both 262 and at 222 nm. In the concentration range between 0.05 to 10 m*M*, the response was linear for all anions. However, there was a difference in detection limits between the two wavelengths. Table I gives the regression parameters obtained from correlating the peak areas to the concentrations of nitrite, nitrate, chlorate and sulfate. Also given in the table are the detection limits of these four solutes in both wavelengths. The better detection limit toward nitrite and nitrate at 222 nm is due to the strong UV absorption by these two anions. Chlorate and sulfate, on the other hand, show better detection limits at 262 nm. It is felt that the high background absorption of BTA at 222 nm hinders in the detection of low amounts of chlorate and sulfate ions.

TABLE I

REGRESSION PARAMETERS OF PEAK AREAS *VS.* CONCENTRATION (m*M*)

Mobile phase contained 4 m*M* benzyltributylammonium chloride, 7 m*M* phosphate buffer (pH 5), and 0.14 m*M* hexane sulfonate in water. Flow-rate was 2 ml/min. Detection wavelengths were 222 and 262 nm. Temperature was 35±0.1°C. All anions were determined in the present of the other three. a = Slope (divided by 10^4); b = intercept (divided by 10^3); R = correlation coefficients; n = number of different concentrations used to calibrate; DL = detection limits.

Anion	*a*	*b*	*R*	*n*	*DL (ppm)*
Wavelength 222 nm					
Nitrite	17.2	−3.87	0.9997	10	2.8
Nitrate	12.4	−4.07	0.9985	10	0.8
Chlorate	0.85	−3.48	0.9906	6	21.3
Sulfate	1.15	−4.80	0.9930	5	28.4
Wavelength 262 nm					
Nitrite	1.03	−1.11	0.9996	5	48.3
Nitrate	1.16	−2.01	0.9974	7	8.5
Chlorate	1.05	−2.59	0.9976	7	10.7
Sulfate	1.59	−3.23	0.9973	7	14.2

Concentration effects of the solutes

It has been reported[6,9] that the retention times of the solutes can be a function of their injected concentration. In general, as the concentration of the solute increased the retention time decreased. This effect was observed in our system for the sulfate anion and it caused a problem in the identification and quantification of sulfate ions in samples where its concentration was not known (as is the case in explosive residues). To overcome this problem, we have used the sulfate calibration curve and another curve which is obtained by plotting the retention time of sulfate as a function of the injected concentration. Fig. 9 shows the dependence of the area and of the retention time of sulfate on the concentration of that anion. The two curves are used as follows: The concentration of a suspected sulfate peak is ascertained by extrapolation from the retention time. This concentration is correlated to the peak area from

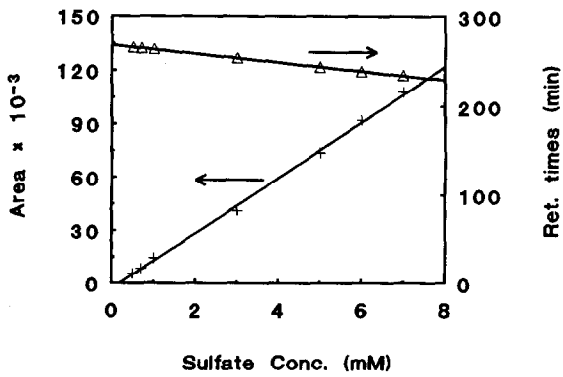
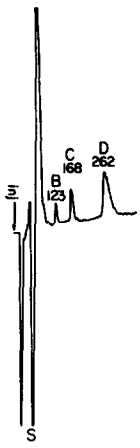


Fig. 9. Dependence of sulfate peak area (+) and retention time (△) on that anion concentration.



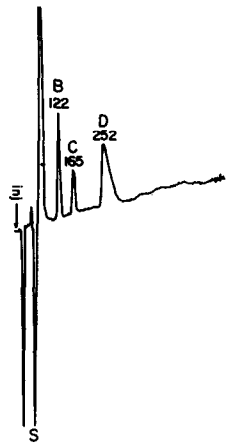


Fig. 10. Chromatogram of water extract from sample 1. The chromatographic conditions are given in Table I. Peaks: S = system peak; B = nitrate; C = chlorate; D = sulfate.

Fig. 11. Chromatogram of water extract from sample 2. Conditions as in Fig. 3. Peaks: S = system peak; B = nitrate; C = chlorate; D = sulfate.

the calibration curve. The calculated peak area is then compared with the experimental value. Agreement between the two values established the concentration and identification of the sulfate anion. In the present work it was found that, at 222 nm, the line correlating the retention to the concentration is described by the equation:

$$t_R = 267 - 5.24C$$

and at 262 nm:

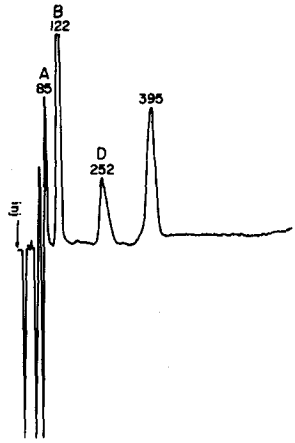
$$t_R = 269 - 4.84C$$

In these two expressions, the retention time t_R is given in s and the concentration C in mM. The correlation coefficients are 0.995 and 0.979, respectively.

Applications of the method

The utility of the chromatographic method for identification and quantitation of post-explosive residues was examined by analyzing several explosive residues from actual cases. Fig. 10 shows the chromatogram obtained from the remains of an explosive charge which was found and detonated by the police. The remains from the explosion were sent for an analysis. Spot test identified potassium chlorate and sulfur. The chromatogram confirmed the presence of these anions. In addition, the presence of nitrate was found as well.

Fig. 11 shows the chromatogram of another sample which was obtained from the remains of a bomb, again, discovered and detonated by the police. The sample was extracted from parts of a pipe which made up the bomb. Conventional analysis found only traces of $KClO_3$. The chromatogram confirmed the presence of chlorate



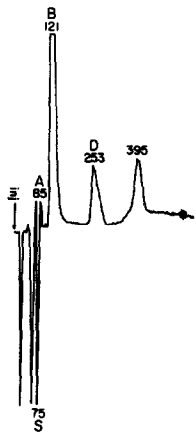


Fig. 12. Chromatogram of water extract from sample 3. Conditions as in Fig. 3. Peaks: S = system peak; A = nitrite, B = nitrate; D = sulfate.

Fig. 13. Chromatogram of water extract from sample 4. Conditions as in Fig. 3. Peaks: S = system peak; A = nitrite; B = nitrate; D = sulfate.

ion. The chromatography was also able to detect the presence of nitrate and sulfate ions.

Fig. 12 shows a chromatogram of a third example. It was obtained from the center of an explosion. Spot test analysis indicated the presence of minute amounts of KNO_2 and sulfur. the chromatographic analysis confirmed the presence of these two ions as well as of nitrate ion. Spot tests and IR techniques cannot differentiate easily between nitrate and nitrite. No such difficulty exists in the method described here. The presence of sulfate, nitrate and nitrite may be indicative of black gunpowder.

Fig. 13 shows a chromatogram of a fourth example. The sample collected from

TABLE II

QUANTITATIVE ANALYSIS OF EXPLOSIVE RESIDUES

See Table I for conditions. Results are in % of the total sample weight.

No. of sample	*Nitrite*	*Nitrate*	*Chlorate*	*Sulfate*
Wavelength 222 nm				
1 (Fig. 10) –	0.11	1.70	5.27	
2 (Fig. 11) –	0.27	3.15	11.07	
3 (Fig. 12)	0.53	6.61	–	18.85
4 (Fig. 13)	0.12	3.27	–	23.27
Wavelength 262 nm				
1 (Fig. 10) –	–	1.68	4.82	
2 (Fig. 11) –	0.69	3.73	12.22	
3 (Fig. 12) –	22.54	–	22.13	
4 (Fig. 13)	0.63	29.89	–	20.63

the site of an explosion. Spot test analysis found traces of potassium nitrite and sulfur. the chromatogram shows nitrite, nitrate and sulfate ions. In Figs. 12 and 13 chlorate ion is missing. However, in both figures there is an unidentified peak which elutes at 395 s.

A comparison between Figs. 10 and 11 and Figs. 12 and 13 shows that different explosives were used in the two groups of explosions. In all chromatograms there is a clear indication of the various anions, and identification is immediate and without interferences. The sulfate anion was identified and quantified according to the method described above.

Table II shows the results of the quantitative analysis of the samples. The analysis was based on the calibration curves that were described in Table I. The quantitation was done both at 262 and 222 nm. It is of interest to note that the quantitative results for chlorate and sulfate agree quite well at both wavelengths. The agreement in the results of nitrite and nitrate is less satisfactory. The reasons for the disagreement in the quantitation of nitrite and nitrate at the two wavelengths is not clear to us.

CONCLUSIONS

The new chromatographic method for the analysis of nitrite, nitrate, chlorate and sulfate ions in explosive residues is fast, selective and sensitive. The system has a linear response for all these anions. It shows selectivity toward nitrate and chlorate, which presents a problem in conventional ion chromatography. There is no need for elaborate sample preparation and the sample can be injected into the chromatograph immediately after water extraction from the bulk sample. Samples from explosive residues yield clean chromatogram with no interferences. A chromatographic system that will identify and quantify thiocyanate and perchlorate is now under development.

ACKNOWLEDGEMENT

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