

**SIMULTANEOUS SEPARATION AND DETERMINATION OF
SIX INORGANIC ANIONS BY NON-SUPPRESSED ION
CHROMATOGRAPHY AND ITS APPLICATION ON
SOME MEDICINAL PLANTS (PART 2)**

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ABSTRACT

A sensitive and simple analytical method for simultaneous separation and determination inorganic anions (fluoride, chloride, bromide, nitrate, iodide and sulphate) was developed by non-suppressed ion chromatography. The separation was achieved on an anion exchange column (Shim pack IC A1, 4.6 mM IDX 100mm) with a mobile phase consisting of 2.2 mM phthalic acid, 2.7mM tris (hydroxymethyl) aminomethane and 0.2mM N-{{(ethylamino) thioxomethyl}} hydrazinocarbonyl methyl} trimethyl ammonium chloride (ETHTC), a flow rate of 1.5 ml/min and 40°C.

The aim of this work is to improve and characterize the method for simultaneous determination of six inorganic anions in medicinal plants by non-suppressed ion chromatography, using optimization procedures, in order to be applied to the routine analysis. The proposed method has numerous advantages over the other widely used non-suppressed ion chromatography methods; higher sensitivity, shorter analysis time, lower quantization and detection limits .

The performance characteristics of the method were established by determining the following validation parameters: precision, accuracy, linearity and detection limits.

Also, the proposed method was successfully applied for the separation of some inorganic anions from some medicinal plants including Mentha Vridis, Mentha Longifolia, Origanum Majorana and Cymbopogon Winterisanus which are existing in different places at Saudi Arabia.

1-INTRODUCTION

Ion chromatography (IC) is a powerful technique for the separation, identification, and quantification of organic and inorganic ions. As a consequence, IC is widely used in the environmental, electronics, petrochemical, power, food and beverage and pharmaceutical industries. [Barkley, et al. (1992); Buldini, et al., (1993); Cox, et al., (1985); Toofan, et al., (1997) and Vanatta, (2001)].

Small et al. (1975) reported the first method for separation and quantitative determination of inorganic ions by high-performance liquid chromatography. This technique is called ion chromatography (IC) and used a combination of analytical column and suppressor column to decrease the conductivity of the mobile phase for conductometric detection. Since then significant drawbacks from the chromatographic point of views have been reported regarding this approach, most arising from the suppressor itself. Butytenhuys [Butytenhuys, (1981)] reported that the number of injections is restricted by the capacity of the suppressor column (if it is used); the suppressor introduces extra band broadening, which results in lower resolution.

Special equipment is needed for ion chromatography; only those buffers can be applied which, after passage through a suppressor, result in low electrical background conductivity. These deficiencies have provided an impetus for the development of chromatographic methods that do not require use of a suppressor column.

Some investigations have been undertaken in an attempt to overcome these disadvantages of suppressed ion chromatography. In particular Gjerde and co-workers [Gjerde & Fritz (1979) and Gjerde, et al., (1980)] demonstrated that the suppressor column is not absolutely necessary for high-performance ion chromatography with electric conductivity detection. They used an ion-exchanger having a low exchange capacity and eluent having a very low conductivity. In this way the background conductivity is sufficiently low to allow the separated anions to be detected with a simple conductance detector.

A number of alternative methods have been reported in the literature, all of which use an analytical separator column without suppressor. These methods differ from each other chiefly in the mode of detection employed. Inorganic anions may be separated on a reversed-phase system either by prior formation of organic derivatives or by using ion-pair formation with subsequent direct detection of UV-absorbing ions

at low UV wavelengths (210-220 nm). Cortes and Stevens [Cortes & Stevens (1984)] applied these same approach to an amino column, however these methods are not widely applicable since only limited number of inorganic ions show UV absorbance. Papp and Fehervary (1988) and Chauret and Hubert (1989) proposed the more general methods using reversed-phase ion-pair high-performance ion chromatography with UV-absorbing pairing ions, where samples gave positive or negative peaks, depending on their charge and retention relative to a UV-absorbing pairing ion. Frenzel et al. (1993) and Reidmann and Glatz (1994) reported that among these techniques, conductometric detection remains the mainstay of high performance ion chromatography.

Compared to non-chromatography techniques, ion chromatography methodology has the advantages of separation before detection, increased sensitivity, simple sample preparation and shorter analysis time [Dasgupta, (1992) and Romano & Krol (1992)]. It finds general application in power plants, semiconductors, detergents, medicine, food, agricultural, and more importantly in the environmental fields [Woods & Rowland (1997); Buldini, et al., (1997) and Fernandez-Boy, et al., (1998)]. Thus, it has been certified and adopted by the American Society for Testing and Materials (ASTM) and the US Environmental Protection Agency (EPA) (1991) in the USA, and Croatian standards HRN EN ISO 10304-3 [State Office for Standardation and Metrodology, Republic of Croatia (1997)] and HRN EN ISO 1030-4 [State Office for Standardation and Metrodology, Republic of Croatia (1997)].

In the present paper a non-suppressed chromatographic method with conductometric detection is described for the simultaneous determination of fluoride, chloride, bromide, nitrate, iodide and sulphate. The aim of this work is to improve performance characteristic of the method by using optimization of chromatographic parameters (eluent flow-rate, mobile phase) in order to be applied to the routine analysis of medicinal plants .

The validity of the method was established by determining the following validation parameters: precision, accuracy, linearity and detection limits .

2-EXPERIMENTAL

2-1 Apparatus:

The ion chromatography was carried out using H1C-6A type (Shimadzu, Japan) consisting of an LC- 10 AD liquid delivery pump, a DGU- 12 A Degasser, Rheodyne (77251) injection valve with a 20 μ l sample loop, CTO -10 AVP column oven, CDD-6A conductivity detector and SCL 10 AVP system controller. The anion exchange column (Shim pack 1C A1, 4.6 mm IDX 100 mm), was from Shimadzu. The column oven was maintained at 40 °C. Data acquisition and treatment were accomplished using a Shimadzu data system C-R7A chromatopac.

2-2-1 Reagents:

All the inorganic anions used in this study were of analytical reagents grade. All chemicals were purchased from BDH chemicals LTD poole England. N-[[[(ethylamino) thioxomethyl] hydrazinocarbonylmethyl] trimethy ammonium chloride (ETHTC) was prepared as reported earlier [Mostafa, unpublished results]. Double distilled deionized water was filtered through 0.2 μ m Whatman membrane. A stock solution of 1000 mg l⁻¹ was prepared for each inorganic anion.

All standard solutions, eluents and reagents were prepared in double distilled deionized water and filtered through 0.2 μ m Whatman membrane filter.

2-2-2- Sample Preparation:

Twenty grams each from the four samples under studies named *Mentha Viridis*, *Mentha Longifolia*, *Origanum Majorana* and *Cymbopogon Winterianus* was dissolved in 70ml of double distilled deionized water, the mixture was heated at different temperatures (60,70,80 and 90°C) for different times 5,10, 15, 20, 25 and 30 min. After cooling, it was filtered through a 0.2 μ m Whatman membrane filter and then the filtered solution was transferred to 100ml flask and double distilled deionized water was then added to the mark. This sample solution was injected into the ion chromatograph directly. Each sample was run ten times .

2-3- The Optimum Conditions :

The optimum analytical conditions have been established in order to separate six inorganic anions simultaneously using Shim pack IC A1 column with 2.45 mM phthalic acid and 2.35 mM tris (hydroxymethyl) aminomethane at pH 3.40; flow rate 1.5 ml/min and at 40°C. The data

obtained were then compared with the eluent solution containing mixture of 2.2 mM phthalic acid + (2.7mM tris + 0.2 mM ETHTC) at pH 3.70 and a flow rate 1.5 ml/min .

3- RESULTS AND DISCUSSION

3-1- Effect of phthalic acid concentration :

The effect of phthalic acid concentration in eluent on the retention volume (VR) of the inorganic anions was investigated . Fig.1 shows that the VR values of inorganic anions increase with increasing the acid concentration. The data indicate that the background of eluent concentration increases with increasing the concentration of phthalic acid. The optimum concentration was found to be 2.45mM.

3-2- The effect of flow rate on the retention time :

The effect of flow rate on retention time was carried out by applying different flow rate ranging from (0.9 to 1.6 ml/min) in order to achieve a good and fast separation, table 1 and figure 2 revealed that the resolution time decreased sharply by increasing the flow rate. Also, the results showed that the most efficient separation of inorganic anions under studies was obtained with flow rate of 1.5 ml/min and it gave more repeatability than other flow rate investigated. In addition, there is long retention time between the separation of the first four anions (F^- , Cl^- , Br^- and NO_3^-) and the last two anions (I^- , SO_4^{2-}). The solution of this problem will be discussed in section(5). In addition, figure 2 indicated that the separation after 1.5 ml/min will be difficult and overlapped for (F^- , Cl^- , Br^- and NO_3^-) except (I^- and SO_4^{2-})

3-3- The effect of eluent pH on the retention behavior of inorganic anions.

Different pH values were used in this study ranging from (2.82-4.84) in order to achieve the optimum resolution time. From table 2 and figure 3. It can be concluded that the retention volumes of inorganic anions decreased with increasing the pH of the eluent, this is due to an increase of eluent strength of phthalic acid by promoting the degree of dissociation (pK_{a1} 2.95 and pK_{a2} = 5.41 for phthalic acid), also the results indicate that the value of pH 3.40 gave a powerful separation for the species under studies, in addition the results revealed that the peaks overlap has been obtained by using pH above 3.4 and the overlap reaches its maximum at pH 4.84 more than that observed at pH 3.73.

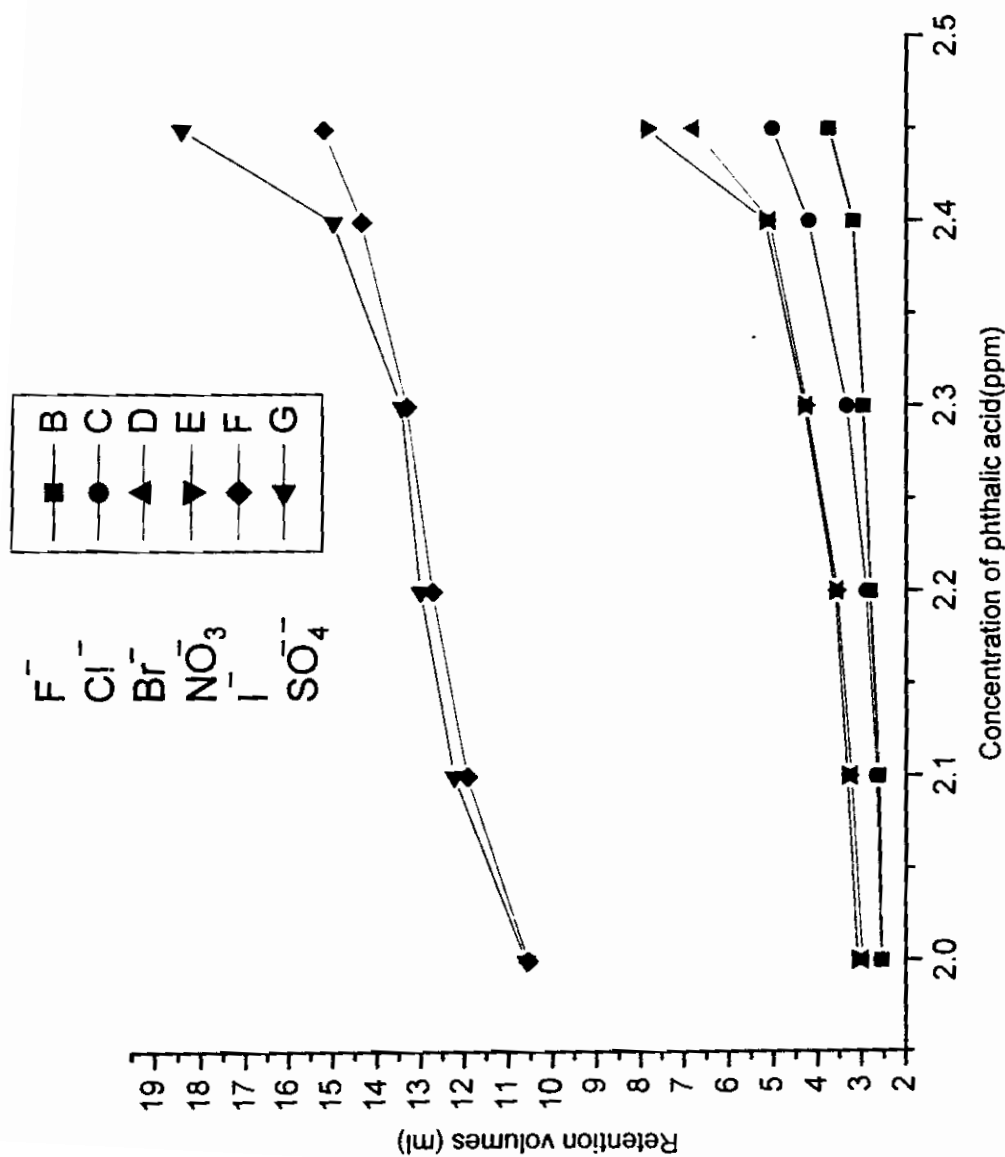


Fig. 1. Effect of phthalic acid concentration: in the eluent on the retention volumes of inorganic anions.

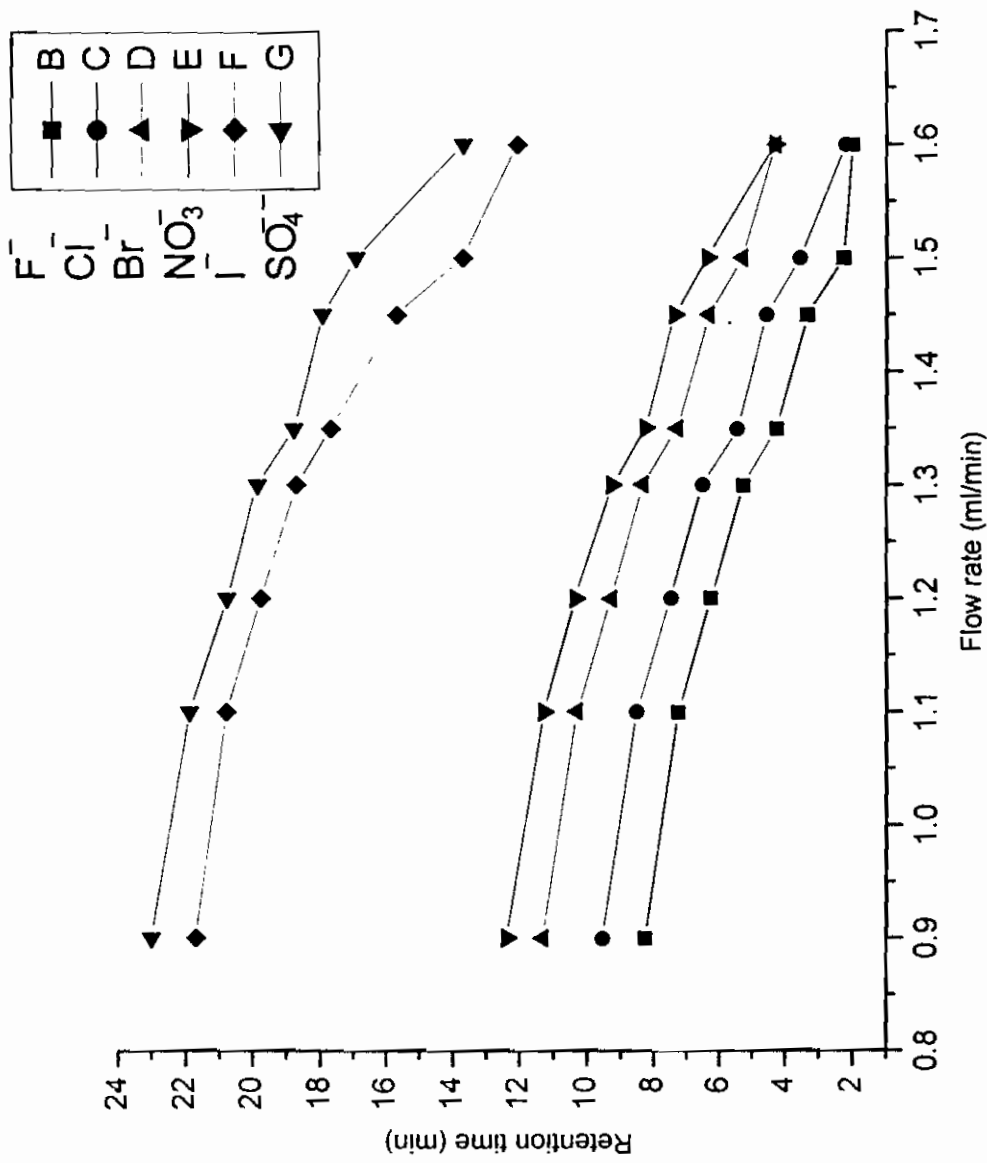


Fig. 2. The effect of flow rate on retention time of investigated inorganic anions.

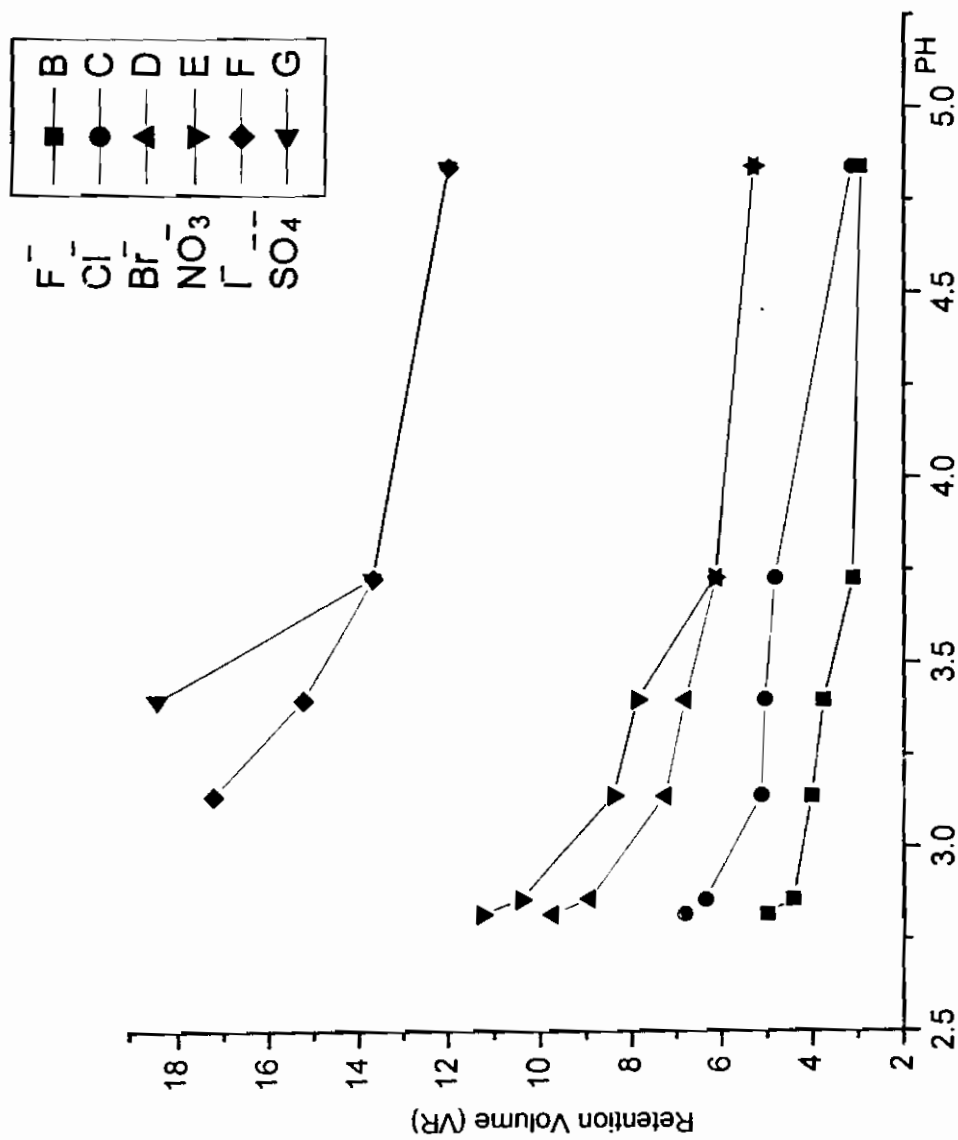


Fig.3.Effect of the eluent PH on retention behavior of inorganic anions.

Table . 1 . The effect of flow rate on retention time (t_R) of investigated inorganic anions.

Flow rate ml / min	F ⁻	Cl ⁻	Br ⁻	NO ₃ ⁻	I ⁻	SO ₄ ⁻²
0.9	8.265	9.549	11.321	12.388	21.721	22.997
1.1	7.276	8.54	10.33	11.298	20.799	21.899
1.2	6.266	7.501	9.299	10.388	19.77	20.799
1.3	5.263	6.532	8.368	9.288	18.724	19.889
1.35	4.259	5.455	7.316	8.299	17.702	18.796
1.45	3.336	4.565	6.319	7.387	15.699	17.939
1.5	2.265	3.549	5.313	6.387	13.721	16.929
1.6	2.011	2.21	4.301	4.32	12.098	13.698

Table . 2 .
The effect of the eluent pH on retention behavior (t_R) of investigated inorganic anions.

pH	F ⁻	Cl ⁻	Br ⁻	NO ₃ ⁻	I ⁻	SO ₄ ⁻²
2.82	4.974	6.813	9.721	11.251	--	--
2.86	4.415	6.351	8.932	10.421	--	--
3.14	4.022	5.121	7.257	8.412	17.221	--
3.4	3.765	5.049	6.813	7.887	15.221	18.429
3.73	3.115	4.826	6.122	6.16	13.674	13.701
4.84	2.952	3.151	5.316	5.299	11.988	12.021

Table . 3 .
The relation between concentration of phthalic acid and t_R at different temperatures.

°C	Concentration (mM)	t_R					
		F ⁻	Cl ⁻	Br ⁻	NO ₃ ⁻	I ⁻	SO ₄ ⁻
25°C	2	2.398	3.367	5.345	6.659	15.678	16.965
	2.1	2.678	4.589	6.786	8.298	17.345	18.569
	2.2	3.989	4.987	8.567	10.453	18.986	20.679
	2.3	4.989	5.897	10.221	12.432	20.598	22.369
	2.4	5.897	6.778	12.987	14.921	22.45	24.648
	2.45	6.968	8.854	14.659	16.998	24.998	26.978
35°C	2	1.923	1.998	3.397	4.212	13.345	14.223
	2.1	2.156	2.545	3.989	5.297	14.996	15.989
	2.2	2.459	2.996	4.989	7.099	16.985	17.789
	2.3	2.576	3.534	5.778	9.201	18.887	19.789
	2.4	2.598	4.297	7.889	11.768	19.998	21.765
	2.45	3.598	5.275	9.996	12.889	21.983	23.998
40°C	2	1.593	1.601	2.697	2.701	8.031	10.131
	2.1	1.698	1.705	3.453	3.503	9.345	11.366
	2.2	1.897	1.903	3.971	4.021	10.968	13.01
	2.3	1.995	2.103	4.562	4.61	11.801	13.999
	2.4	2.198	2.202	4.901	5.021	12.723	14.756
	2.45	2.265	3.549	5.313	6.387	13.721	16.929

3-4- The effect of column temperature and eluent strength on the retention time .

The effect of column temperature and eluent strength on the retention time are shown in figures (4a, 4b, and 4c) the results allotted in table (3). At all three temperatures (25, 35, 40 °C) the retention times of all the inorganic anions under studies increased as the eluent concentration increased from 2.0 to 2.45 mM phthalic acid .

The retention times at all six strength decreased when the column temperature increased from 25 to 40 °C. It can be seen from figures (4a, 4b, and 4c) that the resolution at concentration 2.45 mM of phthalic acid at 40°C gives the shortest retention time. The optimum condition proposed in this work enables us to reduce the whole resolution time for all six inorganic anions from 26.978 to 16.929 min.

3-5- The effect of ETHTC on retention time t_R and sensitivity:

The effect of adding ETHTC to the mobile phase on the retention time of inorganic anions under investigation, shows that the degree of resolution of inorganic anions is improved by adding ETHTC to the eluent mixture. Different concentrations of ETHTC (0.2 mM- 2.0 mM) were used. The results indicate that the efficiency of separation process is increased with decreasing the concentration of ETHTC, also the results revealed that the best concentration of phthalic acid and tris (hydroxymethyl) aminomethane in the presence of ETHTC found to be 2.2mM, 2.7 mM respectively. In addition the results indicate that the retention time sharply decreased when the ETHTC are contained in the composition of mobile phase. Figs. 5 and 6 pointed out that the retention time have been reduced from 16.929 to 11.880 min. The decrease in retention time for F^- and Cl^- was slightly decreased where for Br^- and NO_3^- was obvious, but the decrease for I^- and SO_4^{2-} where sharp especially for SO_4^{2-} (from 13.721 to 11.880 min for I^- and from 16.929 to 10.614 min for SO_4^{2-}).

In addition SO_4^{2-} was eluted after I^- , in the absence of ligand (ETHTC), while SO_4^{2-} was eluted faster than I^- , in the presence of ligand ETHTC the detection limits (S/N=3) for various inorganic anions are given in Table (4) the detection limits obtained by using the mixture of 2.2mM phthalic acid and [2.7mM (hydroxymethyl) aminomethane + 0.2mM ETHTC, pH 3.70] were several times lower than those obtained by 2.45mM phthalic acid + 2.35mM tris (hydroxymethyl) aminomethane pH 3.40. Also, the data show that the conductivity of the mobile phase

containing 2.2mM phthalic acid + mixture of [2.7 mM tris (hydroxymethyl) aminomethane + 0.2mM ETHTC] is 101.7 $\mu\text{s}/\text{cm}$ while the conductivity of 2.45 mM phthalic acid+2.35mM tris (hydroxymethyl) aminomethane is 107.8 $\mu\text{s}/\text{cm}$. These results mean that the presence of ligand (ETHTC) in the composition of mobile phase decreases the background conductivity which leads to increase in detection sensitivity, Figure.7. reveals the sensitivity of F^- before and after adding ETHTC .

Table (4) shows the calibration graph of peak areas for all analytes are linear, with regression coefficient (r^2) of (0.9970-0.9997) .

3-6- Application :

The results (peak area) of each analyte are different at the different temperatures (60,70,80 and 90°C) for the six heating times (5, 10, 15, 20, 20, 25 and 30 minutes) with relative standard deviation (R.S.D) below 2%. Table (5) shows that the amount of extraction increases with increasing the temperature and also the time of heating. According to the results it can be concluded that the most suitable temperature and the heating time is 90°C and 30 min. In addition, the results indicated that all samples under investigation contain only five ions (F^- , Cl^- , NO_3^- , I^- , and SO_4^{2-}) and the absence of Br^- ion.

Table .4 .
 Detection limit (S/N=3), linear range of inorganic anions and regression coefficient.

Compound	Detection limit (mg / L)		Linear Range(mg/L)	(r ²)
	2.45mM Phthalic acid + 2.35mM tris	2.2mM Phthalic acid+ (2.7mM tris + 0.2mM L)		
F ⁻	0.11	0.003	0.5-2500	0.9970
Cl ⁻	0.01	0.0022	0.5-2500	0.9990
Br ⁻	0.10	0.0022	0.5-2700	0.9990
NO ₃ ⁻	0.01	0.0022	0.5-2000	0.9990
I ⁻	0.12	0.003	0.5-2500	0.9997
SO ₄ ⁻	0.11	0.0035	0.5-5000	0.9997

Tris: (hydroxymethyl) aminomethane
 L : ETHTC

Table . 5 .
The concentration of inorganic anions in the medicinal plants at different temperatures(ppm).

Temp.	Sample	F ⁻				Cl ⁻				Br ⁻				NO ₃ ⁻			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
60	5	a	47.3	10.2	55.3	ND	3517	851	3087	327.8	ND	ND	ND	40.7	715	1617	12.8
		b	0.39	0.69	0.37	ND	1.98	1.87	1.90	1.93	ND	ND	ND	0.96	0.83	0.79	0.97
	10	a	49.6	11.3	58.6	ND	3652	879	3118	332.5	ND	ND	ND	53.8	822	1745	19.2
		b	0.37	0.67	0.36	ND	1.91	1.85	1.86	1.90	ND	ND	ND	0.93	0.81	0.75	0.95
	15	a	51.8	12.7	60.7	ND	3722	922	3175	334.4	ND	ND	ND	77.6	945	1833	23.2
		b	0.37	0.66	0.35	ND	1.88	1.86	1.85	1.87	ND	ND	ND	0.90	0.77	0.72	0.92
	20	a	53.2	13.5	62.6	ND	3844	973	3227	338.6	ND	ND	ND	83.3	1002	1924	29.2
		b	0.35	0.64	0.34	ND	1.85	1.86	1.83	1.85	ND	ND	ND	0.86	0.74	0.68	0.90
25	a	55.4	14.6	64.7	ND	3935	1001	3266	340.8	ND	ND	ND	91.5	1061	2039	34.2	
	b	0.34	0.62	0.31	ND	1.82	1.81	1.81	1.83	ND	ND	ND	0.85	0.71	0.63	0.87	
30	a	57.7	15.4	68.5	ND	4073	1039	3312	346.2	ND	ND	ND	105	1125	2111	36.5	
	b	0.32	0.61	0.31	ND	1.80	1.79	1.78	1.79	ND	ND	ND	0.83	0.69	0.61	0.84	
70	5	a	69.4	17.7	78.7	ND	4185	1146	3448	349.3	ND	ND	ND	174	1212	2217	75.2
		b	0.29	0.58	0.30	ND	1.79	1.77	1.75	1.76	ND	ND	ND	0.76	0.61	0.54	0.80
	10	a	72.3	18.6	80.2	ND	4298	1189	3511	350.8	ND	ND	ND	204	1242	2331	79.5
		b	0.27	0.56	0.28	ND	1.73	1.70	1.69	1.70	ND	ND	ND	0.71	0.58	0.47	0.72
	15	a	74.7	19.5	81.8	ND	4362	1219	3568	353.7	ND	ND	ND	262	1284	2427	81.3
		b	0.27	0.55	0.28	ND	1.70	1.67	1.65	1.67	ND	ND	ND	0.65	0.51	0.40	0.67
	20	a	76.6	20.8	83.6	ND	4410	1254	3611	356.2	ND	ND	ND	302	1304	2545	87.4
		b	0.25	0.54	0.27	ND	1.67	1.63	1.61	1.65	ND	ND	ND	0.60	0.45	0.37	0.62
25	a	77.2	21.7	85.2	8.3	4476	1282	3655	358.1	ND	ND	ND	361	1358	2630	91.3	
	b	0.23	0.53	0.27	0.85	1.63	1.60	1.58	1.59	ND	ND	ND	0.51	0.40	0.31	0.55	
30	a	79.7	22.3	86.7	9.7	4532	1302	3728	359.4	ND	ND	ND	432	1422	2705	96.8	
	b	0.22	0.52	0.26	0.83	1.60	1.57	1.53	1.55	ND	ND	ND	0.49	0.38	0.29	0.50	

Samples: 1 (*Mentha Viridis*) 2 (*Mentha Longifolia*) 3 (*Oreganum Majorana*) 4 (*Cymbopogon Winterianus*)

Table (5) Contd.

Temp. °C	Time/min	Area %	F ⁻				Cl ⁻				Br ⁻				NO ₃ ⁻			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
80	5	a	85.7	24.8	69.5	11.5	4612	1377	3975	362.4	ND	ND	574	1566	2803	174.3		
		b	0.20	0.50	0.24	0.81	1.57	1.50	1.47	1.48	ND	ND	0.45	0.34	0.27	0.47		
	10	a	86.2	24.7	91.7	12.4	4677	1409	4049	365.3	ND	ND	669	1624	2864	179.5		
		b	0.19	0.49	0.20	0.79	1.55	1.48	1.45	1.46	ND	ND	0.41	0.30	0.25	0.43		
	15	a	87.3	26.3	94.8	13.5	4721	1441	4113	367.1	ND	ND	735	1669	2976	181.1		
		b	0.17	0.46	0.17	0.76	1.53	1.46	1.41	1.43	ND	ND	0.37	0.28	0.24	0.40		
	20	a	88.6	27.4	97.3	14.3	4785	1488	4199	369.6	ND	ND	801	1736	3021	184.8		
		b	0.15	0.44	0.17	0.75	1.50	1.44	1.38	1.39	ND	ND	0.34	0.26	0.20	0.38		
	25	a	89.7	28.2	99.8	15.4	4811	1513	4251	372.8	ND	ND	887	1788	3155	187.5		
		b	0.15	0.42	0.15	0.75	1.48	1.41	1.34	1.36	ND	ND	0.30	0.23	0.19	0.34		
	30	a	90.4	29.7	102.5	16.6	4899	1549	4332	374.2	ND	ND	954	1819	3217	189.3		
		b	0.14	0.41	0.14	0.73	1.46	1.39	1.30	1.33	ND	ND	0.28	0.20	0.18	0.30		
80	5	A	92.4	30.1	108.1	18.6	5078	1622	4452	377.6	ND	ND	1098	1872	3371	293.7		
		B	0.12	0.39	0.11	0.69	1.43	1.36	1.27	1.29	ND	ND	0.21	0.18	0.17	0.29		
	10	A	93.0	30.7	109.8	19.4	5188	1665	4511	379.4	ND	ND	1175	1906	3482	295.4		
		b	0.12	0.37	0.11	0.67	1.40	1.33	1.25	1.26	ND	ND	0.19	0.16	0.15	0.25		
	15	A	93.5	31.8	110.3	20.7	5297	1712	4572	381.2	ND	ND	1209	1966	3554	297.5		
		b	0.11	0.36	0.10	0.67	1.38	1.30	1.22	1.24	ND	ND	0.17	0.13	0.14	0.21		
	20	A	94	32.6	112.9	21.8	5324	1776	4689	382.6	ND	ND	1297	2016	3662	299.4		
		b	0.11	0.33	0.10	0.63	1.36	1.28	1.20	1.21	ND	ND	0.15	0.12	0.13	0.18		
	25	A	95	33.2	115.3	22.6	5490	1823	4766	384.7	ND	ND	1378	2073	3754	300.3		
		b	0.10	0.30	0.10	0.61	1.33	1.25	1.18	1.19	ND	ND	0.13	0.11	0.12	0.15		
	30	A	97	34	117	23.4	5531	1886	4837	386.3	ND	ND	1413	2140	3839	302.6		
		b	0.10	0.29	0.10	0.58	1.30	1.23	1.16	1.17	ND	ND	0.11	0.10	0.11	0.13		

Table (5) Contd:

Temp. C°	Time/ min	Mean and RSD %	SO ₄ ²⁻				I ⁻				T _{cm} P. C°	SO ₄ ²⁻				I ⁻			
			1	2	3	4	1	2	3	4		1	2	3	4	1	2	3	4
50	5	a	429	121	3612	584	14.3	62.3	44.1	57.6	80	1216	975	4518	1277	28.8	275	106.3	111.7
		b	0.33	0.61	0.28	0.31	0.81	0.63	0.78	0.66		0.17	0.25	0.15	0.17	0.60	0.39	0.52	0.46
	10	a	537	163	3738	677	15.4	69.9	51.4	69.4		1262	1031	4597	1321	29.6	307	109.7	113.2
		b	0.33	0.57	0.27	0.29	0.79	0.60	0.77	0.65		0.16	0.24	0.15	0.17	0.59	0.38	0.49	0.44
	15	a	629	296	3821	729	16.8	73.8	62.5	73.5		1315	1085	4661	1373	30.8	349	111.4	115.4
		b	0.29	0.55	0.26	0.28	0.77	0.58	0.75	0.63		0.16	0.22	0.14	0.16	0.55	0.38	0.47	0.43
	20	a	725	342	3887	768	17.3	7.55	71.4	79.9		1366	1135	4714	1423	31.7	385	113.8	117.6
		b	0.27	0.51	0.25	0.27	0.75	0.56	0.73	0.61		0.15	0.20	0.14	0.16	0.53	0.37	0.45	0.42
25	a	763	395	3948	828	18.1	89.4	81.7	84.8	1437	1177	4772	1488	32.8	422	115.4	119.8		
	b	0.26	0.48	0.24	0.26	0.73	0.53	0.72	0.59	0.14	0.19	0.13	0.15	0.51	0.37	0.43	0.41		
30	a	818	433	4016	878	19.4	94.3	83.3	86.2	1492	1225	4816	1528	33.1	473	118.3	121.6		
	b	0.25	0.44	0.22	0.25	0.72	0.51	0.70	0.57	0.13	0.17	0.12	0.15	0.50	0.35	0.40	0.40		
90	5	a	917	580	4155	935	21.5	99.5	87.5	89.7	1576	1313	4921	1587	34.9	524	121.8	124.3	
		b	0.24	0.40	0.20	0.24	0.70	0.49	0.69	0.55	0.12	0.15	0.11	0.14	0.49	0.34	0.39	0.39	
	10	a	954	619	4207	984	22.7	113	91.6	93.2	1617	1373	4979	1638	35.7	552	122.7	125.6	
		b	0.23	0.39	0.19	0.23	0.68	0.47	0.67	0.53	0.12	0.13	0.11	0.14	0.47	0.33	0.38	0.38	
	15	a	1019	663	4275	1038	23.9	141	93.8	96.6	1685	1414	5013	1697	36.1	584	123.9	127.7	
		b	0.22	0.37	0.19	0.22	0.67	0.46	0.63	0.52	0.11	0.12	0.10	0.13	0.45	0.32	0.37	0.37	
	20	a	1073	731	4318	1097	24.6	156	95.7	97.5	1706	1474	5058	1722	36.8	604	125.1	129.8	
		b	0.20	0.32	0.18	0.20	0.65	0.43	0.60	0.51	0.11	0.12	0.10	0.13	0.43	0.30	0.35	0.35	
25	a	1112	772	4379	1134	25.3	179	97.2	99.7	1765	1523	5117	1774	37.3	639	126.6	131.2		
	b	0.19	0.29	0.18	0.19	0.63	0.42	0.57	0.49	0.10	0.11	0.11	0.12	0.42	0.29	0.33	0.34		
30	a	1145	823	4421	1186	26.2	214	99.4	101.7	1820	1595	5180	1863	38.5	670	128.1	134.8		
	b	0.18	0.27	0.17	0.19	0.62	0.40	0.54	0.48	0.10	0.11	0.10	0.11	0.40	0.27	0.32	0.33		

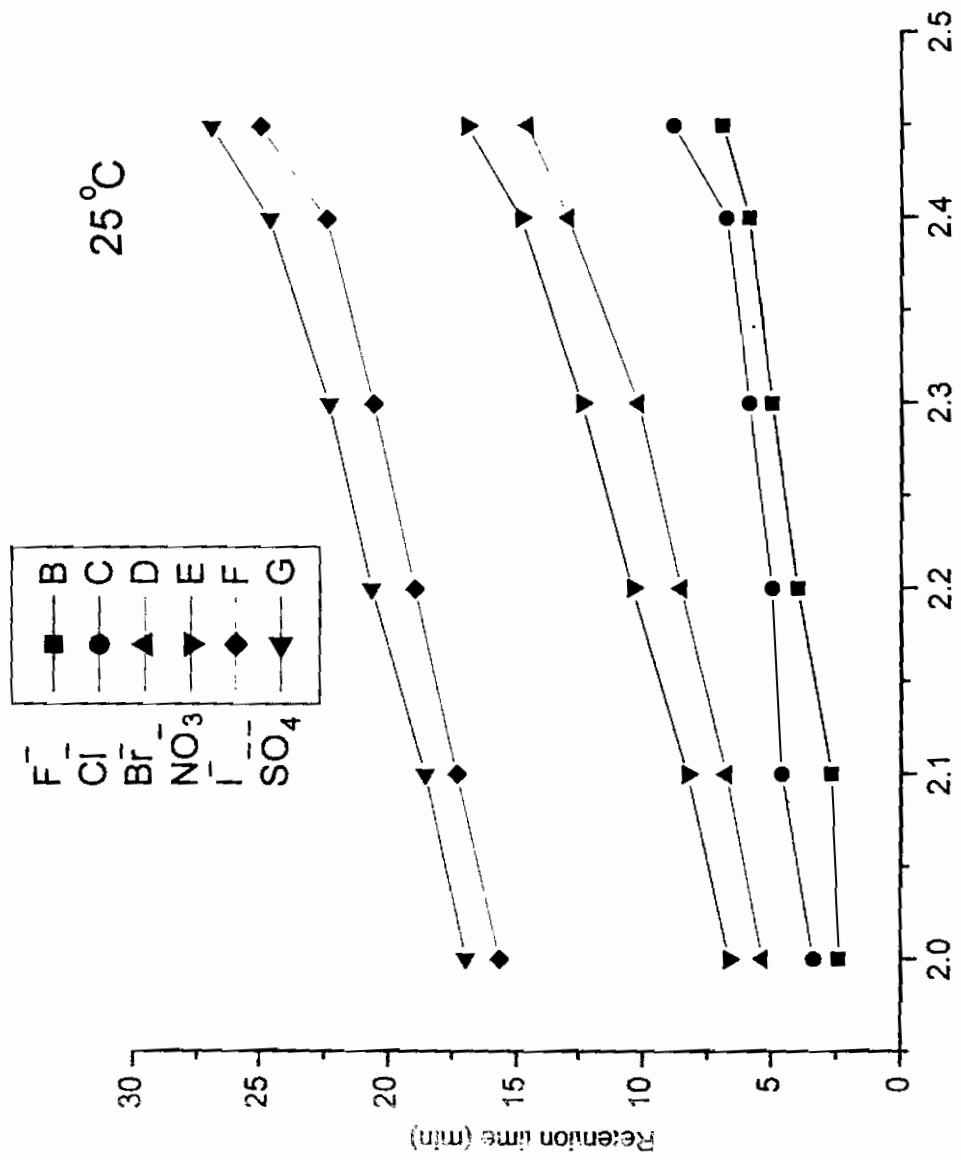


Fig.4a. The effect of column temperature and eluent strength on the retention time of inorganic anions .

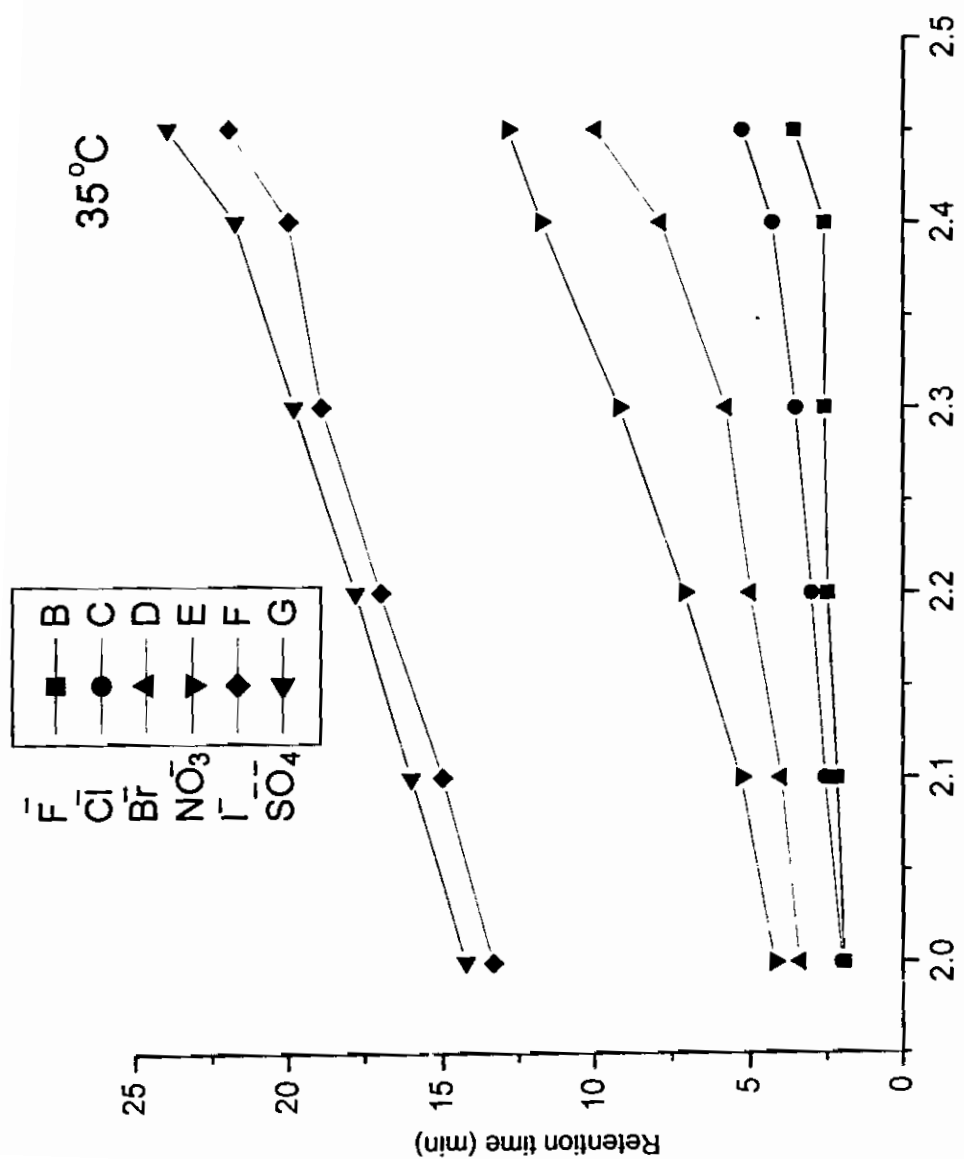


Fig.4b. The effect of column temperature and eluent strength on the retention time of inorganic anions .

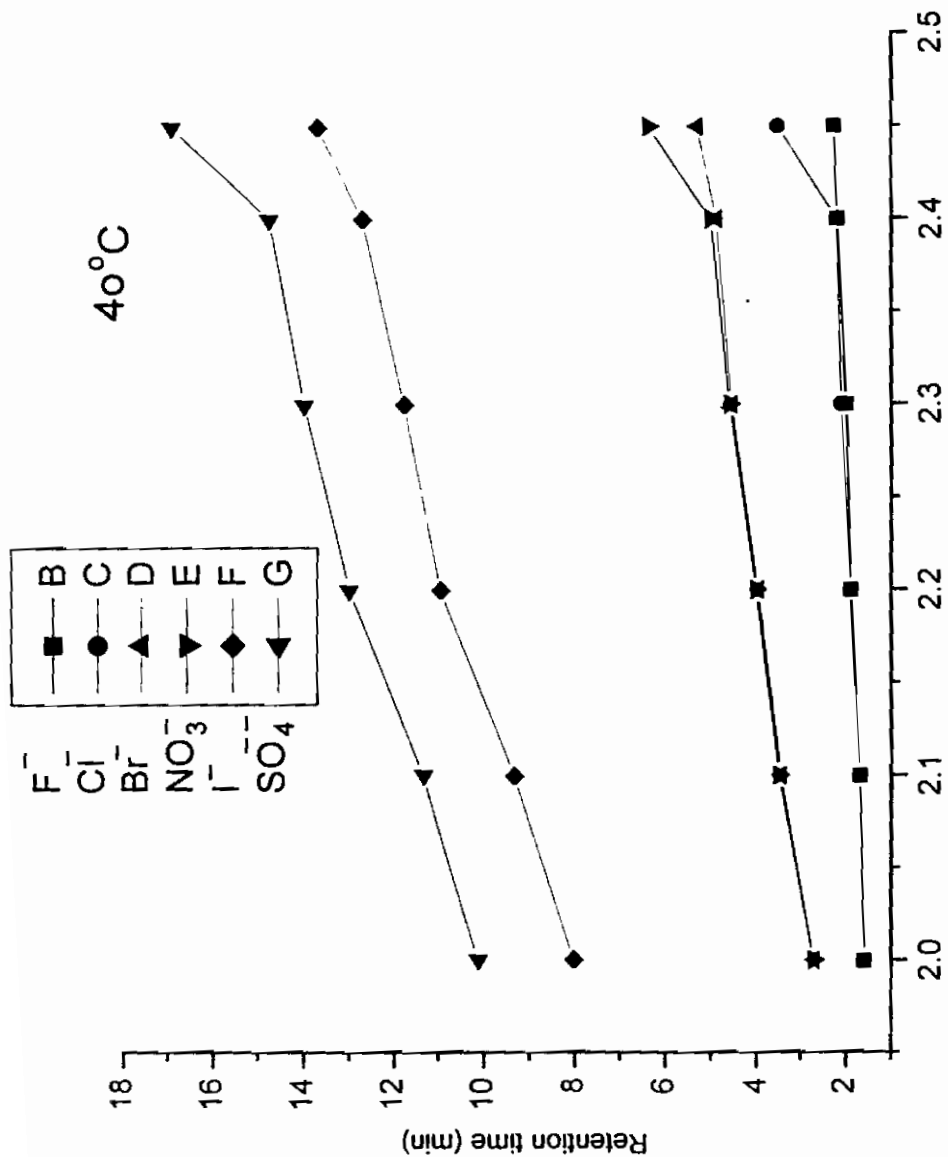


Fig.4c. The effect of column temperature and eluent strength on the retention time of inorganic anions.

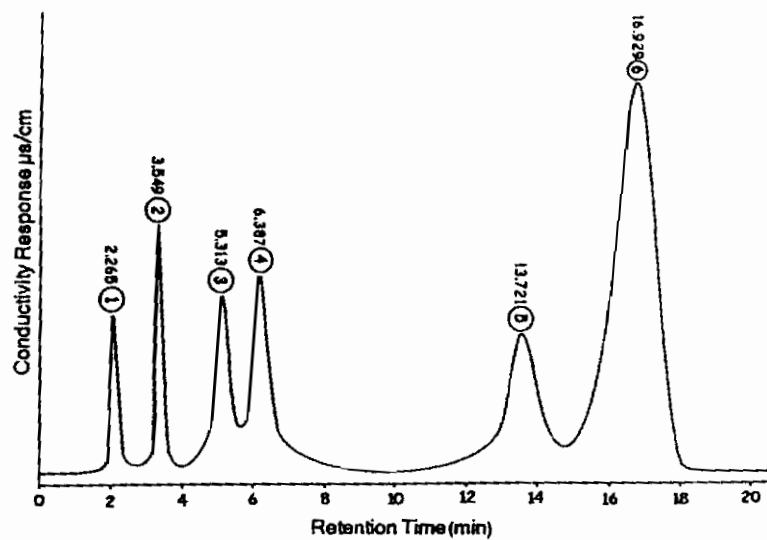


Fig .5. Typical resolution for a mixture of inorganic anions. column , shim – pack IC A1, temperature 40°C , eluent mixture of 2.45 mM phthalic acid and 2.35 mM tris (hydroxymethyl) aminomethane (pH 3.40) ; flow rate 1.5ml/min .
Peaks;1= F⁻ ;2 = Cl⁻ ;3 = Br⁻ ;4= NO₃⁻ ;5 = I⁻ ;6 = SO₄⁻.

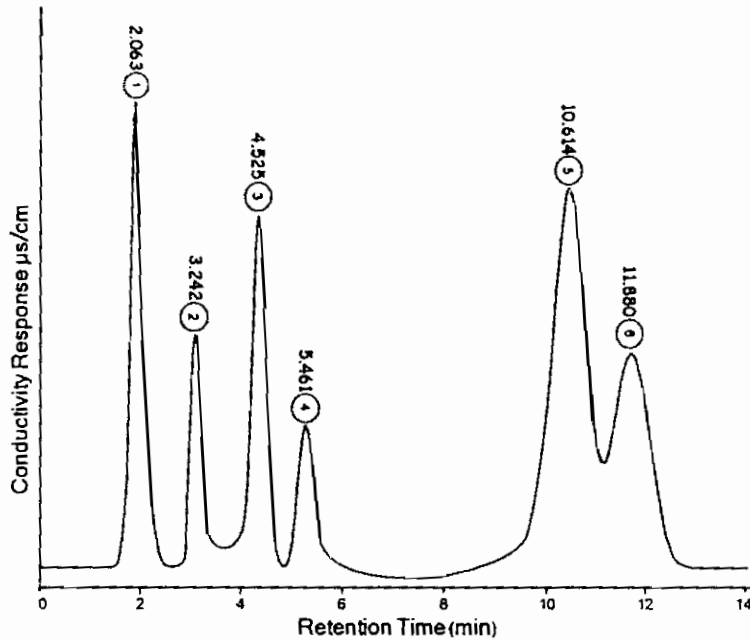


Fig .6. Typical resolution for a mixture of inorganic anions. column , shim – pack IC A1, temperature 40°C , eluent mixture of 2.2 mM phthalic acid and 2.7 mM tris (hydroxymethyl) aminomethane + 0.2mM N{[(ethylamino) thioxomethyl] hydrazinocarbonylmethyl} trimethyl ammonium chloride (pH 3.70) . flow rate 1.5ml / min . peaks ; 1 = F⁻ ; 2 = Cl⁻ ; 3 = Br⁻ ; 4 = NO₃⁻ ; 5 = SO₄⁻ ; 6 = I⁻ .

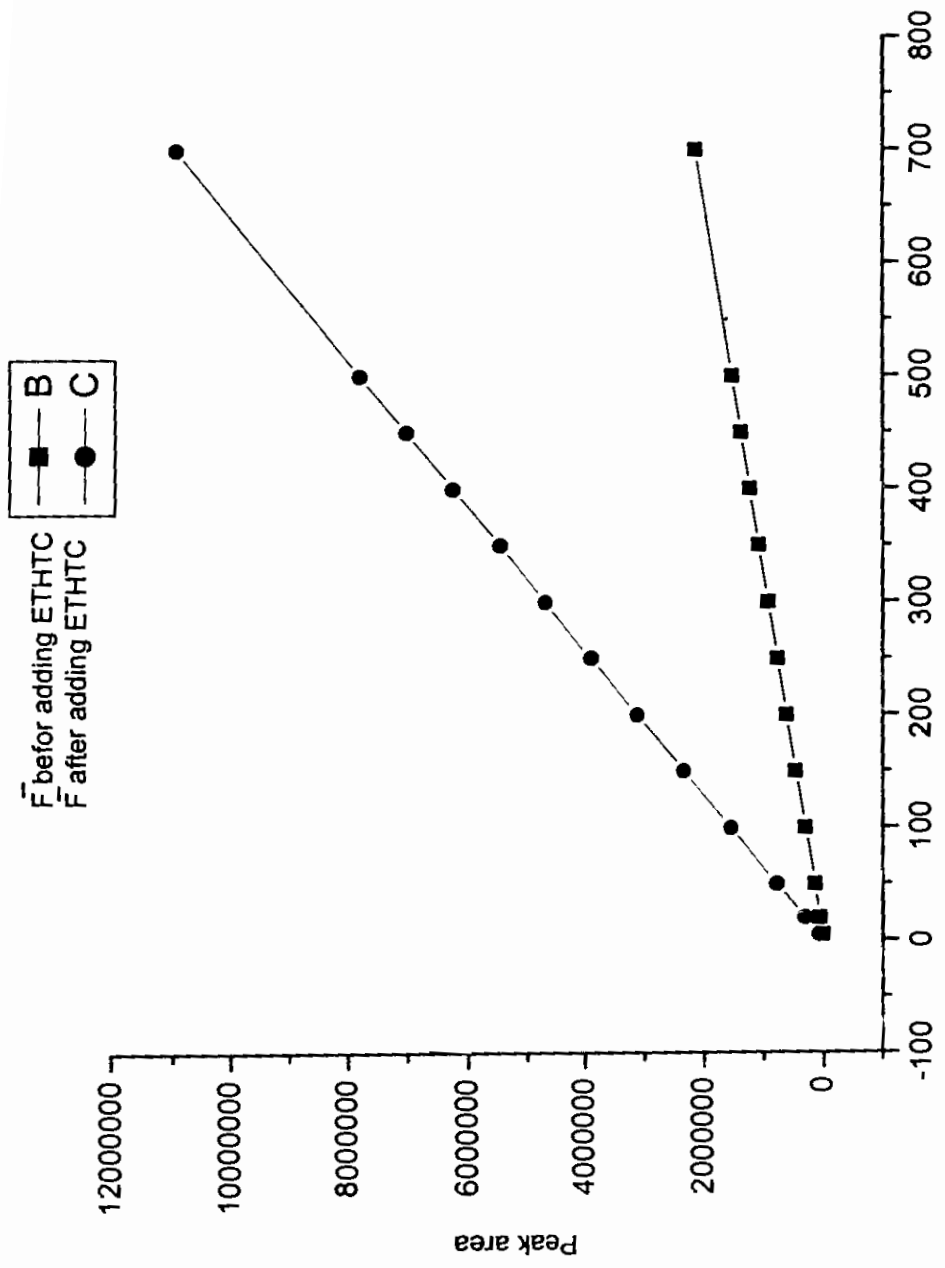


Fig.7. The relation between concentration and peak area (sensitivity) for F⁻.

4-CONCLUSION

The applicability of the proposed method for determination of inorganic anions using non suppressed ion chromatography has been established. The method has been fully optimized to determine simultaneously six inorganic anions in some medicinal plants but could be applicable to different species. The optimal conditions for separating inorganic anions by using 2.45 mM phthalic acid, 2.35mM tris (hydroxymethyl) aminomethane, 40°C, flow rate 1.5 ml/min and pH 3.40 were found to be favorable. The selection of this conditions enables us to reduce the whole resolution time (from 26.978 to 16.929 min). In order to increase the sensitivity and decrease the analysis time the previous conditions mentioned above have been developed, the 2.2mM phthalic acid+mixture of [2.7 mM tris (hydroxymethyl) aminomethane + 0.2mM ETHTC] at pH 3.70 was adopted. This new eluent gives more advantages, excellent results, and less quantities of tris (hydroxymethyl) aminomethane, and phthalic acid in comparison to that mentioned above in absence of ETHTC. On using this ligand ETHTC a typical analysis was completed in less than 12 min.

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Separation-Determination-Inorganic Anions-Ion Chromatography 109

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الملخص العربي

تم تطوير طريقة تعتمد على كروماتوجرافيا الأيونات تتيح التعيين المتزامن لأكثر الأيونات غير العضوية شيوعاً (فلوريد ، كلوريد ، بروميد ، نترات ، أيوديد وكبريتات).
تم الفصل على عمود تبادل أنيوني باستخدام طور متحرك مناسب ومعدل إنسياب = ١٥ مل / دقيقة عند درجة ٤٠ م . ويمكن استخدام هذه الطريقة في التحاليل الروتينية وفي المراكز الطبية وقد تم التأكد من صلاحيتها من خلال تعيين المقاييس الآتية .. الدقة - الحساسية - الإنضباط الكمي - حدود التعيين الدقيق.