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Improved Method for the Determination of Inorganic Anions in Roselle (*Hibiscus sabdariffa* L.) by Suppressed Ion Chromatography

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ABSTRACT

A smpile, rapid and accurate method for the determination of main mono and divalent inorganic anions in Roselle (*Hibiscus sabdariffa* L.) has been developed. The quantitative determination of anions F^-, Cl^-, NO^-_2, Br^-,NO^-_3, HPO^{2-}_4,SO^{2-}_4, and I^- was accomplished by suppressed conductimetric ion chromatography (IC).

The separation is achieved on shim-pack IC-SA_2 250 mmL X 4.0 mm ID Peak. column operated at 30°C within 12.21 minutes by isocratic elution with 1.8 mM potassium carbonate, 12 mM Potassium bicarbonate and 0.0125mM of N- {[(ethylamino) thioxomethyl] hydrazine carbonlmethyl} trimethyl ammonium chloride (ETHTC) and pH 9.25 as eluent at flow rate of 1ml/min. The method does not need a special sample treatment.

Key words: Optimization, determination, inorganic anions, ETHTC, Roselle (*Hibiscus sabdariffa* L) suppressed ion chromatography.

INTRODUCTION

Ion chromatography (IC) is one of the simplest and most effective techniques to determine both anions and cations owing to its high sensitivity, rapidity and ease of operation[1-3].

The presence of ligand in the composition of mobile phase and its effect on the resolution has been reported by several authors[4-14].

It has been reported that the inclusion of EDTA in the mobile phase yields increased resolution and peak symmetry[10].

The addition of 18-crown-6 to an eluent has been known to be very effective in improving peak resolution between monovalent cations[9,15,16]. This behavior can be explained by stability constant of complexation of alkali metal ions with 18-crown – 6 absorbed on cation-exchange resion of separation column[17].

Also the use of ligand assist too much in solving the problem of overlapping of some inorganic anions and organic acids[4,13,14]. Moreover the presence of ligand in composition of mobile phase increase the sensitivity detection of the separated ions as well as reducing the retention time of the separated ions[11-14].

The first objective of the present work to contribute more information about the effects of added N- {[(ethylamino) thioxomethyl] hydrazine carbonylmethyl} trimethy ammonium chloride (ETHTC) on the optimization, sensitivity and detection limits of some inorganic anions by suppressed ion chromatography determination and their separation in the presence of ETHTC. Roselle (*Hibiscus sabdariffa* L.), an annual shrub, is commonly used to make jellies, jams, and beverages. The brilliant red color and unique flavor make it a valuable food product.

Nowadays, a great interest exists in the crop of Roselle due to the high antioxidant properties of the flowers calyxes. Which have been extensively evaluated[18-22]. Also the nutritional composition (proximate composition, amino acids, fibre and minerals) of Roselle has been reported[23]. Because of the importance of Roselle it was planned to study and contribute more information on the simultaneous determination of inorganic anions in Roselle as the second objective since no attention has been paid for the separation and determination of inorganic ions in Roselle. (*Hibiscus sabdariffa* L.)

EXPERIMENTAL

Apparatus

In this study analysis and data collection were performed by LC Solution software using a HIC-20A SUPER ion chromatograph purchased from shimadzu consisting of an LC-20 AD_{sp} liquid delivery pump, a DGU-20 A, Degasser, Rheodyne (77251) injection valve with a 20 mL sample loop, CTO-20 AC_{sp} column oven, CDD-10 A_{sp} conductivity detector, and SCL - 10 A_{sp} system controller. The anion exchange column (Shim-pack IC-SA 2-250 mmL X 4.0 mm ID PEEK). The column oven was maintained at 30°C.

2-2 Reagents:

All the inorganic anions in this study were of analytical reagents and were purchased from BDH chemical poole England.

N- {[(ethylamino) thioxomethyl] hydrazine carbonylmethyl} trimethyl ammonium chloride (ETHTC) was prepared as reported earlier[27]. Double distilled deionized water was filtered through 0.2mm whatman membrane. All standard solution eluents and reagents were prepared in double distilled deionzed water and filtered through 0.2mm whatman membrane filter.

Sample preparation

20g of Roselle (*Hibiscus sabdariffa* L.) was plased in a flask containing 70 ml double distilled dionized water. The mixture was heated at different temperature (60, 70, 80 and 90°C) for 5, 10, 15, 20, 25 and 30 minutes. After cooling the solution was filtered through 0.2mm whatman membrane filter and then the filtered solution was transferred to 100 ml flask and double distilled deionized water added to the mark. This sample solution was injected into the ion chromatograph. This sample was run ten times.

The optimum conditions

The optimum analytical conditions have been established in this method in order to separate, and determine eight inorganic anion simultaneously using isocratic method with 1.8 mM potassium carbonate and 12 mM potassium bicarbonate at pH 9.24, flow rate 1ml/min and at 30°C the data obtained was compared with the eluent solution containing 0.0125 mM ETHTC in addition to the above mentioned eluent at pH 9.25 and flow rate 1 ml/min.

RESULTS AND DISCUSSION

Effect of concentration of potassium carbonate on retention volumes VR of mono and divalent anions.

The effect of concentration of potassium carbonate in the eluent on the retention volumes of mono and divalent anion F^-, Cl^-, NO^-_2, Br^-,NO^-_3, HPO^{2-}_4,SO^{2-}_4, and I^- was investigated for the simultaneous separation of these anions.

Fig 1: Shows the relationship between the concentration of potassium carbonate in the eluent and the retention volumes of these mono and divalent anions. From fig 1 it can be concluded that the retention volumes of these anion decreased drastically for Br^-, NO^-_3, HPO^{2-}_4,SO^{2-}_4, and I^- while it is slightly decrased for F^-, Cl^- and NO^-_2, with increasing the concentration of potassium carbonate, also the shortest retention volumes was obtained with the concentration of 1.75 mM potassium carbonate for

Table 1: The influence of flow rate on the retention of analytes

Flow rate	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	$HPO_4^{—}$	$SO_4^{—}$	I^-
0.45	6.572	7.284	9.021	10.451	13.879	14.896	17.141	20.468
0.65	5.594	6.014	7.986	8.895	11.917	13.965	14.693	17.883
0.85	4.372	5.01	6.859	7.685	8.893	10.112	11.601	15.764
0.9	4.103	4.628	6.697	7.232	8.399	8.812	10.659	15.102
1	3.122	4.444	5.181	6.116	6.755	6.755	8.409	14.373

Table 2 : The effect of the eluent pH on retention behavior of anions

pH	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	$HPO_4^{—}$	$SO_4^{—}$	I^-
8.99	3.207	6.451	10.171	15.723	19.101	21.457	23.702	29.273
9.04	3.191	5.927	9.133	13.921	16.829	18.219	19.976	26.478
9.07	3.137	5.939	7.927	12.107	13.672	15.217	17.227	24.213
9.14	3.162	5.307	6.968	10.531	11.623	12.829	15.141	21.237
9.18	3.146	4.912	6.207	8.731	9.378	10.712	12.211	18.131
9.21	3.129	4.523	5.373	6.769	7.526	8.216	9.321	15.568
9.24	3.122	4.444	5.181	6.116	6.755	6.755	8.409	14.373

Table 3 : The influence of column temperature.

Temp °C	Conc. (mM)	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	$HPO_4^{—}$	$SO_4^{—}$	I^-
25	0.75	5.301	6.322	6.921	8.012	9.123	10.201	11.251	15.321
	0.95	5.365	6.401	7.011	8.271	9.237	10.272	11.323	15.468
	1.15	5.372	6.462	7.112	8.372	9.371	10.362	11.401	15.598
	1.35	5.415	6.484	7.213	8.481	9.472	10.478	11.521	15.701
	1.55	5.502	6.524	7.322	8.571	9.592	10.512	11.626	15.792
	1.75	5.612	6.631	7.417	8.678	9.674	10.633	11.701	15.837
	1.8	5.736	6.747	7.498	8.798	9.877	10.723	11.812	15.992
28	0.75	4.123	5.241	6.101	7.123	7.311	8.212	9.332	14.121
	0.95	4.234	5.251	6.21	7.214	7.456	8.345	9.452	14.229
	1.15	4.341	5.341	6.321	7.345	7.566	8.461	9.562	14.391
	1.35	4.456	5.421	6.455	7.501	7.639	8.581	9.675	14.521
	1.55	4.569	5.501	6.567	7.599	7.787	8.672	9.821	14.639
	1.75	4.691	5.592	6.612	7.627	7.824	8.778	9.989	14.781
	1.8	4.821	5.624	6.701	7.798	7.978	8.99	10.012	14.901
30	0.75	2.611	3.43	4.498	5.211	5.421	6.123	7.541	12.323
	0.95	2.741	3.601	4.527	5.498	5.632	6.341	7.797	12.421
	1.15	2.872	3.71	4.622	5.643	5.736	6.47	7.846	12.556
	1.35	2.86	3.73	4.737	5.747	6.102	6.561	7.958	12.675
	1.55	2.901	4.198	4.877	5.832	6.472	6.631	8.135	13.021
	1.75	2.998	4.236	4.901	5.889	6.552	6.651	8.216	13.521
	1.8	3.122	4.444	5.181	6.116	6.755	6.755	8.409	14.373

the all anions under investigation, in addition the optimum concentration was found to be 1.8 mM potassium carbonate and overlapped for NO^-_3 and HPO^{2-}_4 appeared at the concentration of 1.8 mM potassium carbonate. The solution of this overlap will be discuss in the ETHTC effect section.

The effect of flow rate of eluent on retention behavior of mono and divalent anions

Several flow rate values ranging from (0.45 -1 ml/min) were used in this study in order to achieve optimum resolution time. Table 1 and fig 2 indicate that the retention volumes decrease with increasing the flow rate, Also the shortest retention volume was obtained with the flow rate 0.9 ml/min for all the ions under studies, moreover the results revealed that the excellent separation of inorganic anions under investigation obtained by using flow rate 1 ml/min for F^- , Cl^- , NO^-_2 , Br^-, SO^{2-}_4 and I^- and it gave more repeatability than other flow rate studied, in addition the separation at flow rate 1 ml/min revealed that there are overlap between NO^-_3 and HPO^{2-}_4 ,section 3.5 will discuss the solution of this problem.

Effect of pH of eluent on retention behavior of mono and divalent anions

In this study different pH values were examined ranging from (8.99 – 9.24) In order to get a good and fast separation. Fig 3 and table 2 shows the relationship between the pH of eluent and the retention volumes of the mono and divalent anions. The data obtained indicates that the retention volumes of the anions under investigation decreased with increasing the pH of the eluent. Also the results revealed that the most efficient separation of inorganic anions under studies (except NO^-_3 and HPO^{2-}_4) was obtained with the pH 9.24. The solution of the overlap between NO^-_3 and HPO^{2-}_4 will be discuss in section 3.5.

The effect of column temperature

The effects of column temperature and eluent strength on the retention volumes of analytes are shown in fig 4a, 4b and 4c. The results are allotted in table 3. At all three temperature, the retention volumes of all the inorganic anions increase with increasing the eluent concentration from 0.75 up to 1.8 mM. The magnitude of the increases was in the following descending order: I^-, $>SO^{2-}_4>HPO^{2-}_4>NO^-_3>Br^->NO^-_2>Cl^->F^-$.

Also, the retention volumes for the seven eluent strength decrease when the column temperature increase from 25 to 30°C and the magnitude of the decrease was in the following descending order: I^-, $>SO^{2-}_4>HPO^{2-}_4>NO^-_3>Br^->NO^-_2>Cl^->F^-$ .The response of the retention volumes of all the inorganic anions to the change in two factors (column temperature and eluent strength) were not liner, moreover the results indicate that the resolution at 30°C and concentration 1.8 mM for potassium carbonate gives the shortest retention volume. On comparing our results with Qiu's method[24] we observed that the process of selection of temperature and eluent strength is very easy. In

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

Compound	Detection limit (mg / L)Linearity		Regression Range (mg/L)	Coefficient(r^2)
	1.8mM K_2CO_3+ 12mM $KHCO_3$	1.8mM K_2CO_3 +12mM $KHCO_3$+ 0.0125 mM ETHTC		
F^-	0.10	0.0032	0.5-3100	0.9991
Cl^-	0.01	0.0023	0.5-3100	0.9997
NO^-_2	0.13	0.12	0.5-2400	0.9992
Br^-	0.10	0.0023	0.5-2900	0.9992
NO^-_3	-	0.0023	0.5-3100	0.9997
$HPO^{—}_4$	-	0.0025	0.5-2600	0.9992
$SO^{—}_4$	0.11	0.0037	0.5-4100	0.9995
I^-	0.11	0.0032	0.5-3400	0.9995

Table 5:The concentration of inorganic anions (ppm) in hibiscus at different temperature (ppm).

Temp (°C)	Time (min)	Meanand RSD%	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	$HPO_4^{—}$	$SO_4^{—}$	I^-
60	5	A	ND	ND	ND	ND	ND	ND	ND	ND
		b	ND	ND	ND	ND	ND	ND	ND	ND
	10	A	ND	ND	ND	ND	ND	ND	65.2	ND
		b	ND	ND	ND	ND	ND	ND	1.21	ND
	15	A	ND	ND	ND	ND	ND	ND	72.1	ND
		b	ND	ND	ND	ND	ND	ND	1.22	ND
	20	A	ND	4.5	ND	ND	ND	ND	84.2	ND
		b	ND	0.94	ND	ND	ND	ND	1.13	ND
	25	A	ND	5.1	ND	ND	ND	ND	93.4	ND
		b	ND	0.93	ND	ND	ND	ND	1.12	ND
	30	A	ND	5.9	ND	ND	ND	21.1	107.6	ND
		b	ND	0.91	ND	ND	ND	1.22	1.16	ND
70	5	A	ND	35.6	ND	ND	ND	32.2	202	ND
		b	ND	0.96	ND	ND	ND	0.56	0.46	ND
	10	A	ND	48.7	ND	ND	ND	38.9	246	ND
		b	ND	0.71	ND	ND	ND	0.76	0.43	ND
	15	A	5.5	88.7	ND	ND	ND	44.3	261.4	ND
		b	1.22	0.32	ND	ND	ND	0.83	0.39	ND
	20	A	7.3	125.2	ND	ND	ND	54.7	277.3	ND
		b	1.10	0.95	ND	ND	ND	1.31	0.53	ND
	25	A	11.2	152.7	ND	ND	ND	66.3	287.5	ND
		b	1.21	0.69	ND	ND	ND	1.20	0.39	ND
	30	A	17.6	193.2	ND	ND	ND	74.7	291.2	ND
		b	0.72	1.20	ND	ND	ND	1.41	0.51	ND
80	5	A	36.3	249.4	ND	ND	ND	98.7	375.2	6.10
		b	0.67	1.10	ND	ND	ND	1.32	1.43	0.92
	10	A	45.6	287.5	ND	ND	25.2	122.7	425.3	9.52
		b	1.0	1.20	ND	ND	1.48	1.24	1.22	0.83
	15	A	61.3	319.7	ND	ND	37.4	174.2	472.1	12.7
		b	1.22	0.90	ND	ND	1.61	1.18	1.50	0.63
	20	A	78.5	344.2	ND	ND	51.4	199.7	501.2	18.9
		b	0.91	1.09	ND	ND	0.87	0.75	0.54	0.89
	25	A	89.3	361.6	ND	ND	74.7	245.6	572.7	29.2
		b	1.03	1.03	ND	ND	1.27	0.78	0.54	0.82
	30	A	98.6	394.7	ND	ND	94.5	289.2	623.2	37.6
		b	1.30	0.94	ND	ND	1.0	0.37	0.78	1.11
90	5	A	137.9	485.3	ND	ND	156.7	470.5	693.2	48.7
		b	0.47	0.37	ND	ND	1.0	0.39	0.79	1.10
	10	A	168.7	535.6	ND	ND	199.7	520.9	773.2	57.8
		b	0.54	0.35	ND	ND	0.92	0.75	0.82	0.96
	15	A	196.3	596.4	ND	ND	243.3	590.2	842.4	71.6
		b	0.62	0.30	ND	ND	1.11	0.78	0.91	0.89
	20	A	244.7	646.5	ND	ND	289.6	670.7	966.6	94.3
		b	0.75	0.43	ND	ND	0.95	0.87	0.78	0.63
	25	A	284.8	698.7	ND	ND	351.7	780.3	1041.2	121.8
		b	0.32	0.45	ND	ND	0.96	0.93	0.47	0.72
	30	A	355.4	773.7	ND	ND	466.3	890.7	1175.5	137.4
		b	0.31	0.44	ND	ND	0.89	0.90	0.57	0.85

(a) Mean; (b) RSD%; (ND):Not detected

addition our results of the optimum conditions enable us to reduce the retention volumes for the eight inorganic anions form 15.992 to 14.373.

The effect of ETHTC on retention volume VR and sensitivity

The addition of ETHTC to the eluent was carried out to improve Peak resolution between inorganic anions under investigation. Different concentration of ETHTC (0.0120 , 0.0125 and 0.013 mM) where added to 1.8 mM K_2CO_3 + 12 mM $KHCO_3$ mobile phase in an attempt to effect the retention of inorganic anion under study. These results were compared to those obtained with the same column but without the addition of ETHTC. Fig 5 Shows that at all the three different concentration (0.0120 , 0.0125 and 0.013 mM) of ETHTC add to the same mobile phase the overlap between NO^-_3 and HPO^{2-}_4 has been solved and the concentration of 0.0125 mM ETHTC gaves the shortest retention volumes. On comparing our results with data of Lamb *et al* [4], Pera *et al* [25] and Kumar *et al* [26] we found our results more satisfactory. J.D. lamb *et al* [4], investigated the separation of eight anions include F^-, Cl^-, Br^-, NO^-_3, SO^{2-}_4, I^-,HPO^{2-}_4 and ClO^-_4 using 15 mM KOH + 2.5 mM 18- crown – 6 as eluent. The results revealed that the time of separation is around 20 minutes.

Pera *et al* [25] also studied the separation of eight anion include F^-, Cl^-, NO^-_3, Br^-, PO^{3-}_4, SO^{2-}_4 and I^- using 3.12 mM Na_2CO_3 + 3.25 mM $NaHCO_3$ + 2% acetone as eluent. The results indicate that the time of separation is around 14 minutes. Kumar *et al* [26] also investigated the separation of seven anions include and using 1.3 mM Na_2CO_3 + 2 mM $NaHCO_3$ as eluent. The results revealed that the time of separation is around 35 minutes.

On the other hand our studies involve the separation of eight anion include F^-, Cl^-, NO^-_2, NO^-_3, Br^-, PO^{3-}_4, SO^{2-}_4 and I^- and the time of separation is 12.21 minutes. The results suggest that our method gives better results than that done by Lamb *et al*[4], Pera *et al*[25] and Kumar *et al*[26]. Also in our previous papers[14,28] we investigated the separation of eight anions include F^-, Cl^-, NO^-_2, NO^-_3, Br^-, PO^{3-}_4, SO^{2-}_4 and I^- using 1.8 mM K_2CO_3+ 12 mM $KHCO_3$ + 5% ACN and 1.8 mM K_2CO_3 + 12 mM $KHCO_3$ + 0.05 mM 18 – crown -6 as eluent respectively, the results revealed that the time of separation is 12.126 and 11.517 respectively. This means that the results obtained by both two papers [14,28] and recent work, the use of 18-crown -6 gives better results than use of ACN and ETHTC as a composition of eluent but the use of ETHTC gives better results than the use of ACN as a composition of eluent.

Figs (6) and (7) shows the simultaneous separation of inorganic anions before and after adding ETHTC to the eluent while Fig.(8) shows the sensitivity of before and after adding ETHTC.

In conclusion the presence o ETHTC increase the sensitivity and decrease the retention time from 14.373 to 12.21 minutes. The detection limit (S/N = 3) for eight inorganic anions ore given in table (4). The detection limit obtained by using the mixture of 1.8 mM K_2CO_3+ 12 mM $KHCO_3$+ 0.0125 mM ETHTC, pH 9.25 are lower several time than those obtained by using 1.8 mM K_2CO_3+12 mM $KHCO_3$, pH 9.24.

Table (4) shows that the calibration graph for all inorganic anions under studies were linear with regression coefficient (r^2) of 0.9991 – 0.9997.

Application

From our acquaintance in the literatures, it has been appeared that there is no study in ion chromatography take up the point of separation and determination of inorganic anions in Roselle (*Hibiscus sabdariffa* L.) by using ion chromatographic technique. Therefore, the new established method by using the mobile phase consisting of 1.8 mM Potassium carbonate + 12 mM Potassium hydrogen carbonate + 0.0125 mM ETHTC has been successfully applied to the separation and determination of inorganic anions in Roselle (*Hibiscus sabdariffa* L.) .

Table (5) summarizes the determination and reproducibility of anion (n = 10) in Roselle (*Hibiscus sabdariffa* L.) sample. So, the data shows that the peak area of each analyte were different at the different temperature (60, 70, 80 and 90°C) for the six heating times (5, 10, 15, 20, 25 and 30 min) only six anions were found in the sample include F^-, Cl^-, NO^-_3, HPO^{2-}_4, SO^{2-}_4, $SO2^-_4$ and I^-) and it was appeared at different temperature and different time,

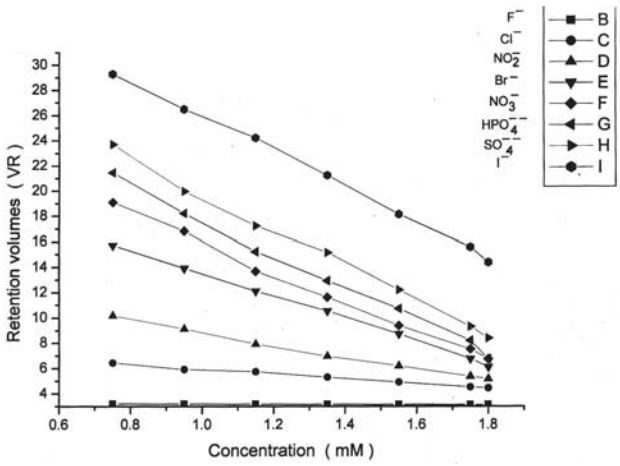

Fig. 1: Effect of potasium carbonate concentration in the eluent on the reaction volumes of inorganic anions

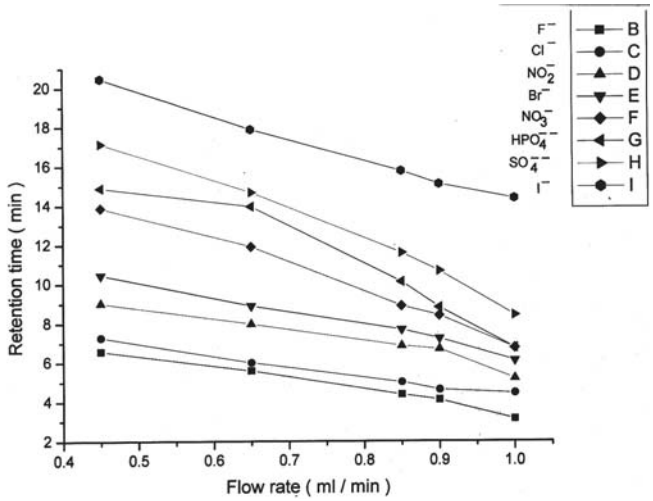

Fig. 2: Effect of flow rate on the retention time of investigated inorganic anions

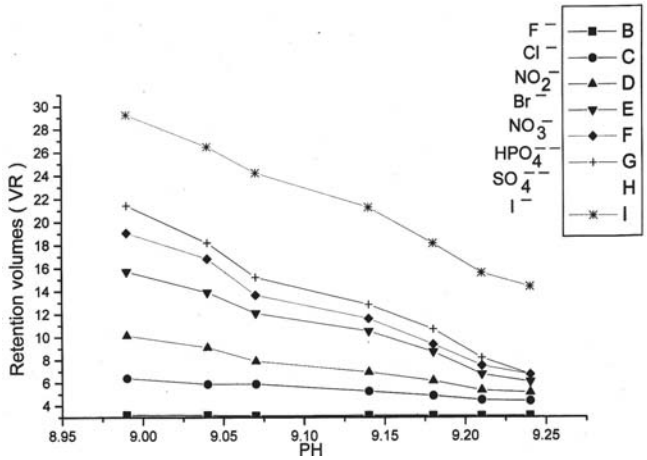

Fig. 3: The effect of eluent pH on retention behaviour of inorganic anions

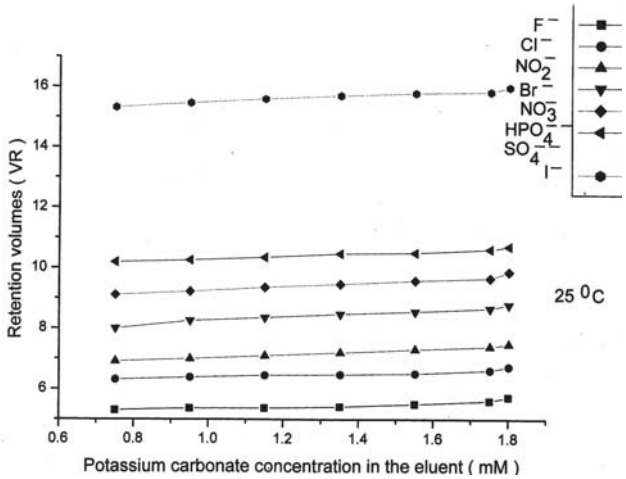

Fig. 4(a): The effect of column temperature and eluent sterngth on the retention volumes of inorganic anions

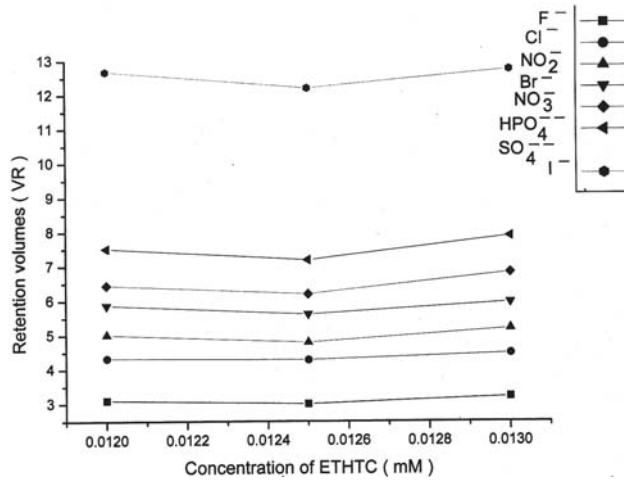

Fig. 4(b): The effect of column temperature and eluent sterngth on the retention volumes of inorganic anions

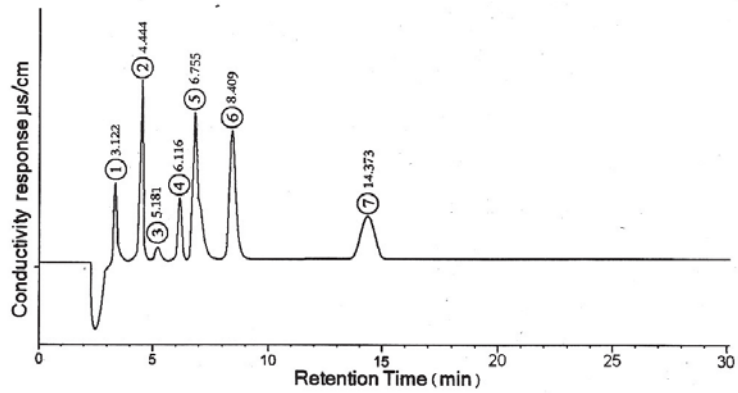

Fig. 4(c): The effect of column temperature and eluent sterngth on the retention volumes of inorganic anions

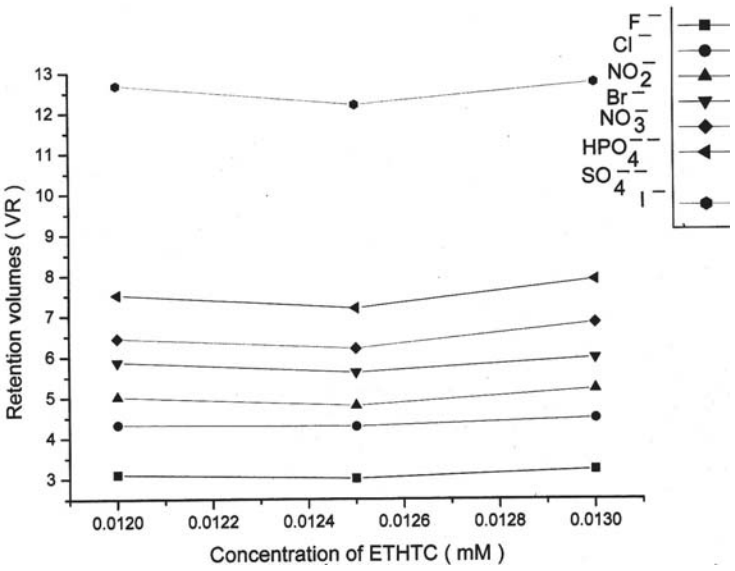

Fig. 5: The effect of ETHTC in the eluetn on the retention volumes of inorganic anions

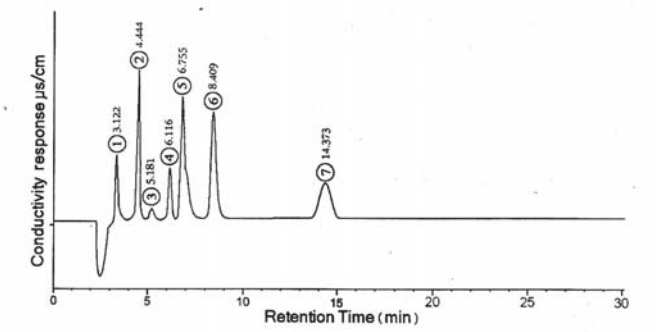

Fig. 6: Tpyical resolution for a mixture of inorganic anions, column, shin - pack IC - SA2, temperature 30°C,eluent mixture of 1.8 mM K_2CO_3 and 12 mM $KHCO_3$ (pH 9.24), flow rate 1ml/min Peaks : 1 = F^-, 2=Cl^-, 3=NO_2^-, 4= Br^-, 5=NO_3 + HPO_4^{2-}, 6= SO_4^{2-}, 7=I^-

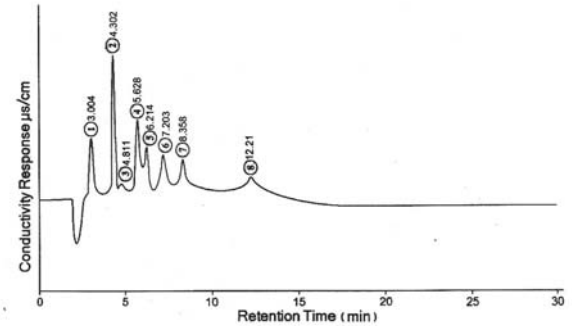

Fig. 6: Tpyical resolution for a mixture of inorganic anions, column, shin - pack IC - SA2, temperature 30°C,eluent mixture of 1.8 mM K_2CO_3 and 12 mM $KHCO_3$ +0.0125m METCH (pH 9.25), flow rate 1ml/min, Peaks : 1 = F^-, 2=Cl^-, 3=NO_2^-, 4= Br^-, 5=NO_3, 6=HPO_4^{2-}, 7= SO_4^{2-}, 8=I^-

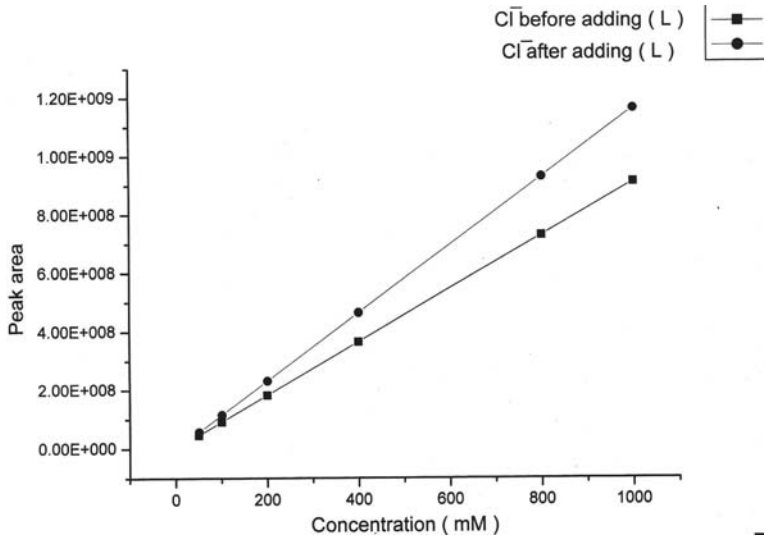

Fig. 8: The relation between concentration and peak area (sensitivty for Cl^-

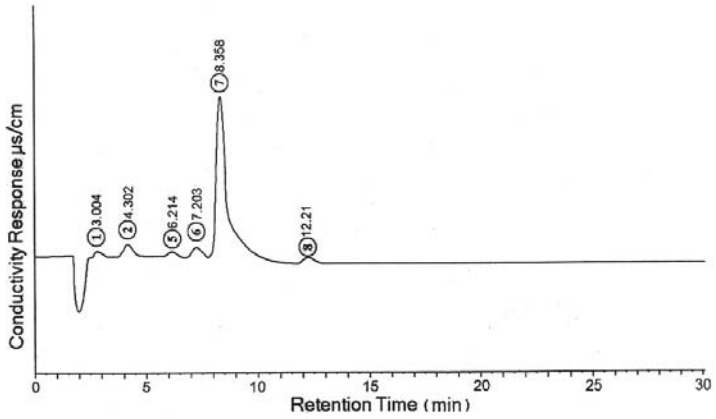

Fig. 8: Tpyical chromatrogram for inorganic anions of hibiscus (conditions the same as in figure 7)

SO^{2-}_4, Cl^- and HPO^{2-}_4 appeared at 60°C at the heating time of 10, 20 and 30 minutes respectively and only appeared at 70°C for a heating time 15 minutes, while I^- and NO^-_3 appeared at 80°C at 5 and 10 minutes respectively in addition the results shows the absence of NO^-_2 and Br^- at variant temperature for variant heating time under our study, probably their concentration in sample were below the limit of detection of the present method. Also the results revealed that the amount of extraction increased with increasing the temperature from 60 to 90°C and also the heating time from 5 to 30 minutes. So, it can be concluded that the most suitable temperature and heating time is 90°C and 30 minutes.

In addition, the results shows the relative standard deviation (RSD%) below 1.62%.

Fig (9) shows the chromatogram of inorganic anions in Roselle (*Hibiscus sabdariffa* L.) and it appears its contain only six ions F^-, Cl^-, NO^-_3, HPO^{2-}_4, SO^{2-}_4 and I^- from eight ions under investigation and the absence of both NO^-_2 and Br^-

CONCLUSION

A simple rapid an ion chromatographic method with conductivity detection has been developed for the simultaneous determination of inorganic anions in Roselle (*Hibiscus sabdariffa* L).

Five ion chromatographic parameters optimized include concentration of both K_2CO_3 and ETHTC in the eluent, temperature of the eluent, flow rate of the eluent and pH of the eluent.

The analytical method proposed showed a high sensitivity and reproducibility and has the advantage of allowing quantitation of the main mono and divalent inorganic anion. The resulting analysis time was 12.21 minutes and the relative standard deviation (RSD%) below 1.62%. The suggested method may be adapted to the separation and determination of main inorganic anions in other food samples.

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