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Optimization of the determination of inorganic anions and organic acids in Roselle (Hibiscus sabdariffa L) by Suppressed ion chromatography

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Abstract

A Simple, sensitive, accurate and rapid analytical method for simultaneous separation and determination of inorganic acions ($F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{-2}, SO_4^{-2}$ and I^-,) and organic acids (acetic acid, formic acid, succinic acid, tartaric acid, malic acid, oxalic acid and fumric acid) was developed by using suppressed conductimetric ion chromatognaphy. The separation was achieved on shim-pack 1C-SA_2 250 mmL × 4.5 mm ID Peak with a mobile phase consisting of 0.6 mM potassium carbonate + 12 mM potassium hydrogen carbonate + 0.0125mM N – {[(ethylamino) thioxomethyl] hydrazine carbonylmethyl} trimethyl ammonium chloride (ETHTC), a flow rate of 1 ml / min and 30°C. Under the optimal conditions the eight inorganic anions and the seven organic acids were completely separated and detected simultaneously within 20. 367 min. The limit of detection (S/ N=3) of inorganic anions and organic acids were in the range of 0.9992 – 0.9998 µg/L and 0.9991 – 0.9998, respectively and the relative standard deviation (RSD %) of all ions found to be below 1.36%.

The method was applied successfully to the analysis of Roselle (Hibiscus sabdariffa L.) sample.

Key words: Optimisation, determination, inorganic anions, organic acids, ETHTC, suppressed ion chromatography Roselle (Hibiscus sabdariffa L.).

1- Introduction

Ion chromatography (IC) remains one of the most powerful instrumental techniques for the determination of inorganic anions and organic acids.

As a consequences, IC is widely used in different environmental samples such as natural water[1-3], snow[4-6], ice[5,7] rain[8,9] and air[10,11], atmosphere monitoring[12], food[13-27], pharmaceutical[28-30], including forensic science[31,32].

In recent years several methods have been proposed for the simultaneous chromatographic separation of inorganic anions and organic acids.

A mixed mode stationary phase was proposed[33] using a silica – based stationary phase bonded with ligands containing both reversed phase and ion exchange function. Anion chromatography with gradient elution[34] has also been proposed and applied to the determination of inorganic anions and organic acids in precipitation samples[35]. Non suppressed ion exchange chromatography with conductivity detection, in which a low conductivity eluent is used, was applied for the simultaneous determination of inorganic anions and organic acids in food samples[24].

In our previous papers[16,17,21] we reported that ETHTC was used successively for the simultaneous separation and determination of some organic acids and inorganic anions. Also, we mentioned earlier that the presence of the ligand leads to increase the sensitivity as well as the changing of the positions. Moreover, the analysis time for separation has been effectively reduced in case of anions salts while the separation is slightly lower in case of organic acids. The main purpose of this study is to establish an efficient suppressed ion chromatographic method for the simultaneous separation and determination of a mixture of organic acids and inorganic anions by optimization of chromatographic parameters (eluent flow rate, temperature, pH, mobile phase).

2- Experimental

2-1: Apparatus:

In this study analysis and data collection were performed by LC solution software using a HIC-20A SUPER ion chromatograph purchased from shimadzo consisting of an LC-20AD$_{SP}$ liquid delivery pump, a DGU-20A$_3$. Degasser, Rheodyne (77251) injection valve with a 20 µl 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-SA2 – 250 mmL X 4.0 mm ID PEEK). The column oven was maintained at 30°C.

2-2. Reagents:

All the inorganic anions and organic acids in this study were of analytical reagents and were purchased from BDH chemical poole England, while N-{[(ethylamino) thioxomethyl] hydrazine carbhonylmethyl}trimethyl ammonium chloride (ETHTC) was prepared as reported earlier[36]. Double distilled deionized water was filtered through 0.2 µm whatman membrane. All standard solution eluents and reagents were prepared in double distlled deionzed water and filtered through 0.2 µm whatman membrane filter.

2.3. Sample preparation:

20 g of Roselle (Hibiscus sabdariffa L.) was placed in a flask containing 70 ml double distilled deionized water. The mixure

was heated at 90°C for 30 minutes. After cooling, the solution was filtered through 0.2 µm whatman membrane filter and then the filtrate was transferred to 100 ml flask and double distilled deionzed water added to the mark. This sample solution was injected into the ion chromatograph directly. Each sample was run ten times.

2-4. The optimum conditions:

The optimum analytical conditions have been established in this study in order to separate and determine eight inorganic anions ($F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}$, SO_4^{2-} and) and seven organic acids (acetic acid, formic acid, succinic acid, tartaric acid, malic acid, oxalic acid and fumaric acid) simultaneously using isocratic method with 0.6 mM potassium carbonate and 12 mM potassium hydrogen carbonate at pH 8.97, flow rate 1 ml/min and at 30°C.

The data obtained was compared with the eluent solution containing of 0.0125 mM ETHTC in addition to the above mentioned eluent at pH 9.98, flow rate 1 ml/min and at 30°C.

3- Results and Discussion

3.1- The effect of potassium carbonate concentration:

In order to achieve simultaneous separation of inorganic anions and organic acids, potassium carbonate and potassium hydrogen carbonate were used as mobile phase. Effect of potassium carbonate concentration on the retention behaviour was examined with the concentration of potassium hydrogen carbonate fixed at 12 mM. The relation between the retention volume of analyte and the concentration of potassium carbonate is shown in fig. (1), it can be seen that the retention volumes decreased with increasing the potassium carbonate concentration, the decreased in retention volumes was linear for the following anions F^-, acetic, formic, Cl^-, NO_2^-, Br^- and NO_3^-, also the decrease in retention volume wehre sharply for the following anions, succinic, tartaric, SO_4^{2-}, malic oxalic, fumaric and I^-, while the decrease in retention volume wehere stdeay for the following anions, F^-, acetic, formic, Cl^-, NO_2^-, Br^- NO_3^- and HPO_4^{2-}. As a result, the optimum potassium carbonate concentration was determined to be 0.51 mM, based on the resolution and retention volume for each analyte, while after this concentration three overlapped has been observed, the first overlap between succinic and tartaric, the second overlap between malic and oxalic and I^-, the third overlap between fumaric and the three mentioned overlap appeared at concentration of 0.6 mM potassium carbonate, section 3.5 will discuss the solution of these overlapp.

3.2: The effect of flow rate:

The influence of flow rate on the retention volume of inorganic anions and organic acids under investigation was carried out by applying different flow rate including 0.45, 0.65, 0.85, 0.9 and 1.0 ml/min in order to achieve good and fast separation. Table (1) and figure (2) revealed that the retention volumes of each anion decrease with increasing the flow rate from 0.45 – 1.0 ml/ min.

The greatest effet was eviedent for I^- while the lowest was for acetic. It is quite evident from the table 1 and fig. (2) that there are big variations in retention volume of anions under investigation, even in the retention volume of individual anions.

This leads to different sensitivities. The relationship between the flow rate and retention volume according to results obtained can be divided into two division, the first division which has sharp decrease in retention volume can be devidied into three subdivision.

The first subdivision showed that there is sharp decrease in retention volum between 0.45 – 0.65 ml/min for only F^-. This subdivision extended to 0.85 ml/min including formic acid, $Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}$, succinic acid, SO_4^{2-} and malic acid, whereas the second subdivision showed sharp decrease in retention volume between 0.9–1.0 ml/min this includes NO_3^-, HPO_4^{2-}, Succinic acid, tartaric acid, SO_4^{2-} and malic acid, the third subdivision has unique quality because its covers all the values of flow rate under stadies (0.45, 0.65, 0.85, 0.9 and 1.0 ml/min) this includes oxalic acid, fumaric acid and I^- The second division which has a steady decrease in retention volume can also be divided into three subdivision, the first subdivision revealed that the steady decrease in retention volume was between 0.45 – 1.0 ml/ min This covers all the fifteenth anions under investigation. Whereas the second subdivision showed steady decrease in retention volume between 0.65-1.0 ml/min for only F^-, and the third subdivision revealed that the steady decrease in retention volume was between 0.85 – 0.9 ml/min. This include six anion NO_3^-, HPO_4^{2-}, succinic acid, tartaric acid, SO_4^{2-} and malic acid. This subdivision extended to 1.0 ml/min including, formic acid, Cl^-, NO_2^- and Br^-.

3.3: Effect of the eluent pH on retention behavior of inorganic anions and organic acids:

Several pH values were applied in this study including 8.65, 8.75, 8.80, 8.88 and 8.97 pH, to study the effect of the eluent pH on retention volume of inorganic anions and organic acids.

The data presented in table (2) and fig. (3) shows the relationship between the pH of the eluent and the retention volumes of inorganic anions and organic acids. The retention volumes of these anions decreased with increasing the pH of the eluent. The greatest effect was eviedent for I^- while the lowest was for acetic acid. As it can be seen there are big variation in retention volume of inorganic anions and organic acids under investigation as function of pH values in the mobile phase, and the retention volumes were slightly decreased for some anions such as F^-, acetic acid, formic acid, Cl^-, NO_2^-, Br^- and NO_3^-, whereas the decrease in retention volumes for the other anion were sharply such as HPO_4^{2-}, succinic acid, tartaric acid, SO_4^{2-}, malic acid, oxalic acid, fumaric and I^-, and the suitable pH selected was 8.88, providing a good separation at reasonable elution time, also the data revealed that the separation after pH 8.88 will be impossible due to three the overlaps first between HPO_4^{2-}, succinin acid and

tartaric acid, second the overlap between malic acid and oxalic acid, and the third orerlap between fumaric acid and I^-, these three overlaps has been appeared at pH 8.97, the solution of these three overlapped will be discuss in section 3.5.

3.4: Effect of column temperature and eluent strength on the retention volume:

The effect of column temperature and eluent strength on the retention volume are shown in figures (4a, 4b and 4c) and the results are allotted in table (3). At the three temperature (25, 28 and 30°C) the retention volume of all inorganic anions and organic acids under investigation is increased as the eluent concentration increases from 0.27 to 0.6 mM of potassium carbonate.

The magnitude of retention volume increases in the following descending order: I^- > fumaric acid > oxalic acid > malic acid > SO_4^{2-} > tartaric acid > succinic acid > HPO_4^{2-} > NO_3^- > Br^- > NO_2^- > Cl^- > formic acid > acetic acid > F^-.

The retention volumes at the different five eluent strength decreased when the column temperature is increased from 25 to 30°C and the magnitude of retention volume decreases was in the following descending order I^- > fumaric acid > oxalic acid > malic acid > SO_4^{2-} > $NO_3^- > Br^-, NO_2^-, Cl^-$ > formic acid > acetic acid > F^-, the increase in the retention volumes for organic acids and inorganic anions was steady and linear at the temperature 25 and 28°C while the increases in retention volumes at 30°C not linear, and the increase was sharply at the concentration between 0.51 and 0.6 mM potassium carbonate for the following anions HPO_4^{2-}, succinic acid SO_4^{2-}, malic acid, oxalic acid, fumaric acid and I^-. Moreover the results indicate that the resolution at concentration 0.51 mM for potassium carbonate and 30°C gives the shortest retention volume, also the data revealed that the separation after 0.51 mM potassium carbonate will be impossible due to the three overlap first between HPO_4^{2-}, succinic acid and tartaric acid, second between malic acid and oxalic acid and third between fumaric acid and I^-, these three overlap has been appeared at 0.6 mM potassium carbonate and at 30°C. The optimum condition proposed in this study enables us to reduce the whole retention volumes for the all separated organic acids and inorganic anion from 35.422 to 22.411min.

3.5: 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 and organic acids under studies. Different concentration of ETHTC (0.0120, 0.0125 and 0.013 m)) where added to 0.6 mM K_2CO_3+ 12 mM $KHCO_3$ mobile phase in an attempt to effect the retention of inorganic anions and organic acids under investigation. These results were compared to those obtained with same column but without the addition of ETHTC. The results revealed that the degree of resolution of inorganic anions and organic acid is improved by adding ETHTC to eluent mixture and the efficiency of separation process is increased with decreasing the concentration of ETHTC and the most efficient

Table 1. The effect of flow rate on retention volume (VR)
of investigated inorganic anions and organic acids

Flow rate ml/min	F^-	Acetic acid	Formic acid	Cl^-	NO_2^-	Br^-	NO_3^-	HPO_4^{2-}	Succinic acid	Tartaric acid	Malic SO_4^{2-}	Oxalic acid	Fumaric acid	acid	I^-
0.45	7.187	7.967	11.652	13.242	15.352	17.312	20.495	22.462	23.964	25.353	27.701	30.523	33.397	35.756	41.329
0.65	5.896	6.297	9.398	9.976	10.785	12.892	16.676	18.932	19.897	21.354	23.406	24.877	25.984	29.654	34.315
0.85	4.919	5.386	6.673	7.014	8.465	9.696	12.687	14.724	15.342	16.453	18.316	19.657	21.768	25.413	27.201
0.9	4.896	5.266	5.968	6.711	7.768	8.973	11.546	13.603	14.207	15.456	17.436	18.756	18.976	23.523	24.612
1	3.858	4.176	4.711	5.619	6.632	8.322	9.492	11.637	11.637	11.637	14.47	16.527	16.527	22.411	22.411

Table 2. The effect of the eluent pH on retention volume (VR)
of investigated inorganic anions and organic acids

pH	F^-	Acetic acid	Formic acid	Cl^-	NO_2^-	Br^-	NO_3^-	HPO_4^{2-}	Succinic acid	Tartaric acid	SO_4^{2-}	Malic acid	Oxalic acid	Fumaric acid	I^-
8.65	5.778	6.625	7.587	9.901	11.121	13.597	15.535	17.645	19.367	20.892	22.979	25.213	28.124	30.878	36.213
8.75	4.877	5.768	6.598	7.592	9.798	12.279	13.787	16.238	17.113	18.256	19.597	20.897	23.759	26.874	30.869
8.8	4.479	5.258	6.194	7.372	9.105	11.537	13.187	15.193	16.212	17.233	17.899	19.395	21.976	25.294	28.874
8.88	3.967	4.476	5.194	6.487	7.746	10.097	11.131	12.621	13.012	14.592	15.567	17.289	18.569	23.396	24.497
8.97	3.858	4.176	4.711	5.169	6.632	8.322	9.492	11.637	11.637	11.637	14.47	16.527	16.527	22.411	22.411

Table 3. The relation between concentration of potassium carbonate and retention volume (VR) at different temperature

Temp °C	Concentration (mM)	VR														
		F^-	Acetic acid	Formic acid	Cl^-	NO_2^-	Br^-	NO_3^-	HPO_4^{2-}	Succinic acid	Tartaric acid	SO_4^{2-}	Malic acid	Oxalic acid	Fumaric acid	I^-
25°C	0.27	7.424	8.522	9.489	11.513	13.537	15.612	17.614	19.603	21.563	23.527	25.573	27.609	29.618	33.131	35.016
	0.37	7.513	8.581	9.517	11.561	13.578	15.671	147.681	19.681	21.67	23.633	25.667	27.696	29.721	33.24	35.118
	0.42	7.657	8.624	9.616	11.654	13.613	15.72	17.743	19.779	21.768	23.725	25.758	27.711	29.824	33.339	35.212
	0.51	7.764	8.682	9.725	11.768	13.674	15.798	17.852	19.882	21.864	23.819	25.849	27.815	29.903	33.443	35.319
	0.6	7.898	8.768	9.833	11.899	13.718	15.816	17.94	19.977	21.959	23.924	25.938	27.921	29.984	33.551	35.422
28°C	0.27	4.513	6.124	7.018	9.142	11.115	13.105	15.103	17.017	19.113	20.123	23.102	25.051	27.128	30.117	32.552
	0.37	4.568	6.178	7.147	9.235	11.153	13.173	15.193	17.195	19.211	20.227	23.211	25.162	27.233	30.318	32.649
	0.42	4.611	6.217	7.298	9.326	11.231	13.251	15.281	17.289	19.314	20.326	23.313	25.249	27.325	30.529	32.739
	0.51	4.662	6.283	7.342	9.432	11.275	13.363	15.379	17.378	19.412	20.419	23.415	25.355	27.422	30.717	32.844
	0.6	4.701	6.317	7.416	9.517	11.379	13.442	15.468	17.468	19.51	20.517	23.418	25.463	27.526	30.922	32.938
30°C	0.27	3.127	3.923	4.219	5.192	5.663	7.147	8.465	9.763	10.101	10.996	12.113	13.154	14.129	17.229	19.245
	0.37	3.279	3.984	4.291	5.313	5.781	7.413	8.519	9.822	10.134	11.013	12.152	13.183	14.324	17.831	19.529
	0.42	3.396	4.061	4.328	5.391	6.011	7.731	8.716	9.853	10.142	11.121	12.173	13.219	14.431	17.956	19.876
	0.51	3.576	4.132	4.416	5.532	6.275	7.982	8.921	9.944	10.466	11.358	12.569	13.272	14.612	18.432	19.929
	0.6	3.858	4.176	4.711	5.619	6.632	8.322	9.492	11.637	11.637	11.637	14.47	16.527	16.527	22.411	22.411

Table 4. Detection Limit (S / N = 3), Linear range and regression coefficient of inorganic anions and organic acids

Compound	Detection Limit (mg / l)		Linearity range (mg/L)	Regression Coefficient (r2)
	0.6 mM k_2CO_3 + 12 mM $KHCO_3$	0.6 mM k_2CO_3 +12 mM $KHCO_3$ + 0.0125 mM ETHTC		
F^-	0.11	0.0042	0.5 - 2800	0.9992
Acetic acid	0.15	0.037	0.5 - 2000	0.9991
Formic acid	0.45	0.054	0.5 - 2000	0.9995
Cl^-	0.02	0.0024	0.5 - 2800	0.9993
NO_2^-	0.13	0.12	0.5 - 2100	0.9994
Br^-	0.11	0.0024	0.5 - 2500	0.9998
NO_3^-	0.12	0.0022	0.5 - 2800	0.9997
HPO_4^{2-}	-	0.0025	0.5 - 2400	0.9995
Succinic acid	-	0.0025	1 - 4500	0.9993
Tartaric acid	-	0.0025	1-1800	0.9991
SO_4^{2-}	0.12	0.0036	0.5-3800	0.9998
Malic acid	-	0.024	1-2800	0.9997
Oxalic acid	-	0.024	1-3200	0.9992
Fumaric acid	-	0.006	1-2800	0.9997
I^-	-	0.005	0.5-3000	0.9998

Table 5. The concentration of inorganic anions and organic acids (ppm) in Roselle (Hibiscus sabdariffa L)

Mean and RSD%	F^-	Acetic acid	Formic acid	Cl^-	NO_2^-	Br^-	NO_3^-	HPO_4^{2-}	Succinic acid	Tartaric acid	SO_4^{2-}	Malic acid	Oxalic acid	Fumaric acid	I^-
A	27.7	398	ND	98.9	ND	ND	45.7	127.4	2811	479	189.7	467	487	ND	98.4
B	0.10	0.22	ND	1.22	ND	ND	0.13	1.35	1.33	1.34	0.11	0.74	0.85	ND	0.94

(A) mean (B) RSD % ND: not detected

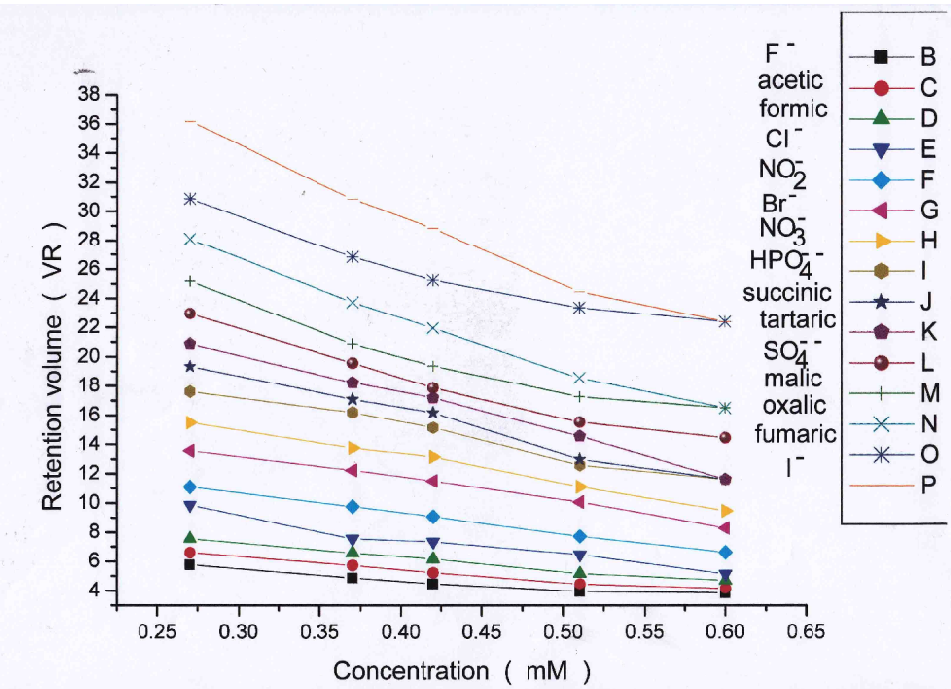


Fig - 1 . Effect of potassium carbonate concentration in the eluent on the retention volumes of inorganic anions anc organic acids .

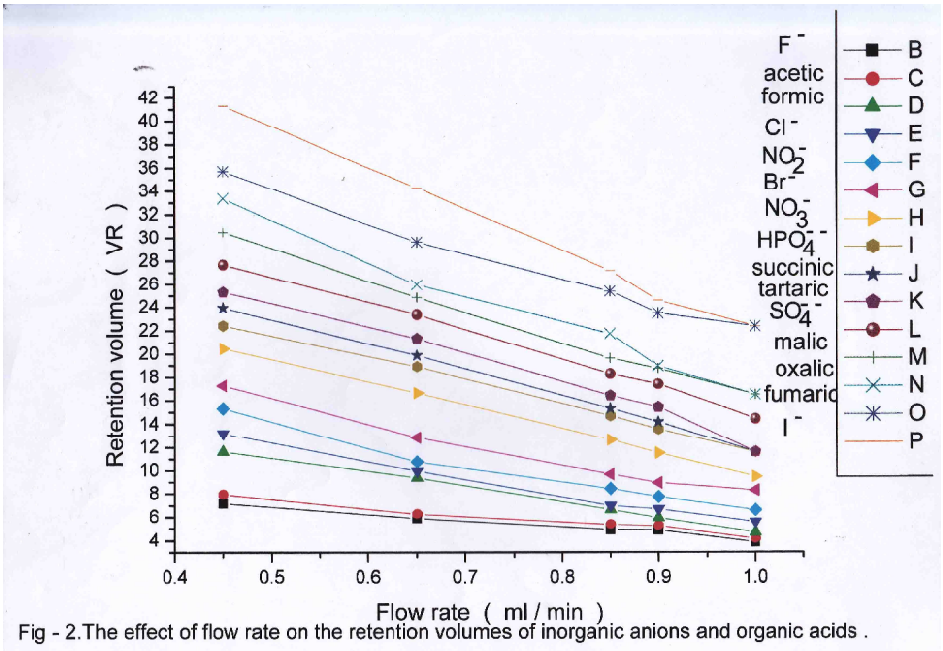


Fig - 2.The effect of flow rate on the retention volumes of inorganic anions and organic acids .

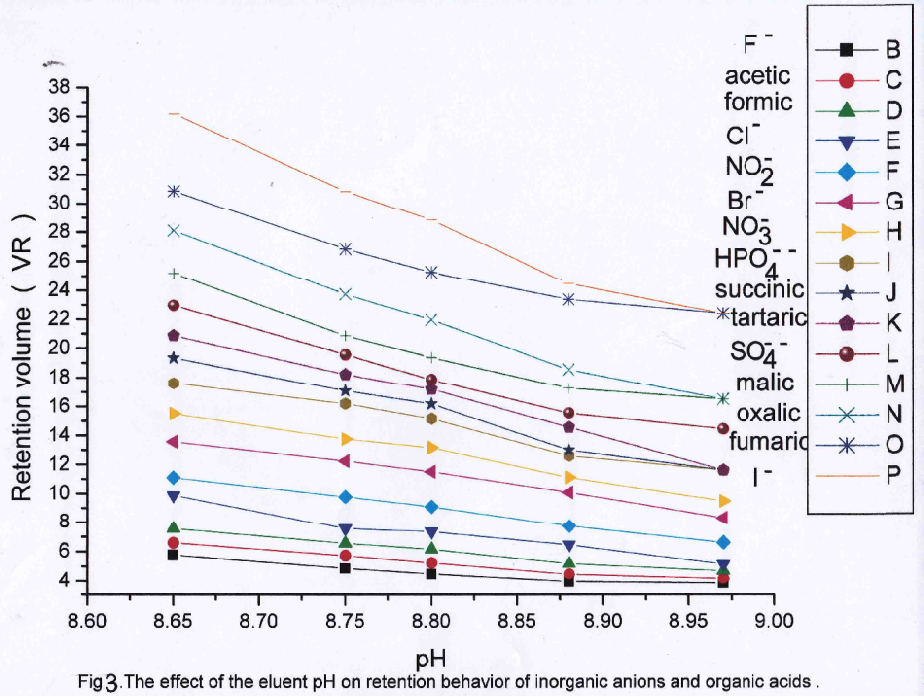


Fig 3. The effect of the eluent pH on retention behavior of inorganic anions and organic acids .

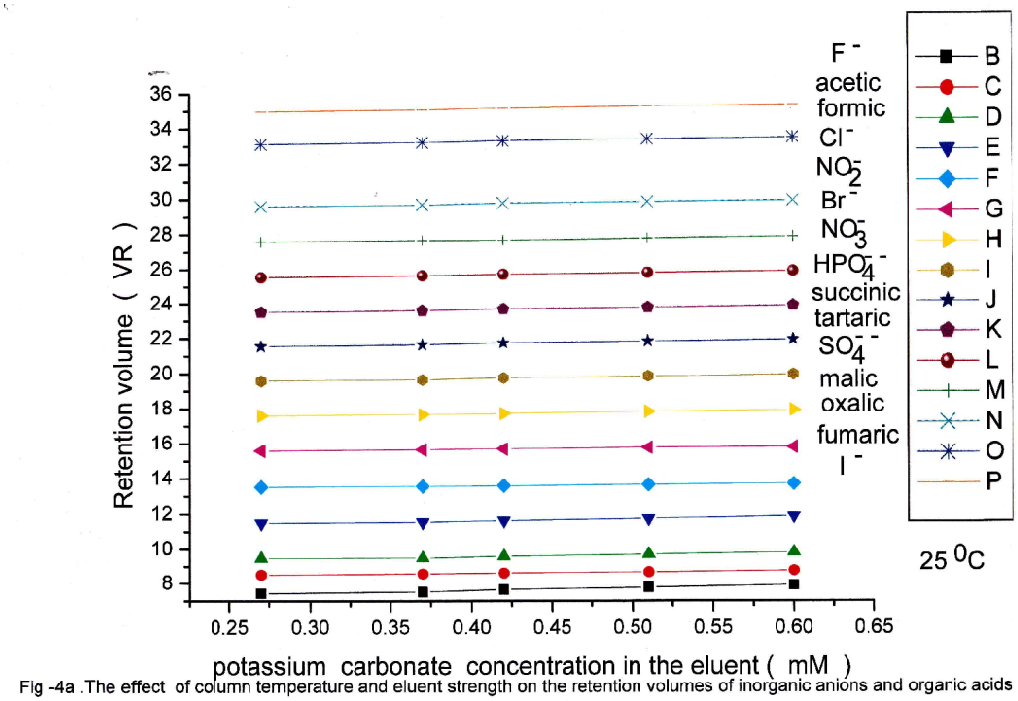


Fig -4a .The effect of column temperature and eluent strength on the retention volumes of inorganic anions and organic acids

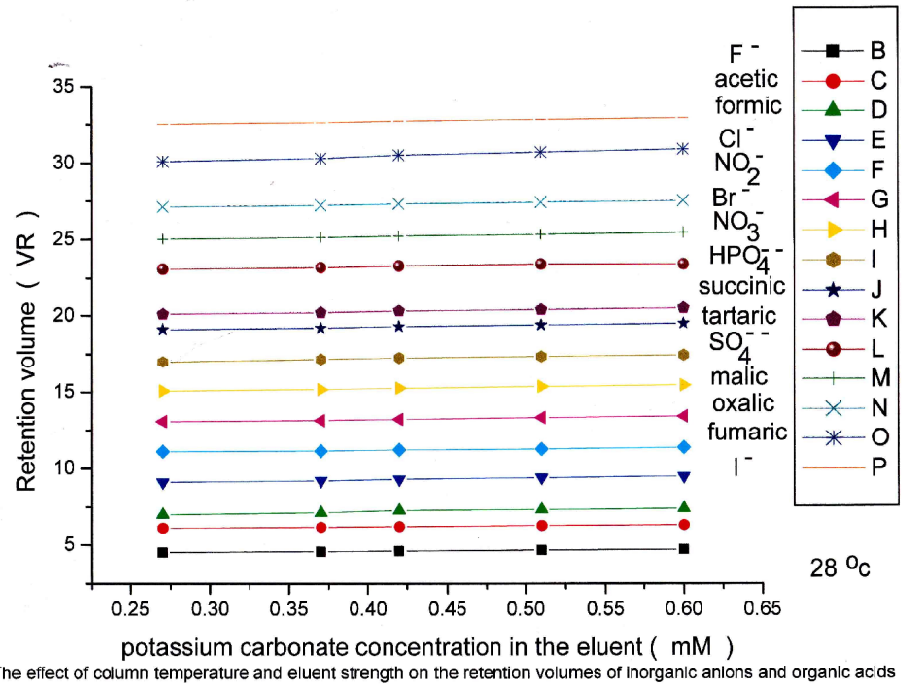


Fig - 4b .The effect of column temperature and eluent strength on the retention volumes of inorganic anions and organic acids

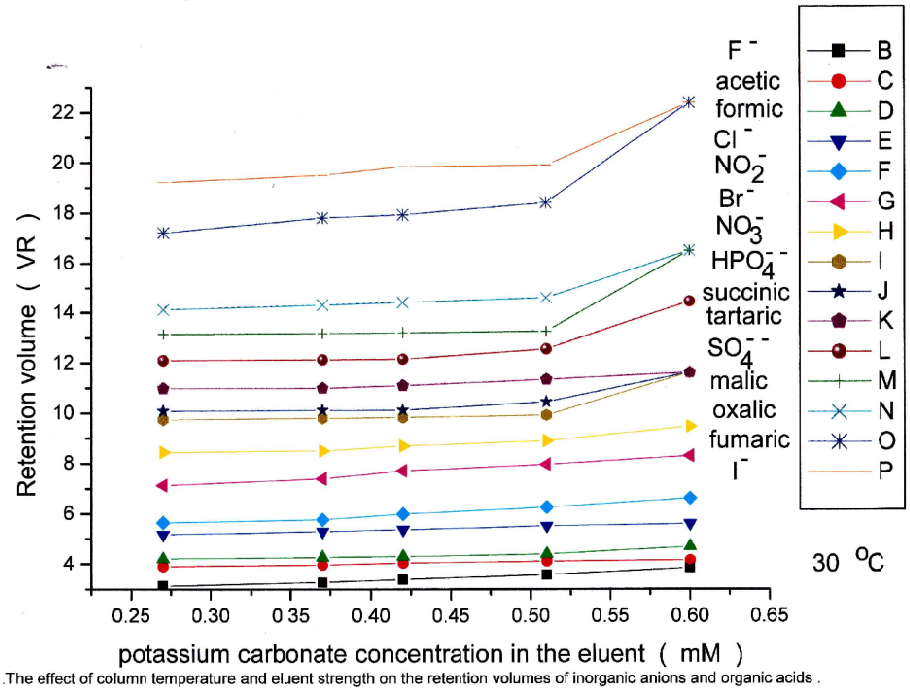
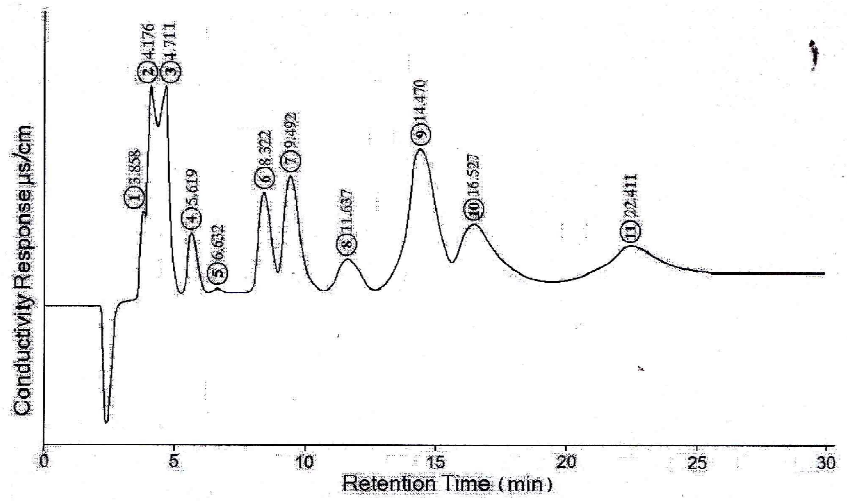
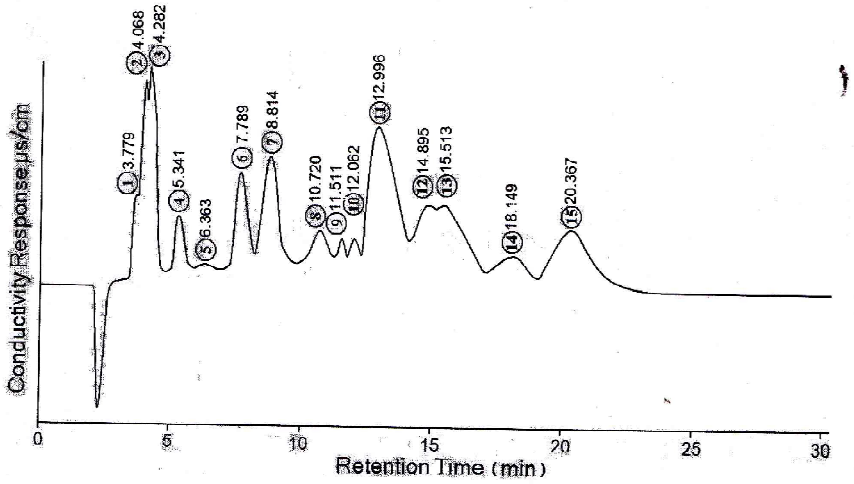


Fig 4c .The effect of column temperature and eluent strength on the retention volumes of inorganic anions and organic acids .



Figure(5): Typical resolution for a mixture of inorganic anions and organic acids . column, shim – pack IC – SA2 , temperature 30℃, eluent , mixture of 0.6 mM K_2CO_3 + 12 mM $KHCO_3$ pH 8.97;flow rate 1ml/min

Peaks : 1=F^-; 2=acetic acid;3=formic acid;4=Cl^-;5=NO_2^-;6=Br^-;7=NO_3^-;8=HPO_4^{2-} +succinic acid + tartaric acid;9=SO_4^{2-};10=malic acid + oxalic acid:11=fumaric acid+I^-



Figure(6): Typical resolution for a mixture of inorganic anions and organic acids column,shim – pack IC – SA2 , temperature 30℃,eluent , mixture of 0.6 mM K_2CO_3 + 12 mM $KHCO_3$ +0.0125 mM ETHTC (pH 8.98);flow rate 1ml/min.

Peaks : 1=F^-; 2=acetic acid;3=formic acid;4=Cl^-;5=NO_2^-;6=Br^-;7=NO_3^-;8=HPO_4^{2-} ;9=succinic acid;10=tartari acid;11=SO_4^{2-};12=malic acid;13=oxalic acid : 14=fumaric acid;15=I^-

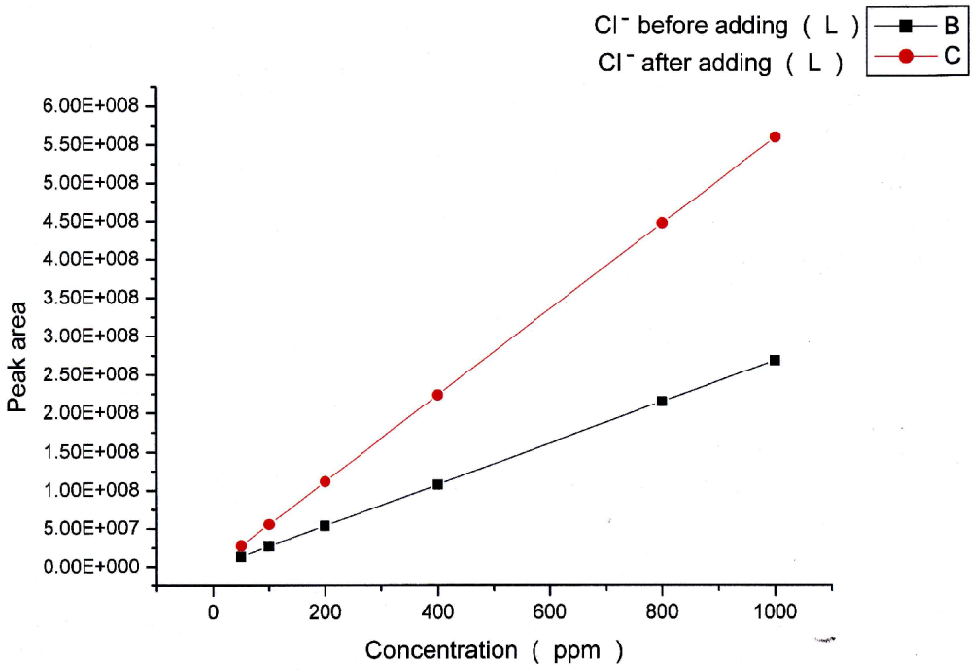
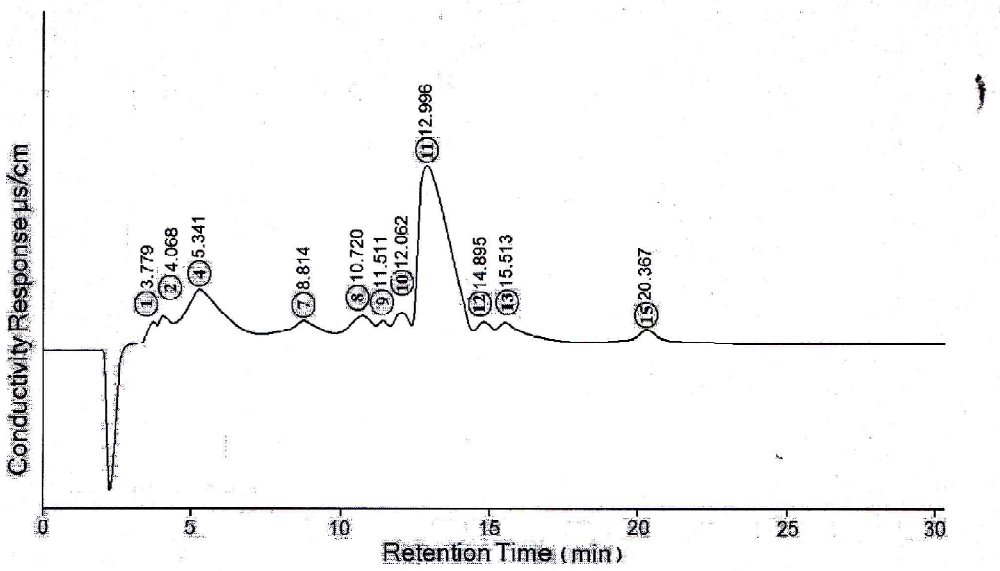


Fig - 7 .The relation between concentration and peak area (sensitivity) for Cl^- .



Figure(8) : Typical chromatogram for mixture of inorganic anions and organic acids of hibiscus (the conditions the same as in figure 7)

and powerful concentration of ETHTC was found to be 0.0125 mM. Also the results indicate that the best concentration of potassium carbonate and potassium hydrogen carbonate in the presence of ETHTC was found to be 0.6 mM and 12 mM respectively.

In addition the results indicate that the ETHTC caused several effects. Which are reducing the retention time (Fig. 5 and Fig. 6) and solving the three overlap mentioned before which is the overlap between HPO_4^{2-}, succinic acid and tartaric acid also the overlap between malic acid and oxalic acid and the overlap between fumaric acid and I^- (Fig. 5 and 6), moreover the sensitivity of inorganic anions and organic acids has been improved. Fig. (7) shows the sensitivity of before and after adding ETHTC, which exhibit that the sensitivity of Cl^- increased by adding ETHTC to the content of mobile phase.

On comparing our results with data of both Yoshikowa *et al.*[12] and Krata *et al.*[6] we found our results more satisfactory. Yoshikawa *et al.*[12] investigated the separation of six organic acids (acetic acids, lactic acid, succinic acid, malic acids, tartaric acid and citric acid) and four inorganic anions (Cl^-, NO_2^-, NO_3^- and SO_4^{2-}) using 2.0 mM benzoic acid + 1.2 mM tris aminomethane as eluent. The results indicate that the time of separation is around 30 minutes.

Krata *et al.*[6] also investigated the separation of two organic acids (acetic acid and formic acid) and seven inorganic anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, and SO_4^{2-}) using mixure of 1.0 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 as an eluent. The results revealed that the time of separation is shorter than 17 minutes.

On the other hand our studies involve the separation of seven organic acids (acetic acid, formic acid, succinic acid, tartaric acid, malic acid, oxalic acid and fumaric acid) and eight inorganic anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-} and I^-) and the time of separation is 20.367 minutes.

The results suggest that our method gives better results than that done by both Yoshikawa *et al.*[12] and krata *et al.*[6].

In addition in our pervious paper[22] we investigated the separation of seven organic acids (acetic acid, formic acid, succinic acid, tartaric acid, malic acid, oxalic acid and fumaric acid) and eight inorganic anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-} and I^-) using 0.6 mM $K_2CO_3^-$ + 12 mM $KHCO_3^-$ + 0.05 mM 18 – crown -6 as eluent, the results revealed that the time of separation 18.588 minutes.

This means that the results obtained by our previous paper[22] gives better results than the use of ETHTC as a composition of the mobile phase.

In conclusion the presence of ETHTC improve the degree of resolution, solving the overlap between the ions, increases the

sensitivity and decreasing the retention time from 22.411 to 20.367 minutes.

The detection limits for various inorganic anions and organic acids are given in table (4). The detection limits obtained by using mixture of 0.6 mM potassium carbonate + 12 mM potassium hydrogen carbonate + 0.0125 mM ETHTC pH 8.98 are lower several times than those obtained by 0.6 mM potassium carbonate + 12 mM potassium hydrogen carbonate pH 8.97. Table (4) shows that the calibration graph for all analytes were linear, with regression coefficient (r^2) of 0.9991 – 0.9998.

3.6: 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 and organic acids in Roselle (Hibiscus sabdariffa L.).

Therefore the new established method by using the mobile phase consisting of 0.6 mM potassium carbonate + 12 mM potassium hydrogen carbonate + 0.0125 mM ETHTC has been successfully applied to the separation and determination of inorganic anions and organic acids in Roselle (Hibiscus sabdriffa L.).

Table (5) summarizes the determination and reproducibility of inorganic anion and organic acids (n = 10) in Roselle (Hibiscus sabdriff L.) sample. It is quiet eviedent from the data presented in table (5) that the Roselle (Hibiscus sabdriffa L) Sample contains six inorganic anions ($F^-, Cl^-, NO_3^-, HPO_4^{2-}$, SO_4^{2-} and I^-) and five organic acids (acetic acid, succinic acid, tartaric acid, malic acid and oxalic acid), with the absence of formic acid, NO_2^-, Br^- and fumric acid, in addation the data revealed that there are variation in concentration of each aralytes presented in the sample and succinic acid was the most abundant analytes while the lowest was for the F^-. In addition the results shows the relative standard deviation (RSD%) below 1.36%.

The chromatogram of inorganic anions and organic acids presented in Roselle (Hibiscus sabdariffa L.) sample is shown in figure (8).

4. Conclusions:

The present study was attained to develop a simple, accurate and rapid analyticl methodology for the simultaneous determination of common inorganic anions, F^-, Cl^-, $NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}, I^-$, and organic acids, acetic acid, formic acid, succinic acid, tartaric acid, malic acid, oxalic acid, fumaric acid in Roselle (Hibiscus sabdriffa L.) The proposed method has several advantages of sensitivity, low detection limit, linearity, reproducibility and the importance of the ligand (ETHTC) in the composition of mobile phase.

The data revealed the nutritional value of Roselle (Hibiscus sabdriff L.).

References

1. P.E. Jackson, D. H. Thomas, B. Donovan, C.A. Pohl, R. E. Kiser, J. Chromatogr. A 920, 51 (2001).
2. R.Garcia–Fernandes, J.I. Garcia–Alonso, A. Sanz-Medel, J. Chromotogr. A 1033,

127 (2004).
3. Shaker J. Azhari, *Ultra Science* Vol. *16 (3)*, 319–328 (2004).
4. J.L. Jaffrezo, N. Calas, M. Bouchet, Atomos. Environ. 32, 2705 (1998).
5. X. Lee, D. Qin, G. Jiang, H. Zhou, Cold Regions Sci. Technol. 34, 127 (2002).
6. Agnieszka Krata, Velichka Kontozova – Deutsch, Laszlo Bencs, Felix Deutsch, Renc Van Grieken, Talanta 79, 16-21 (2009).
7. T. Jauhiainen, J. Moore, P. Peramaki, J. Derome, K. Deroms, Anal. Chim. Acta 389, 21 (1999).
8. Y.S. Fung, K. M. Lau, Talanta 45, 641 (1998).
9. J.A. Morales, L.S. de Graterol, H. Velasquez, M. G. de Nava, B.S. de Borrego, J. Chromatogr. A 804, 289 (1998).
10. S.R. Souza, M. F. M., Tavares. L. R. F., de Garvalho, J. Chromatogr. A 796. 335 (1998).
11. V. Kontozva – Deutsch, A. Krata, F. Deutsch, L. Bencr, F. Van Grieken, Tlanta 75, 418 (2007).
12. K.J. Yoshikawa, M. Okamura, M. Inokuchi, S. Akio, Talanta 72, 305–309 (2007).
13. S. N. Walford, J. Chromatogr. A 956, 187 (2002).
14. Z. Yan, Y. Y. Guo, M. L. Ye, F. S. James, J. Chromatogr. A 1085, 143 (2005).
15. Z.F. Chen, B.W. Darvell, V.W.- H. Leung, Arch. Oral Biol. 49, 863 (2004).
16. Shaker J. Azhari, International Jour. Chemistry, Vol. 15, No 3, 163-178 (2005).
17. Shaker J. Azhari, Mansoura Journal of Chemistry, Vol. 32 (2), December (2005).
18 Shaker J. Azhari, International Jour. Chemistry, Vol. 15 No. 4, 221- 235 (2005).
19. Shaker J. Azhari, *International Jour. Chem.* Vol *18*, No. 3, 117–128 (2008).
20. Shaker J. Azhari, *Ultra Chemistry* Vol. *7(1)*, 51–64 (2011).
21. Shaker J. Azhari, *Oriental Journal of Chemistry* Vol 28 No. 01 (2012).
22 Shaker J. Azhari, *Mansoura Jounal of Chemistry*, Vol. 45(1), (2012).
23. A. Al cazar, P. L. Fernandez – Caceres, M. J. Martin, F. Pablos, A. G. Gonzalez, Talanta 61, 95-101 (2003).
24. M.Y. Ding, P. R. Chem, G.A. Lou, J. Chromatogr. A, 764, 341–345 (1997).
25. P. Masson, J. Chromatogr. A, 881, 387–394 (2000).
26. Jaana M. Kakola, Raimo J. Alen, Jukka Pakka Isoaho, Rose B. Matilainen, J. Chromatogr. A 1190, 150-156 (2008).
27. Xiumei Geng, Sufang Zhang, Qian Wang, Zongbao kent Zhao, J. Chromatogr. A, 1192, 187–190 (2008).
28. L. Valentin – Blasini, B.C. Blount, A. Delinsky, J. Chromatogr. A, 1155 1032 (2007).
29. B.S. Yu, Q. G. Yuan, L.H. Nie, S.Z. Yao, J. Pharm. Biomed. 25, 1027-1032 (2001).
30. H. E. Aribi, Y.J.C.L. Blanc, S. Antonsen, T. Sakuma, Anal. Chim. Acta 567 39 (2006).
31. J.M. Doyle, B.R. McCord, J. Chromatogr. B 714, 105 (1998).
32. T. Kishi, J. Nakamura, H. Arai, Electrophresis 19, 3 (1998).
33. R. Saari – Nordhaus, J.M. Anderson, Jr, Anal. Chem. 64, 2283 (1992).
34. J. Cheam, J. Chromatogr. 482, 381 (1989).
35. V. Cheam, Analyst 117, 1137 (1992).
36. M.M. Mostufa, Spectrochimica Acta Part A 66, 480-486 (2007).