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SIMULTANEOUS DETERMINATION OF INORGANIC ANIONS AND ORGANIC ACIDS IN BASIL (*OCIMUM BASILICUM*; L) BY ION CHROMATOGRAPHY

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ABSTRACT

Supprssed conductimetric detection ion chromatography (IC) is used for the separation and detection of inorganic anions and organic acids by anion-exchange chromatography using potassium carbonate, potassium hydrogen carbonate, 18-crown-6 as mobile phases. The effects of potassium carbonate, concentration of 18-crown-6, flow rate, column temperature and pH of the eluent have been studied. The optimized experimental conditions were applied for the determination of inorganic anions and organic acids in basil (*Ocimum Basilicum*; L) sample.

Keywords: Suppressed ion chromatography, 18-Crown-6, Basil (Ocimum Basilium), Organic acids, Inorganic acids.

INTRODUCTION

"Crown ethers" are the common name for macrocyclic polyethers become valuable tools in organic synthesis as a result of their ability to solvate alkali, alkaline-earth and transition metal ions in nonpolar aprotic solvents [**Pedersen, (1971) Gokel and Durst. et al., (1976) Valentine and Curtis. et al., (1975)**]. The synthesis of crown ethers led to Noble prize in chemistry to Pedersen [**Pedersen et al., (1967)**] 18-crown-6 is an organic compound with the formula, ($C_6H_4O_6$), and the IUPAC name is 1, 4, 7, 10, 13, 16-hexaoxacyclooctadecane. The selectivity of the crown ethers as complexing agent is due to the large size of the crown cavity, which only admits ions of comparable ionic radii. 18-Crown-6 has an estimated cavity diameter of 2.6-3.2 Å which is suitable for complexing with K^+, NH_4^+ and Rb^+. The ionic diameters of these ions are 2.66, 2.86, and 2.94 Å, respectively, **Christensen., 1971 Pedersen., 1965.**

In recent years several methods have been proposed for the simultaneous separation using chromatographic technique of organic acids and inorganic anions [**Nordhaus and Anderson (1992) V. Chem. 1989 and 1992 Masson., (2000)**].

[1] **Ding, Chem and Luo. et al, 1997 Alcazar, et al (2003), Pablos and Bose et al (2004), Ohata., Tanaka., 1999 Kwon et al (1999) Izatt et al, 1985 Mori et al, 2006 Mori., 2003 Goswami, Das., 2008 Lamb et al (2006), Ding et al (2001), Azhari., 2004 Azhari., 2005 Azhari., 2008 Krata et al (2009).** Most of the work reported the use of 18-crown-6 to improve the separation of analyte. Addition of 18-crown-6 to eluent has been known to be very effective in improving peak resolution between monovalent cations [**Ohata, Tanaka, (1999) Kwon et al., (1999a), Kwon et al., (1999b)**]. This behavior can be explained on the basis of the stability constants of the complexes formed between the alkali metal ions and 18-crown-6 absorbed on the cation exchange resin during the separation on a column (log K_{Na} = 0.80, log K_{NH4} = 1.23 and log K_K = 2.03) [**Izatt et al., (1985)**]. K^+ ion forms a very stable complex since the internal cavity size of 18-crown-6 has the same size as K^+ ion. **Mori** et al [**Mori, et al., (2006)**] reported a method to improve the resoluation of monovalent cations using 18-crown-6 which is selective to K^+ ion in the presence of 20 mM succinic acid as eluent. The optimum concentration of 18-crown-6 was 2 mM. Mori et al **(2003)** also investigated the influences on the V_R values of anions and cations by adding 18-crown-6 to the composition of the mobile phase. Complete separation of anions and cations $(Cl^-, NO_3^-, SO_4^{2-}, Na^+, K^+, HN_4^+, Ca^{2+}$ and $Mg^{2+})$ was achieved by elution with 15 mM tartaric acid and 2.5 mM 18-crown-6. Goswami and Das **(2008)** demonstrated that the addition of 18-crown-6 to the composition of the eluent leads to better separation of Na^+ and K^+ ions. On the other hand, **Lamb** et al **(2006)** added 2.5 mM 18-crown-6 to aqueous mobile phase of 15 mM KOH in order to improve the separation of common polarizable anions especially of perchlorate. Recent work in our

laboratory shows that the addition of 0.05 mM 18-crown-6 to 1.8 mM of potassium carbonate and 12 mM potassium bicarbonate (pH=9.24) as eluent, gives several advantages including the decrease of retention time, increasing the sensitivity of detection, linearity, reproducibility and solving the problem of overlapping inorganic anions [**Azhari., (2011)**] The aim of the present study is to establish an efficient suppressed ion chromatographic method for the simultaneous separation and determination of a mixture of inorganic anions and organic acids by optimization of chromatographic parameters (mobile phase, eluent flow-rate, pH and temperature).

EXPERIMENTAL

Apparatus

In this study the data collection were performed by IC 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$_3$. Degasser of the model 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 were used. The anion exchange column of the type Shim-Pack IC-SA2-250 mmL, X 4.0 mm ID Peek. The column oven was maintained at 30 °C.

Reagents

All the inorganic anions and organic acids in this study were of analytical grade and were purchased from BDH. 18-Crown-6 was purchased from Aldrich. Double distilled deionized water was filtered through 0.2 µm Whatman memberane.

All the standard solution eluents and reagents were prepared in double distilled deionized water and filtered through 0.2 µm Whatman membrane filter.

Sample preparation

20 g of basil (*Ocimum Basilicum;* L) was placed in a flask containing 70 ml double distilled deionized water. The mixture 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 measuring flask and double distilled deionized

water was added to the mark. This sample solution was injected into the ion chromatograph directly. Each 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 anions and seven organic acids 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 0.05 mM 18-crown-6 in addition to the above mentioned eluent at pH=8.98, flow rate 1 ml/min and at 30 °C.

RESULTS AND DISCUSSION

Effect of potassium carbonate concentration

In order to separate simultaneously inorganic anions and organic acids, the effect of the concentration of potassium carbonate in the eluent on the retention behavior is investigated. The retention volumes of eight inorganic anions and seven organic acids are studied at five different potassium carbonate concentration (0.26, 0.36, 0.46, 0.56 and 0.6 mM).

Fig. 1 shows the variation of retention volumes for fifteen different analyte as a function of potassium carbonate concentration in the mobile phase. It can be seen that the retention volumes decreased steadily for F^-, acetic, formic acids, $Cl^-, NO_2^-, Br^-, NO_3^-$ and HPO_4^{2-} ions when potassium carbonate concentration is increased, whereas more significant decreases in the retention volumes observed for succinic, tartaric, malic, oxalic, fumaric acids, SO_4^{2-} and I^- ions. The optimum concentration selected was 0.56 mM, for potassium carbonate providing a good separation at reasonable elution time, and after this concentration three overlapped has been observed. The first overlapped between HPO_4^{2-}, succinic and tartaric acids, the second overlapped between malic and oxalic acids, and the third overlapped between fumaric acid and I^- ion. The three mentioned overlapped have been observed at concentration of 0.6 mM for potassium carbonate.

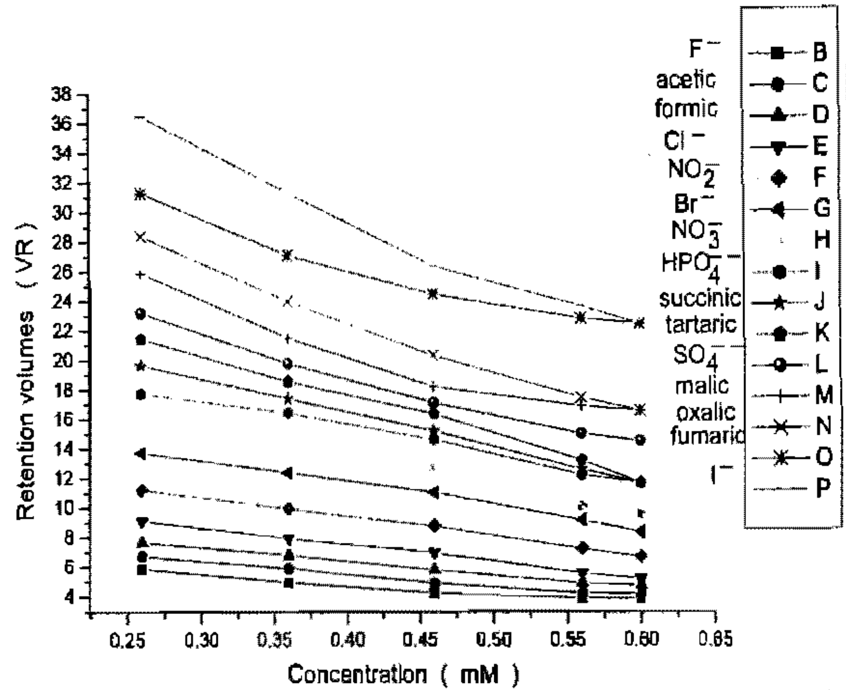


Fig -1Effect of potassium carbonate concentration in the eluent On the retention volumes of inorganic anions and organic acids

Effect of flow rate

Different flow rate values were used in this study ranging from (0.4-1.0 ml/ min) in order to achieve the optimum resolution time. From Fig (2) it can be concluded that the retention volumes of inorganic anions and organic acids under investigation decrease with increasing the flow rate, and the retention volumes were decreased steadily for F^- and acetic acid at all values of flow rate studied (0.4-1.0 ml/min) while the decrease of retention volumes for the following anions formic, tartaric, malic and fumaric acids, Cl^- and NO_2^- ions were sharply decreased at flow rates ranging between 0.4-0.95 ml/min but the decreases were slightly at the flow rates between (0.95-1.0 ml/min) for all the mentioned above anions. Also, the decrease of retention volumes were quite sharp for, I, SO_4^{2-} ions and oxalic acid, at all values of flow rates investigated (0.4-1.0 ml/min). Therefore, it can be concluded that the optimum flow rate is 0.95 ml/min for all anions under investigation since there are three overlapped has been observed after 0.95 ml/min (at 1.0 ml/min.). The first overlap between HPO_4^{2-} , succinic and tartaric acids, the second overlap between malic and oxalic acids, and the third overlap between fumaric acid and I^- ions. The solution of these three overlaps will be discussed in details on using 18-crown-6.

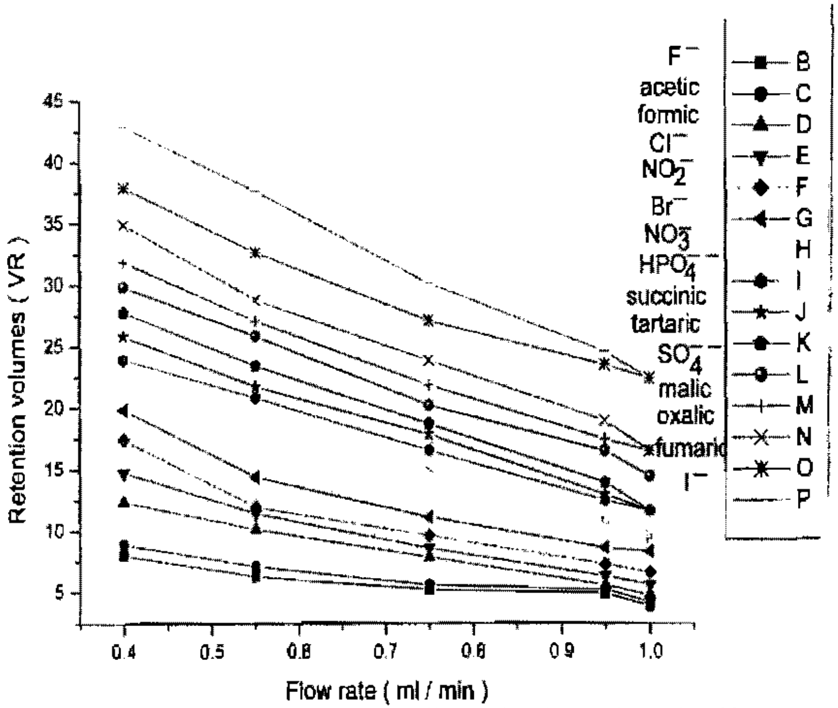


Fig -2.The effect of flowr rate on the retention volumes of inorganic anions and organic acids .

Effect of pH on retention behavior of inorganic anions and organic acids

Fig. 3 shows the relationship between the pH of the eluent and the retention volumes of eight inorganic anions and seven organic acids at five different pH values (8.64, 8.74, 8.84, 8.93 and 8.97). The results show the variation of retention volumes for inorganic anions and organic acids under investigation as a function of the pH values in the mobile phase. The retention volumes were slightly decreased for acetic, formic acids, F^-, $Cl^-, NO_2^-, Br^-, NO_3^-$, and HPO_4^{2-} when the pH is increased, whereas sharp decrease in the retention volumes are observed for succinic, tartaric, malic, oxalic, fumaric acids, I^- and SO_4^{2-} ions. The optimum pH selected was 8.93 which provided a good separation at reasonable elution time while after this there are several overlaps appeared. The first overlapped between HPO_4^{2-}, succinic and tartaric acids, the second overlapped between malic and oxalic acids and the third overlapped between fumaric acid and I^- ion. These three overlaps have been appeared at pH 8.97, the solution of these three overlapped will be discussed on using 18-crown-6.

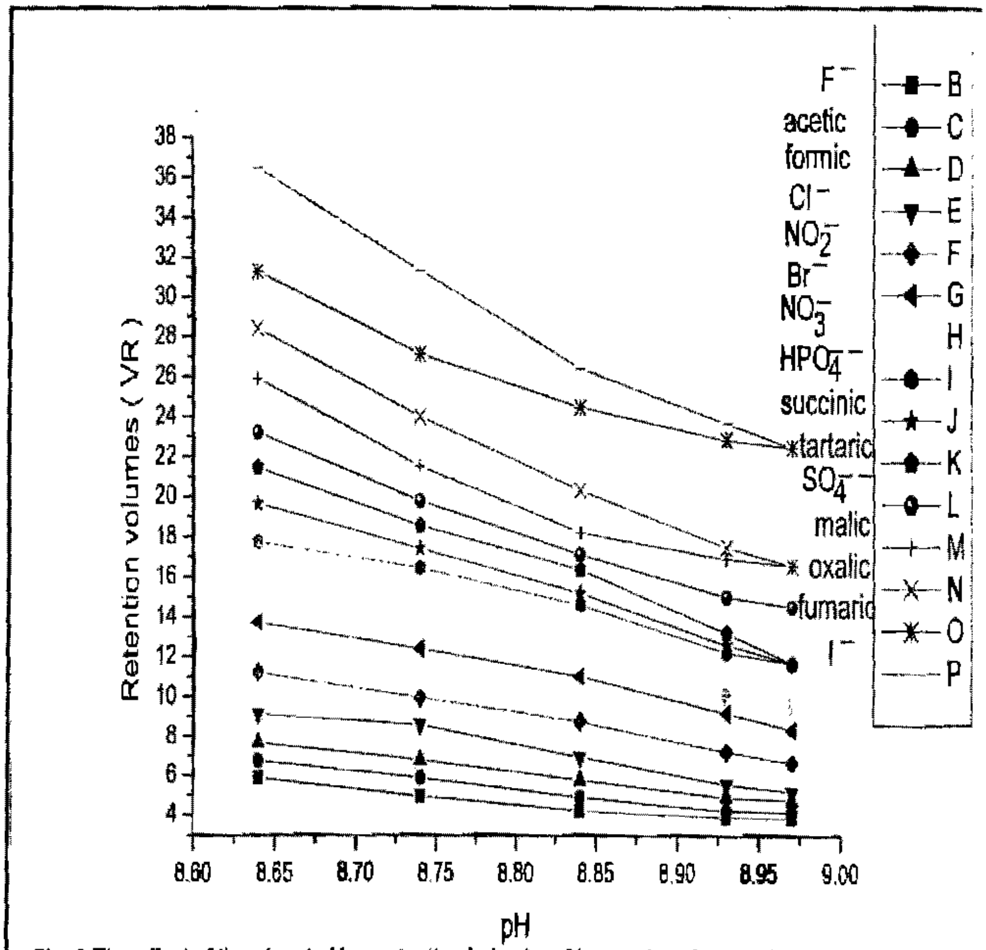


Fig -3.The effect of the eluent pH on retention behavior of inorganic anions and organic acids .

Effect of column temperature and eluent strength on the retention volume

Three different temperatures 24, 27 and 30 °C were used to study the influence of column temperature and eluent strength on the retention volume of the analytes under investigation.

[2] From the data in Table 1 it can be concluded that at all three temperature 24, 27 and 30 °C the retention volumes of all inorganic anions and organic acids under studies increase with increasing the eluent concentration from 0.26 up to 0.6 mM. The magnitude of the increase is in the flowing descending order: I^- > fumaric acid > oxalic acid > malic acid > SO_4^{2-} > tartaric acid > succinic acid > HPO_4^{2-} > NO_3^- > Br^- > Cl^- > formic acid > acetic acid > F^-. Also, the retention volumes for the five eluent strength decrease when the column temperature is increased from 24 to 30 °C and the magnitude of the decrease is in the following descending order, I^- > fumaric acid > oxalic acid > malic acid > SO_4^{2-} > HPO_4^{2-} > tartaric acid > succinic acid > NO_3^- > Br^- > Cl^- > formic acid > acetic acid > F^-. The increase in the retention volumes for inorganic anions and organic acids under investigation are steady at the temperature 24 and 27 °C while the increase in retention volumes at 30 °C is sharp at the concentration between 0.56-0.6 mM for the following anions, HPO_4^{2-}, succinic, SO_4^{2-} malic acid, oxalic, fumaric acids and I^-. Moreover, the results indicate that at concentration of 0.6 mM for potassium carbonate at 30 °C gives the shortest retention volumes and the peaks overlap has been appeared at 30 °C in three positions. The first overlap between HPO_4^{2-}, succinic and tartaric acids, the second overlap between malic and oxalic acids and third overlap between fumaric acid and I^- ion. On comparing these results with **Qiu's** method (2002) it can be observed that the process of selection of temperature and eluent strength is very easy. Furthermore, in the present results enable to reduce the retention volumes for the analytes under investigation from 36.536 to 22.411.

Table (1): 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^-
24°C	0.26	8.342	9.413	10.423	12.513	14.622	16.517	18.517	20.519	22.604	24.513	26.602	28.541	30.603	34.078	36.141
	0.36	8.421	9.472	10.517	12.609	14.675	16.581	18.586	20.618	22.673	24.611	26.69	28.638	30.698	34.181	36.233
	0.46	8.586	9.531	10.631	12.672	14.701	16.687	18.629	20.717	22.781	24.716	26.789	28.744	30.713	34.267	36.345
	0.56	8.669	9.645	10.721	12.721	14.762	16.741	18.733	20.816	22.878	24.814	26.877	28.834	30.816	34.359	36.439
	0.6	8.775	9.725	10.828	12.838	14.842	16.874	18.884	20.92	22.986	24.913	26.968	28.951	30.921	34.447	36.536
27°C	0.26	6.108	7.134	8.11	10.152	12.135	14.011	16.106	18.021	20.041	21.035	24.038	26.045	28.048	31.62	33.541
	0.36	6.131	7.176	8.146	10.213	12.141	14.153	16.184	18.186	20.143	21.134	24.134	26.142	28.132	31.698	33.636
	0.46	6.148	7.251	8.267	10.271	12.253	14.259	16.279	18.279	20.239	21.229	24.229	26.239	28.237	31.722	33.744
	0.56	6.215	7.312	8.313	10.317	12.317	14.368	16.381	18.367	20.337	21.317	24.331	26.329	28.341	31.819	33.833
	0.6	6.316	7.384	8.419	10.473	12.422	14.417	16.477	18.47	20.429	21.416	24.427	26.447	28.446	31.915	33.929
30°C	0.26	3.108	3.911	4.212	5.122	5.651	7.135	8.453	9.749	10.018	10.995	12.109	13.153	14.122	17.213	19.234
	0.36	3.245	3.973	4.287	5.279	5.763	7.319	8.512	9.815	10.132	11.011	12.148	13.172	14.311	17.792	19.463
	0.46	3.437	4.102	4.348	5.465	6.131	7.897	8.761	9.892	10.153	11.224	12.231	13.251	14.513	18.105	19.953
	0.56	3.713	4.152	4.398	5.571	6.425	8.117	9.114	10.317	10.879	11.412	13.139	13.421	15.179	18.828	20.279
	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

Effect of 18-crown-6 on retention volume (V_R) and sensitivity
The effects of adding 18-crown-6 to the mobile phase on the retention volume of inorganic anions and organic acids under investigation are studied. The results show that the degree of resolution of inorganic anions and organic acids are improved by adding 18-crown-6 to the eluent mixture. Different concentrations of 18-crown-6 (0.025-075 mM) are used. The results reveal that the efficiency of separation process is increased with decreasing the concentration of 18-crown-6 and the most efficient and powerful concentration of 18-crown-6 is found to be 0.05 mM. Also, the results indicate that the best concentration of potassium carbonate and potassium hydrogen carbonate in the presence of 18-crown-6 is found to be 0.6 mM and 12 mM, respectively. In addition the results indicate that the 18-crown-6 causes several effects which are reducing the retention time (Figs. 5 and 6) and solving the three overlap mentioned before between HPO_4^{2-}, succinic and tartaric acids. Also, the overlap between malic and oxalic acids as well as the overlap between fumaric acid and I^- ion, (Figs. 5 and 6) and the sensitivity for inorganic anions and organic acids has been increased. Fig. 7 shows of the sensitivity of Cl^- before and after adding 18-crown-6. The results exhibit that the sensitivity of the Cl^- ion increased by adding 18-crown-6 to the content of mobile phase. On comparing our results with the data reported by **Yoshikawa** et al **(2007)** and Krata et al **(2009) Yoshikawa (2007)** investigated the separation of six organic acids (acetic, lactic, succinic, malic, tartaric, citric acids) and four inorganic anions (F^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}) using 2.0 mM benzoic acid + 1.2 mM tris-aminomethane as eluent. Also, the results revealed that the time of separation is about 30 minutes.

Also, **Krata** et al **(2009a) Krata et al (2009b)** studied the separation of two organic acids (acetic, formic) and seven inorganic anions (Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}) using mixture of 1.0 mM $NaHCO_3$ and 3.2 mM Na_2CO_3 as an eluent. The results indicate that the time of separation is shorter than 17 minutes. On the other hand, in the present work, the separation of seven organic acids (acetic, formic, succinic, tartaric, malic, oxalic, and fumaric acids) and eight inorganic anions (F^- , Cl^-, NO_2^-, Br^- , NO_3^-, HPO_4^{2-} , SO_4^{2-} , I^-) and the time of separation was found to be less than19 minutes.

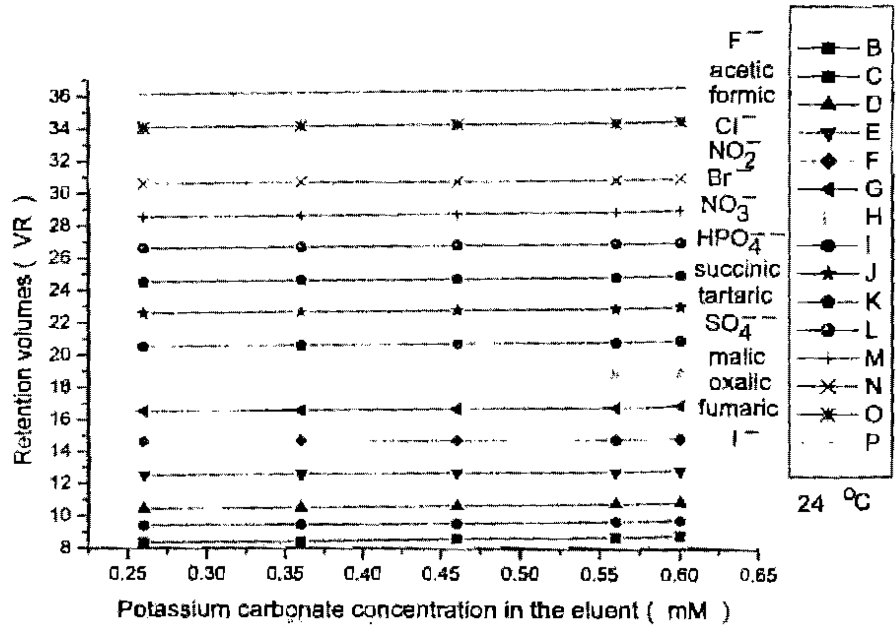


Fig . 4a the effect of column temperature and eluent strength on the retention volumes of inorganic anions and organic acids .

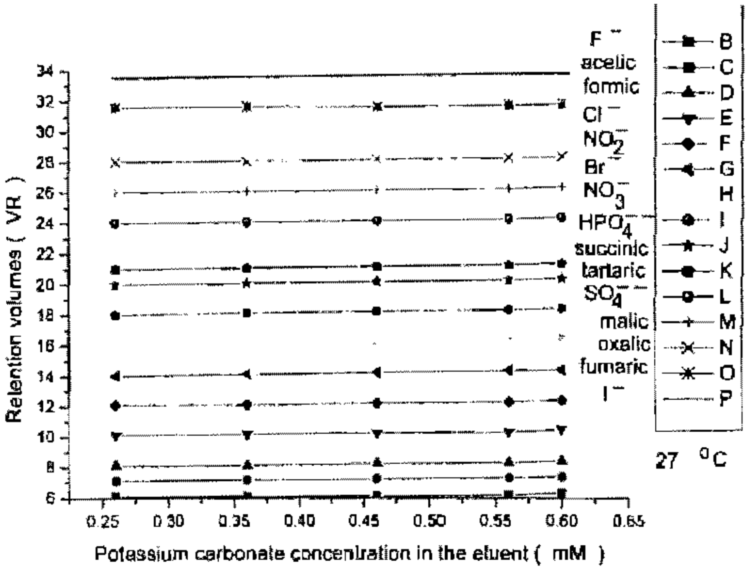


Fig 4b the affect of column temperature and eluent strength on the retention volumes of inorganic anions and organic acids

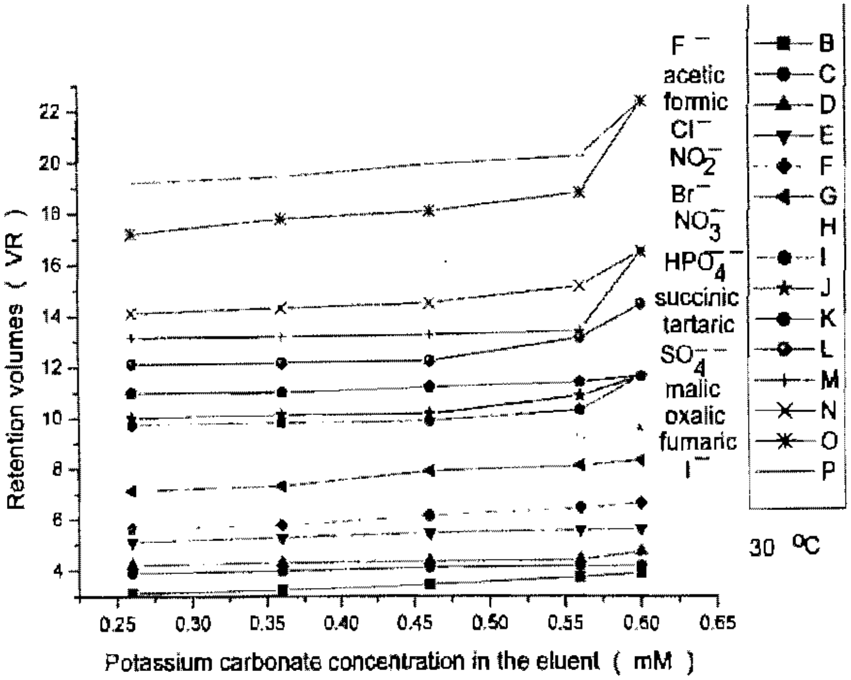
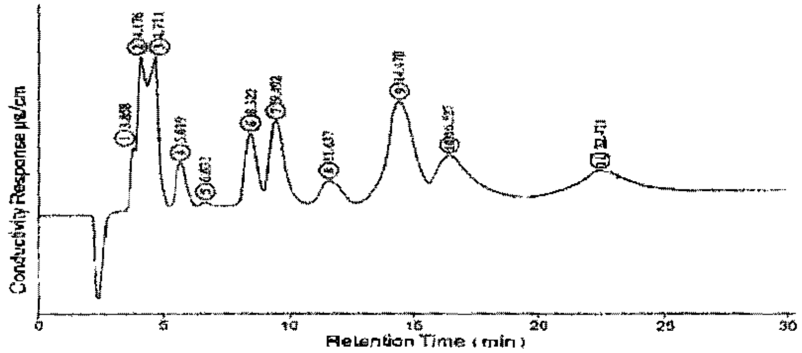
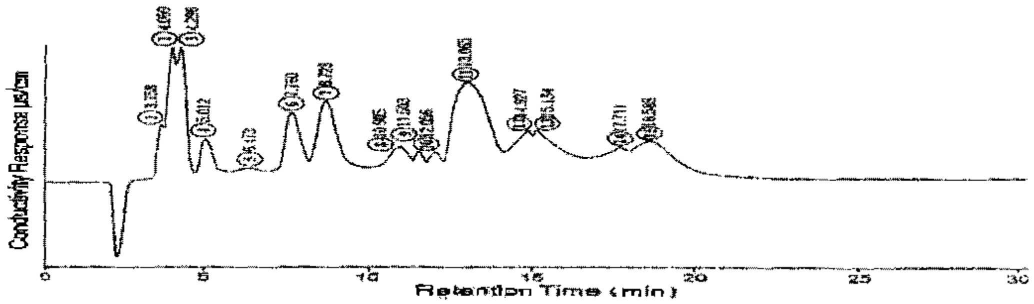


Fig .4c the effect of column temperature and eluent strength on the retention volumes of inorganic anions and organic acids .



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 organicacids . column, shim – pack IC – SA2 , temperature 30℃, eluent , mixture of 0.6 mM K_2CO_3 + 12 mM $KHCO_3$ +0.05 mM 18-crown-6(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^-

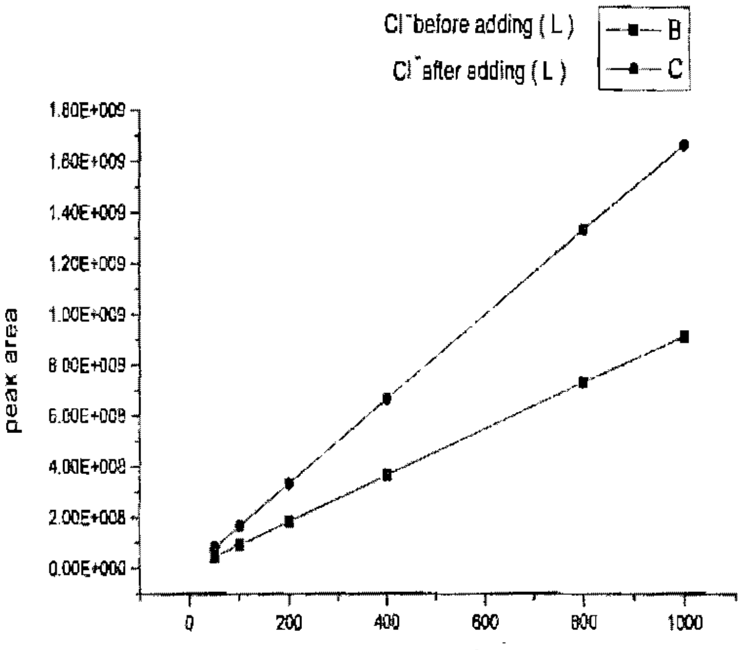


Fig - 7 : The relation between concentration and peak area (sensitivity) for Cl$^-$

In conclusion the presence of 18-crown-6 improves the degree of resolution, solving the overlap between ions, increase the sensitivity and decreasing the retention time from 22.4 to 18.6 minutes. The detection limits for various inorganic anions and organic acids are given in Table (2). The detection limits obtained by using mixture of 0.6 mM potassium carbonate, 12 mM potassium hydrogen carbonate, 0.05 mM 18-crown-6 at pH = 8.98 are lower several times than these obtained by 0.6 mM potassium carbonate + 12 mM potassium hydrogen carbonate at pH=8.97 Table (2) shows the linearity range for all analytes, with regression coefficient (r^2) ranging between 0.9991-0.9998.

Table (2): Detection Limit (S / N = 3), Lineare range and regression coefficient of inorganic anions and organic acids.

Compound	Detection Limit (mg / l)		Linearity range (mg/l)	Regression Coefficient (r^2)
	0.6 mM k_2CO_3 + 12 mM HCO_3	0.6 mM k_2CO_3 + 12 mM $KHCO_3$ + 0.05 mM 18-crown-6		
F^-	0.11	0.004	0.5 - 3000	0.9991
acetic acid	0.15	0.035	0.5 - 2000	0.9991
formic acid	0.45	0.052	0.5 - 2000	0.9994
Cl^-	0.02	0.0022	0.5 - 3000	0.9993
NO_2^-	0.13	0.11	0.5 - 2200	0.9994
Br^-	0.11	0.0022	0.5 - 2800	0.9997
NO_3^-	0.12	0.0021	0.5 - 3000	0.9998
HPO_4^{2-}	-	0.0023	0.5 - 2500	0.9994
succinic acid	-	0.0023	1 - 5000	0.9993
tartaric acid	-	0.0023	1-2000	0.9985
SO_4^{2-}	0.12	0.0034	0.5-4000	0.9997
malic acid	-	0.022	1-3000	0.9998
oxalic acid	-	0.022	1-3500	0.9992
fumaric acid	-	0.005	1-3000	0.9997
I^-	-	0.004	0.5-3200	0.9998

Application

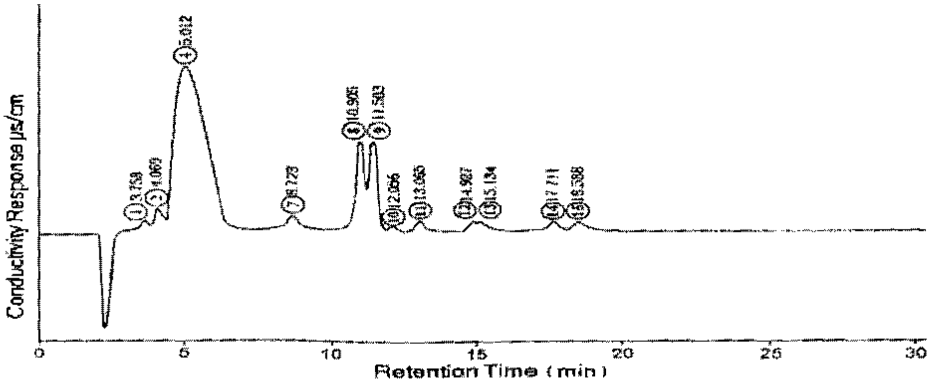
As practical example of the application of the present method, inorganic anions and organic acids in basil (*Ocimum Basilicum*; L) were analyzed. The presence of analyte is confirmed by comparing their retention time with the standard solution.

The data presented in Table (2) indicates that the sample of *basil* contains six inorganic anions (F^-, Cl^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}, I^-) and six organic acids (acetic, succinic, tartaric, malic, oxalic, fumaric acids). So the obtained results indicate that the concentration of formic acid, (F^-, Cl^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}, I^-) and six organic acids (acetic, succinic, tartaric, malic, oxalic, fumaric acids). Hence the proposed method indicates that the concentration of formic acid and NO_2^-, Br^- ions in *Basil* sample is probably lower than the limit of detection. Succinic acid is the most abundant analyte, followed by HPO_4^{2-}, tartaric, oxalic, malic, acetic, fumaric acids, SO_4^{2-}, F^-, I^-, Cl^- and NO_3^- ions. In addition Table (3) shows that the relative standard deviation (RSD) is below 1.56%. Fig (8) shows the chromatogram of inorganic anions and organic acids in *Basil* sample.

Table (3): The concentration of inorganic anions and organic acids (PPm) in basil (ocimum basilicum 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	129.4	450	ND	95.10	ND	ND	3.32	1334.5	2920	509	309.2	480	495	387	109.4
b	0.10	0.2	ND	1.29	ND	ND	0.12	1.55	1.43	1.29	0.95	0.72	0.81	0.71	0.84

(a) mean
(b) RSD %
ND: not detected



Figure(8) : Typical chromatogram for mixture of inorganic anions and organic acids of basil (ocimum basilicum L) (conditions the same as in fig 6)

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الملخص العربي

تم إستخدام تقنية كروماتوجرافيا الأيون في نظام الكشف التوصيلي المخمود لتقدير كل من الانيونات غير العضوية والاحماض العضوية عن طريق استخدام كربونات وبيكربونات البوتاسيوم وإثير الكراون (18-crown-6) كطور متحرك. وتمت دراسة العوامل التي تؤثر على عملية الفصل الكروماتوجرافي مشتملة على كل من تركيز كربونات البوتاسيوم وتركيز إثير الكراون ، ومعدل التدفق ودرجة حراره العمود ، ودرجة الحموضه .

ومن ثم تم تطبيق الشروط العملية المناسبة لتقدير ألايونات غيرالعضويه والاحماض العضويه في نبات الريحان