

**OPTIMIZATION AND SIMULTANEOUS DETERMINATION
OF ORGANIC ACIDS BY NON-SUPPRESSED ION
CHROMATOGRAPHY AND ITS APPLICATION
ON SOME MEDICINAL PLANTS (PART I)**

Shaker J. Azhari

Department of Chemistry, Faculty of Applied Sciences,
Umm Al-Qura University, P.O.Box 7605, Makkah,
Saudi Arabia

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ABSTRACT

A non-suppressed ion chromatography for the determination of some organic acids is proposed. Eight organic acids including, formic, acetic, ascorbic, succinic, lactic, citric, tartaric and oxalic acids were separated and identified. The separation was achieved on an anion exchange column (Shim pack 1C A1, 4.6 mm IDX 100mn) with a mobile phase consisting of 2.45 mM phthalic acid, 2.35 mM of tris- [(hydroxymethyl-) aminomethane and 0.2 mM of N-[(ethylamino) thioxomethyl] hydrazinocarbonylmethyl) trimethyl ammonium chloride (ETHTC). The purpose of this work is to improve the analytical parameters and to characterize the simultaneous determination of organic acids by non-suppressed ion chromatography to routine analysis. The proposed method has numerous advantages over the other widely used: 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, detection and quantization limits.

Also, the proposed method was successfully applied for the separation of some organic acids from some medicinal plants including mentha vridis, mentha longivolia, origanum majorana and cymbopogon winterianus which are existing in different places at Saudi Arabia.

1. INTRODUCTION

The determination of organic acids in medicinal leaves is very important. Their presence and relative ratio, in fact, can affect the chemical and sensorial characteristics of the matrix (e.g., pH, total acidity and microbial stability). In this context, organic acids are measured [Timpa & Burke (1986); Gansedo & Luch (1986); Wilson et al., (2002); Palmer & List (1973); PaLMER, (1955); Bengtsson & Samuelson (1972); Bengtsson & Samuelson (1971) and Saccani et al., (1995)] using enzymatic methods or liquid chromatographic techniques. Also, it has been reported that the negative peak influences the determination of acetic and lactic acids for alcoholic drinks [Ding et al., (1991)] or ascorbic acid for tea samples. In order to resolve these problems, a mixed eluent of 2.7mM of phthalic acid and (2.2mM tris + 0.2 m M of ETHTC) was used in our studies. The traditional HPLC techniques with rejection index or UV detector not always allow the separation of minor organic acids [Timpa & Burke (1986) and Gansedo & Luch (1986)].

The prime goal in this paper is to develop a simple and reproducible HPLC method for rapid separation and quantification of a group of some major organic acids. Also, this method is applied for the separation and determination of these organic acids in some medicinal leaves.

2- EXPERIMENTAL

2-1 Apparatus :

The ion chromatographic measurements were carried out using HIC-6A (Shimadzu, Japan) consisting of an LC- 10 AD liquid delivery pump, a DGU- 12 A Degasser, Rheodyne (77251) injection valve with a 20 µl 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 purchased from Shimadzu. The column oven was maintained at 40°C. Data acquisition and treatment were accomplished using a Shimadzu data system C-R7A chromatopac.

2-1-1 Reagents :

All the organic acids used in this study were of analytical reagents grade. All chemicals were purchased from BDH chemicals Ltd poole England.

N-{{(ethylamino) thioxomethyl} hydrazinocarbonylmethyl} trimethyl ammonium chloride (ETHTC) was prepared in our laboratory [Mostafa, unpublished results]. Double distilled deionized water was filtered through 0.2 μm Whatman membrane . A stock solution of 1000 mg l^{-1} was prepared for each organic acid

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

2-1-2 Sample preparation :

20 g of each one of the four medicinal samples under investigation namely, mentha longifolia (laniaceae) mint (mentha), labjatea (origznum mujorane) and gramineae (cymbopogon witerianus), was placed in a flask containing 70 ml double distilled deionized water. The mixture was heated at different temperatures (60, 70, 80, and 90 $^{\circ}\text{C}$) for 5,10, 15, 20, 25 and 30 min. After cooling, the solution was filtered through a 0.2 μm Whatman membrane filter and then the filtered solution was transferred to 100ml flask and double distilled deionized water added to 100ml. This sample solution was injected into the ion chromatograph directly. Each sample was run ten times.

2-1-3 The optimum conditions :

The optimum analytical condition have been established to separate eight organic acids 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 $^{\circ}\text{C}$. The data obtained was then compared with the eluent solution containing a mixture of 0.2 mM of N {{(ethylamino) thioxomethyl} hydrazioncarbonyl methyl} trimethyl ammonium chloride (ETHTC) in addition to the above mentioned eluent at pH = 3.63 and a flow rate 1.5 ml/min.

3- RESULTS AND DISCUSSION

3-1 Effect of phthalic acid concentration.

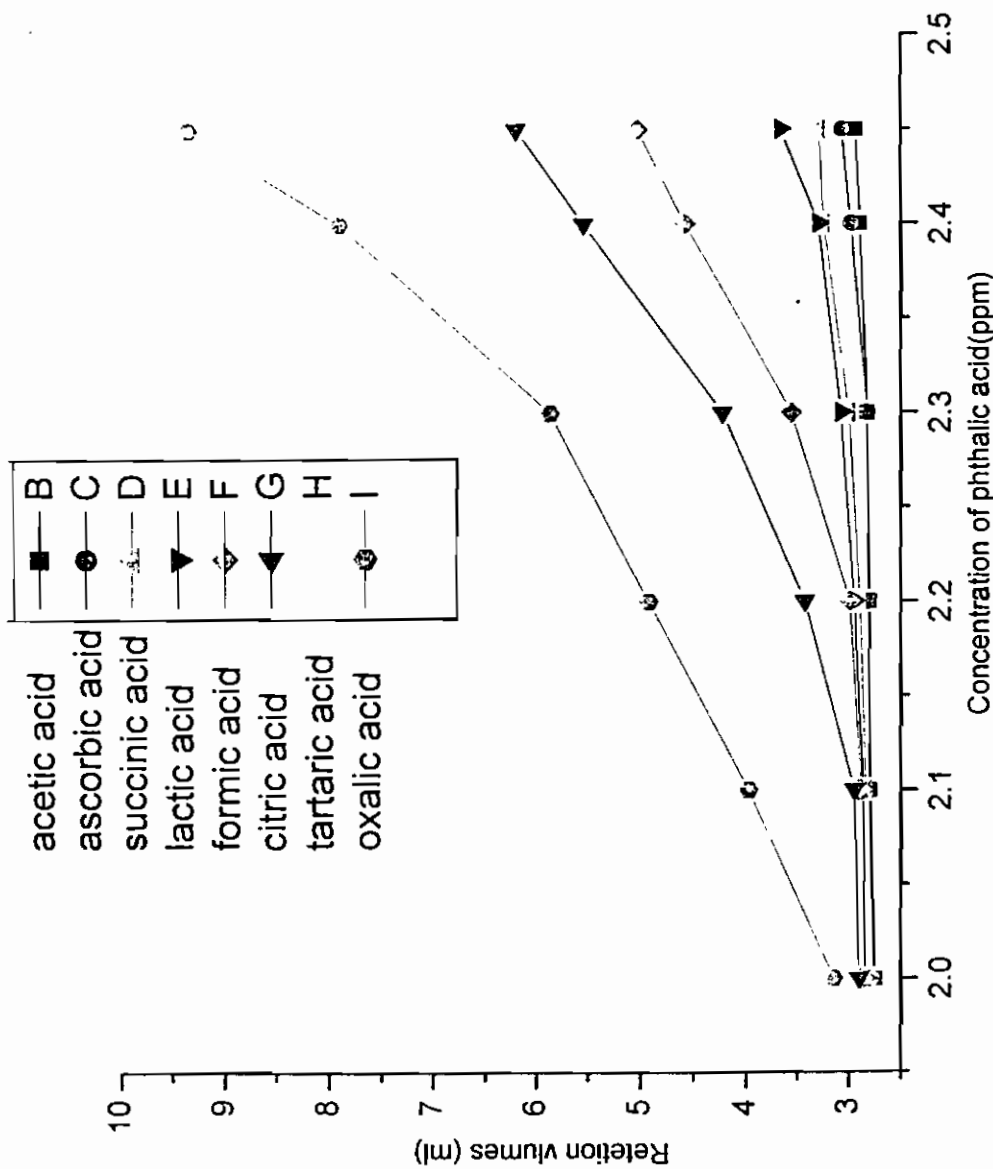
The effect of phthalic acid concentration on the retention volume (V_R) of the organic acids was investigated. Fig.1 shows that the V_R values of organic acids increase with increasing the acid concentration. The data show that the background of eluent concentration increases with increasing the concentration of phthalic acid. The best concentration was found to be 2.45 mmol.

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

The effect of flow rate on the retention time was carried out at different flow rates ranging from 0.9 to 1.5 ml/min (table 1) and represented graphically in Fig.2. The data obtained indicates that the resolution time decreases by increasing the flow rate. Also, the results show that the most efficient separation of organic acids under investigation is obtained using a flow rate of 1.5 ml/min. On increasing the flow than 1.5 ml/min., we observed that the separation process becomes difficult and overlapped.

3-3 Effect of eluent pH on the retention behaviour of organic acids.

Different pH values in the 2.82-4.84 range (table 2) were used in order to achieve the optimum resolution time. Fig.4 shows that the retention volume of organic acids decreases with increasing the pH of the eluent strength for phthalic acid by promoting the degree of dissociation ($pK_a = 2.95$ and $pK_2 = 5.41$ for phthalic acid). The results indicate that an excellent separation for the species under investigation is obtained by using $pH = 3.4$. Also, the peaks overlap has been obtained on using pH above 3.4 and the overlap reaches its maximum at $pH=4.84$ more than that observed at $pH=3.73$. The degree of resolution of organic acids is improved by adding N- {[(ethylamino) thioxomethyl] hydrazinocarbonylmethyl} trimethylammonium chloride (ETHTC). Different concentrations of ETHTC (0.2 mmol- 2.0 mmol) were used. The results indicate that the efficiency of separation process is increased with decreasing the concentration of ETHTC.



F.1.Effect of phthalic acid concentrations in the eluent on the retention volumes of organic acids.

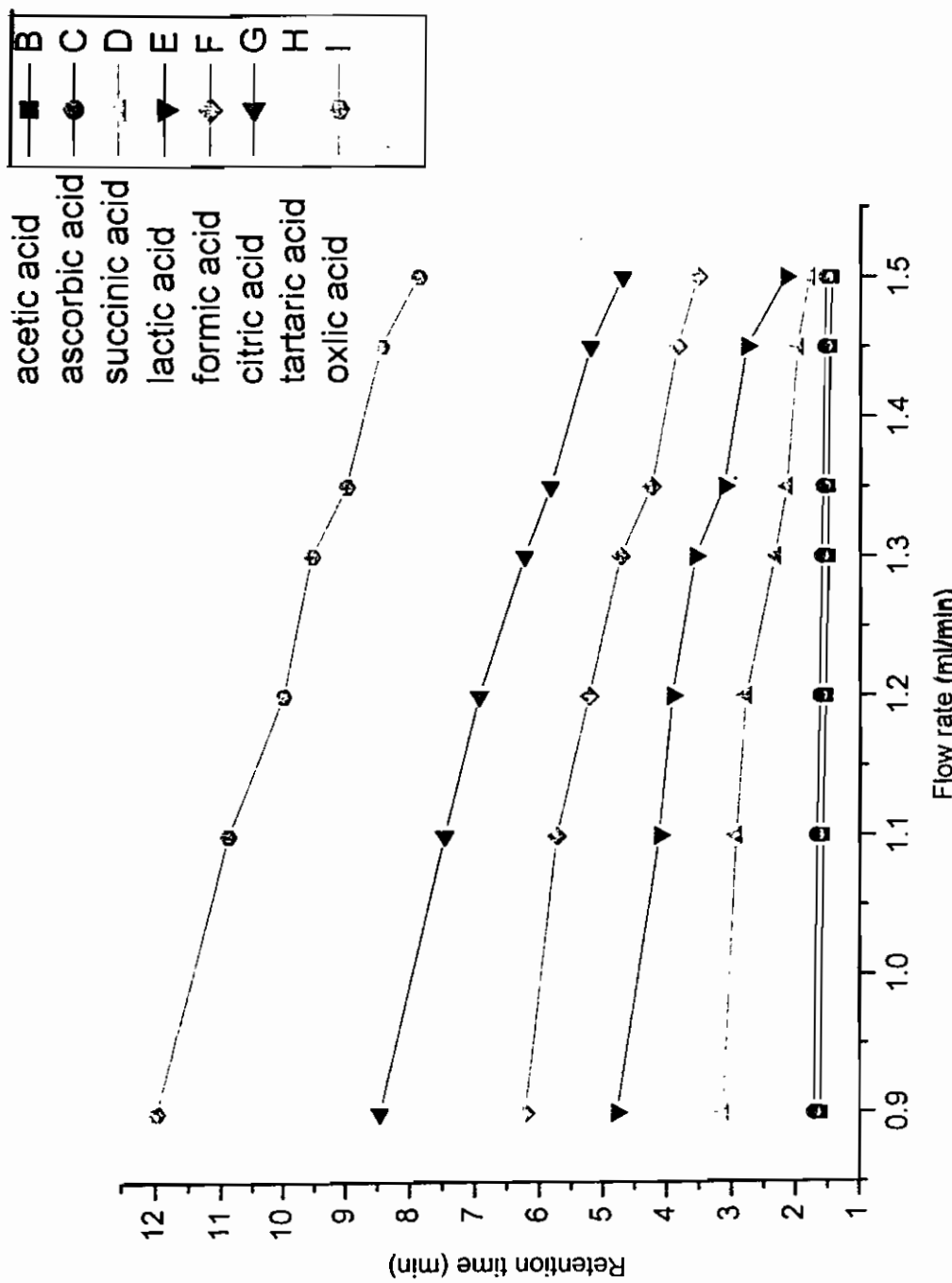


Fig.2. The effect of flow rate on retention time of investigated organic acids.

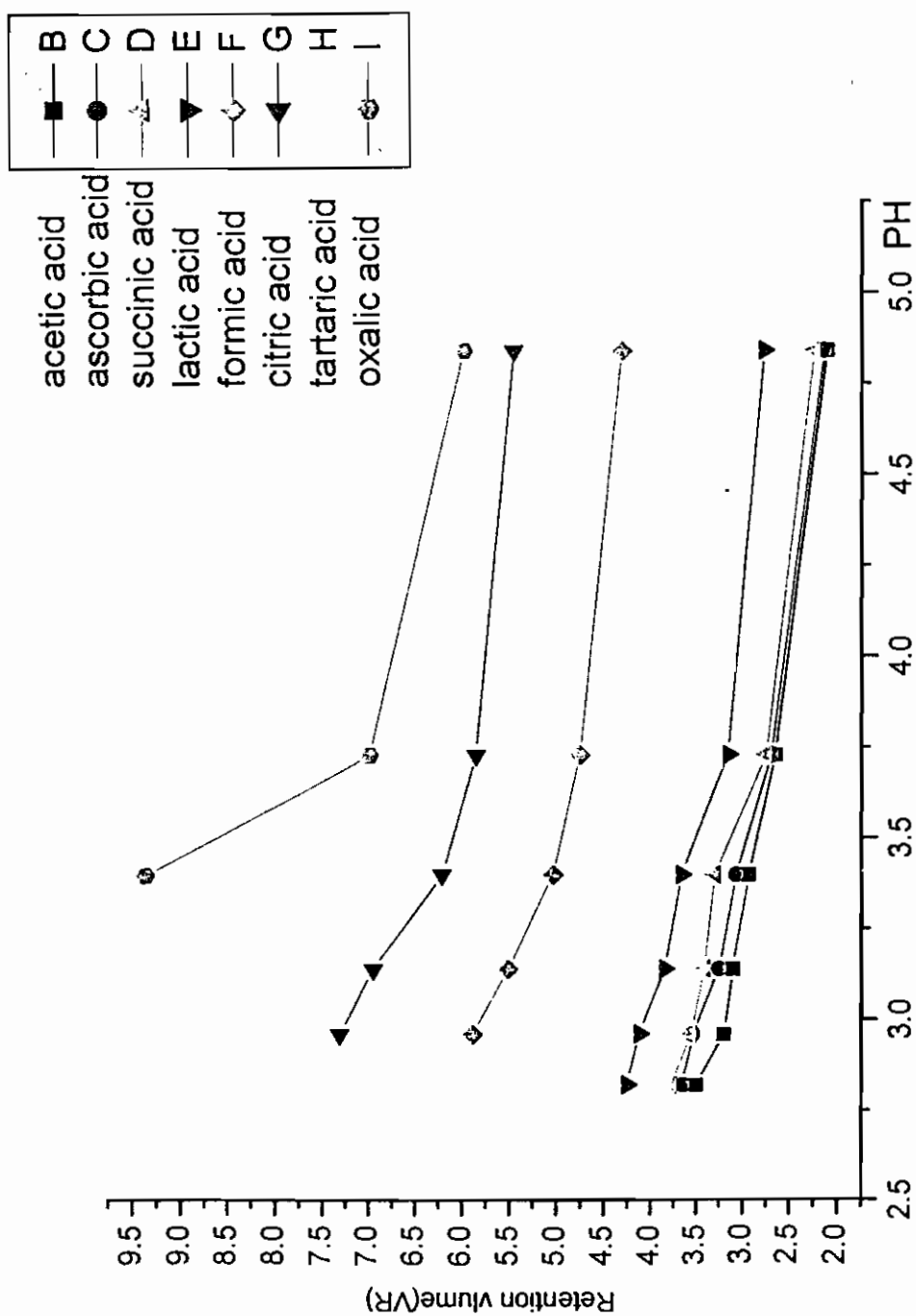


Fig.3. Effect of the eluent PH on retention behavior of organic acid

Table (1): The effect of flow rate on retention time (min R) of investigated organic acids.

Flow rate ml / min	Acetic acid	Ascorbic acid	Succinic acid	Lactic acid	Formic acid	Citric acid	Tartaric acid	Oxalic acid
0.9	1.61	1.713	3.125	4.775	6.219	8.453	10.856	11.979
1.1	1.578	1.672	2.937	4.122	5.738	7.455	9.113	10.885
1.2	1.535	1.63	2.789	3.925	5.236	6.928	8.312	9.99
1.3	1.5	1.61	2.335	3.567	4.736	6.236	7.537	9.535
1.35	1.498	1.583	2.153	3.125	4.253	5.839	6.932	8.986
1.45	1.479	1.563	1.983	2.785	3.856	5.231	6.395	8.452
1.5	1.438	1.537	1.798	2.172	3.54	4.713	5.951	7.87

Table (2): The effect of the eluent pH on retention behavior (V_R) of investigated organic acids.

pH	Acetic acid	Ascorbic acid	Succinic acid	Lactic acid	Formic acid	Citric acid	Tartaric acid	Oxalic acid
2.82	3.5	3.65	3.78	4.26	---	---	---	---
2.96	3.2	3.54	3.56	4.13	5.89	7.312	---	---
3.14	3.1	3.25	3.41	3.85	5.52	6.952	8.346	---
3.4	2.938	3.073	3.298	3.672	5.04	6.213	7.451	9.37
3.73	2.65	2.69	2.75	3.16	4.76	5.86	6.12	7
4.84	2.11	2.14	2.24	2.78	4.33	5.47	5.7	6

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

The effects of column temperature and eluent strength on the retention time are shown in Fig.4 and represented in Table 3. The retention times of organic acids increase with increasing the eluent concentration from 2.0 upto 2.45mM and with decreasing the temperature.

Table .3.The Relation between concentration of phthalic acid and TR at different temperatures.

°C	Concentration (m M)	TR									
		Acetic acid	Ascorbic acid	Succinic acid	Lactic acid	Formic acid	Citric acid	Tartaric acid	Oxalic acid		
25°C	2	1.55	1.76	1.99	2.2	2.42	2.65	2.88	3.65		
	2.1	1.87	2.79	3.23	3.67	3.96	5.22	6.8	7.23		
	2.2	2.45	3.6	3.96	4.99	5.44	6.77	8.9	9.98		
	2.3	3.26	4.75	5.42	6.34	7.59	8.88	10.77	11.44		
	2.4	3.98	5.99	6.6	8.55	9.61	10.98	12.64	13.66		
	2.45	4.998	7.76	8.93	10.22	11.19	12.93	14.54	16.59		
35°C	2	1.35	1.37	1.49	1.59	1.63	1.75	1.98	2.83		
	2.1	1.495	1.62	1.96	2.45	2.76	2.99	3.32	3.87		
	2.2	1.6	1.94	2.55	2.98	3.24	3.59	3.89	4.65		
	2.3	1.92	2.43	3.65	4.11	4.43	4.83	5.201	5.87		
	2.4	2.32	2.65	3.85	4.33	4.76	5.1	5.5	7.22		
	2.45	2.62	2.94	4.41	4.97	5.34	5.77	6.27	9.35		
40°C	2	1.25	1.27	1.29	1.34	1.36	1.4	1.62	1.65		
	2.1	1.295	1.3	1.33	1.36	1.38	1.45	1.8	2.48		
	2.2	1.3	1.31	1.39	1.45	1.49	1.93	2.39	3.43		
	2.3	1.32	1.32	1.5	1.58	2.06	2.73	3.17	4.38		
	2.4	1.395	1.395	1.75	1.8	3.09	4.06	4.48	6.42		
	2.45	1.438	1.573	1.798	2.172	3.54	4.713	5.951	7.87		

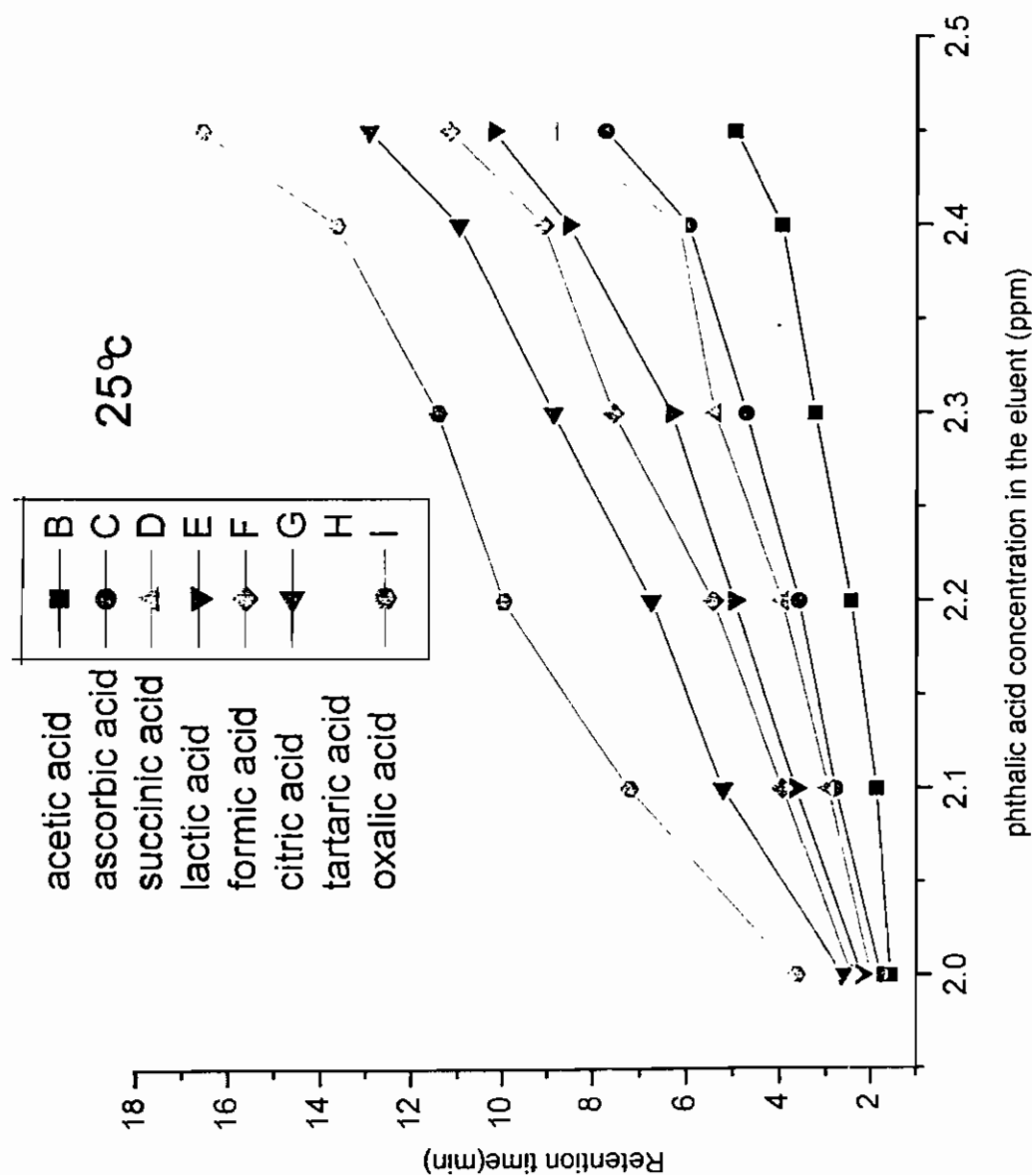


Fig.4a. The effect of column temperature and eluent strength on the retention times of organic acids.

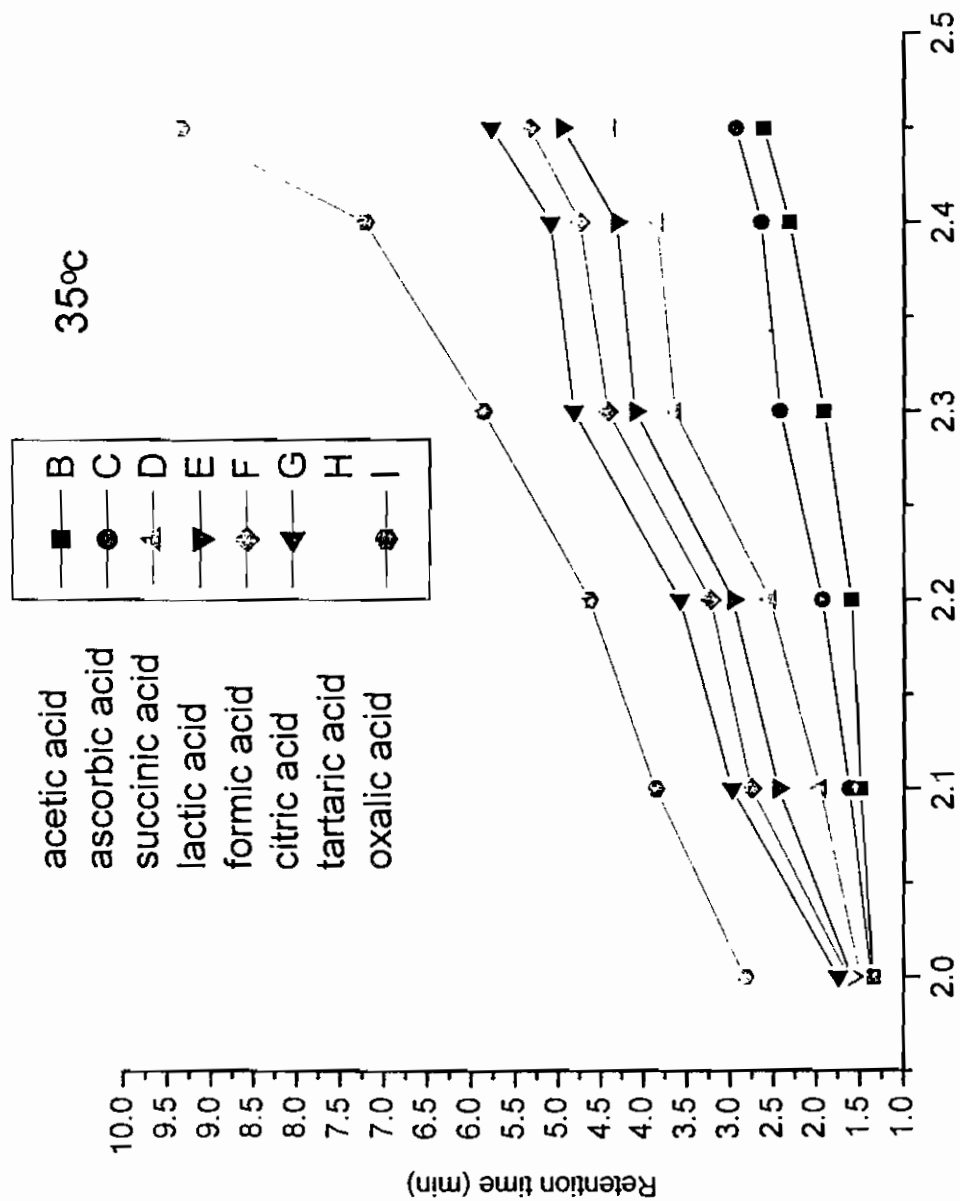


Fig .4b. The effect of column temperature and eluent strength on the retention times of organic acids .

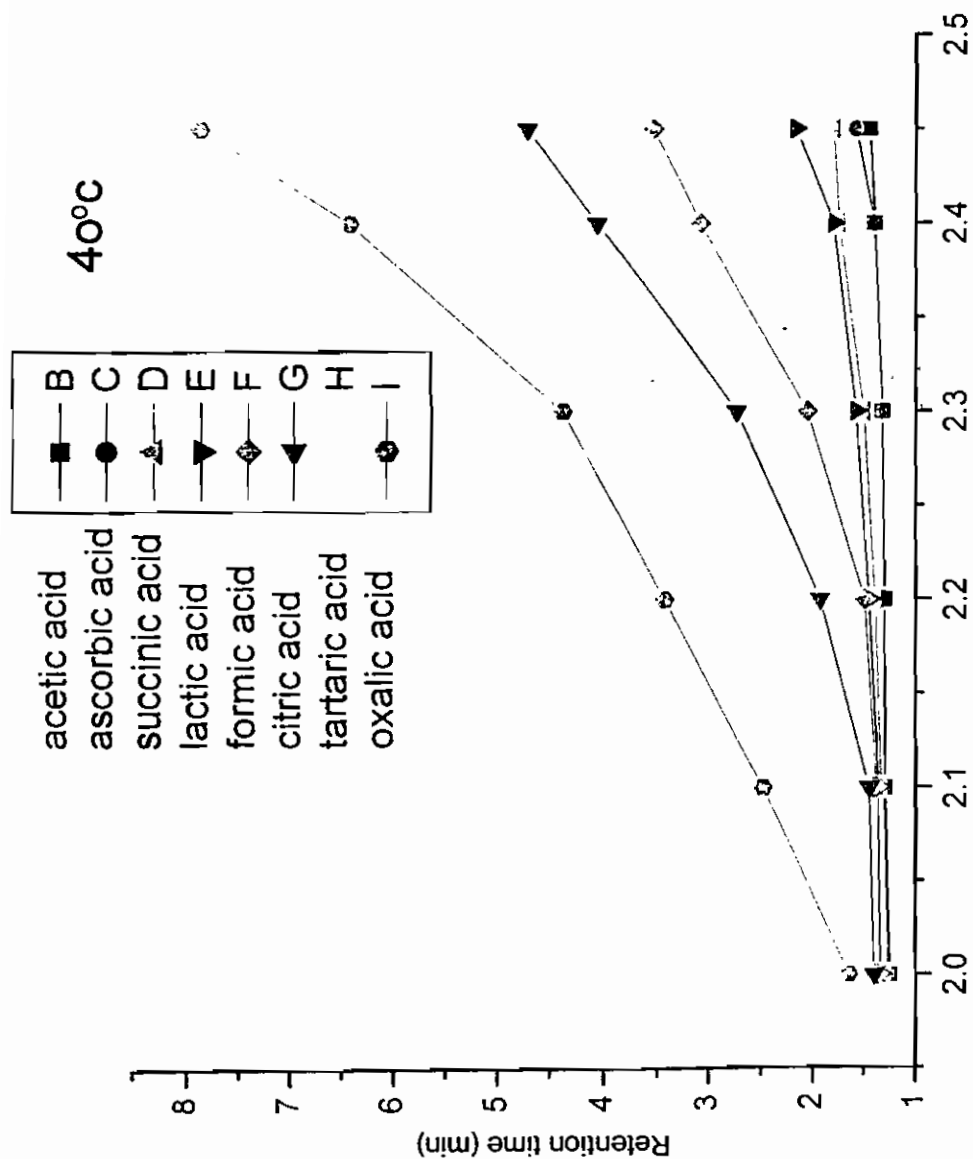


Fig .4c. The effect of column temperature and eluent strength on the retention times of organic acids .

Also, the retention times for the six eluent strength decrease when the column temperatures increase from 25 to 40°C. The results on (Fig.4) show that the resolution at 40°C and concentration 2.45 mM, gives the shortest retention time. On comparing our results with Qiu's method [Qiu & Jin (2002)], we observed that the process of selection of temperature and eluent strength is very easy. In addition, our results of the optimum conditions enable us to reduce the resolution time for the eight organic acids from 16.59 to 7.87 min .

3.5 Effect of ETHTC on tR and sensitivity .

The effect of addition of ETHTC to the mobile phase on the tR shows that the retention time is slightly decreased on adding ETHTC. The detection limits (S/N = 3) for various organic acids are given in Table 4. The results show that the detection limits obtained by using a mixture of 2.45 mM phthalic acid 2.35 mM N-hydroxy methylaminomethan and 0.2 mM of ETHTC at pH = 3.63 are efficiently lowered compared to those those obtained in the absence of ETHTC. Also, the data shows that the conductivity of the mobile phase containing ETHTC is 125.7 μ /cm while the conductivity reaches 107.8 μ /cm in the absence of ETHTC. Such increase in conductivity may be due to the lowering in the background conductivity [Ding et al., (1997)] leads to the increase in detection sensitivity. On the other hand , our results show that the increase of background conductivity will led to increase. In detection sensitivity. This is mainly attributed to the presence and contribution of ETHTC in the composition of the mobile phase. Table 4 shows the calibration graphs of the peak areas for all analytes. This table also indicates the linearity of the peak area with regression coefficient (r^2) of (0.9990 - 0.9997) .

3.6 Application :

The efficiency of extraction of each analyst differs by varying temperature (60, 70, 80 and 90 °C) for the different time values (5,10 15,20, 25 and 30min) with relative standard deviation (R.S.D.) below 3%. Table (5) shows that the most suitable temperature and time for extraction of the organic acids under investigation are 90 °C and 30 min, respectively. Also, the results show that sample numbers 1,3 and 4 contain eight organic acids. Meanwhile, sample (2) contains only six organic acids with the absence of both formic and tartaric acids. Moreover, the results indicate that sample (3) is stable for 3 days, sample

(2) for 2 days while sample 1,4 are stable for only one day if kept in refrigerator. The contents of organic acids start to decompose after the stability period mentioned above. All samples show the obscure of organic acid contents after six days except sample (1) which is totally decomposed after five days. Moreover, the results indicate that both ascorbic and lactic acids are unstable in comparison to the other six acids and decomposed completely after four days for all sample. All the four samples are precipitated after 7 days.

The chromatograms of the standard mixture of organic acids and samples are shown in figs. 1 and 2, respectively .

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

Compound	Detection limit (mg / L)		Linearity Range(mg/L)	Regression Coefficient (r^2)
	Phthalic acid+(tris)	Phthalic acid+(tris+L)		
Acetic acid	0.20	0.095	0.5-500	0.9991
Ascorbic acid	0.14	0.017	0.5-3000	0.9998
Succinic acid	0.32	0.07	0.5-5000	0.9991
Lactic acid	0.23	0.075	0.5-1500	0.9998
Formic acid	0.21	0.067	0.5-1200	0.9993
Citric acid	0.63	0.065	1-2500	0.9991
Tartaric acid	0.41	0.02	0.5-2000	0.9993
Oxalic acid	0.10	0.025	0.5-3000	0.9991

Tris :(hydroxymethyl) aminomethane

L : ETHTC

Table . 5 . The concentration of organic acids in the medicinal plants at different temperatures.

Temp .	Time/ min	Mean and RSD %	Acetic acid				Ascorbic acid				Succinic acid				Lactic acid			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
60	5	a	53	813	1085	49	25.2	303	29	35	230	---	---	---	202	223	817	1020
		b	1.48	2.55	2.71	1.57	1.72	0.94	1.68	1.81	1.45	---	---	---	1.70	1.95	1.64	1.82
	10	a	95	901	1113	67	28.3	345	37	41	360	9	---	---	209	234	874	1034
		b	1.44	2.51	2.49	1.55	1.70	0.91	1.62	1.74	1.42	2.33	---	---	1.69	1.76	1.63	1.73
	15	a	127	947	1193	98	38.8	401	44	67	430	11	---	---	215	256	921	1075
		b	1.40	2.48	2.37	1.53	1.53	0.88	1.48	1.66	1.42	2.31	---	---	1.54	1.27	1.63	1.68
	20	a	145	1004	1278	113	43.2	434	53	84	502	18	---	---	221	271	933	1113
		b	1.38	2.43	2.28	1.49	1.49	0.86	1.43	1.62	1.40	2.29	---	---	1.23	1.37	1.62	1.58
	25	a	175	1062	1321	145	49.7	470	62	117	551	27	8	---	225	294	963	1145
		b	1.29	2.39	2.21	1.47	1.36	0.83	1.33	1.56	1.39	2.27	2.27	2.53	1.10	1.48	1.61	1.52
	30	a	183	1103	1378	157	54.1	502	71	148	591	38	18	9	231	323	984	1167
		b	1.25	2.36	2.17	1.45	1.17	0.78	1.12	1.47	1.37	2.22	2.33	2.40	0.99	1.30	1.59	1.47
70	5	a	195	1171	1427	178	69.2	551	82	197	611	47	23	13	248	341	1019	1189
		b	1.22	2.29	1.18	1.42	0.94	0.74	0.88	1.34	1.30	2.17	2.26	2.13	0.83	1.32	1.58	1.38
	10	a	213	1207	1465	196	74.6	612	99	241	617	54	34	22	257	362	1049	1234
		b	1.19	2.24	1.10	1.39	0.86	0.66	0.81	1.27	1.30	2.11	2.25	1.89	0.79	1.29	1.58	1.33
	15	a	234	1258	1513	214	81.2	654	112	263	623	62	43	31	271	386	1097	1256
		b	1.16	2.21	0.98	1.36	0.84	0.63	0.77	1.21	1.31	1.19	2.22	1.86	0.75	1.21	1.56	1.32
	20	a	257	1305	1546	235	87.3	717	131	299	631	74	52	47	285	401	1114	1288
		b	1.13	2.19	0.95	1.33	0.78	0.57	0.71	1.17	1.27	1.14	1.88	1.72	0.72	1.12	1.41	1.20
	25	a	296	1379	1575	268	91.5	774	152	320	637	83	61	54	302	424	1165	1321
		b	1.11	2.15	0.92	1.28	0.69	0.52	0.65	1.12	1.27	1.11	1.76	1.67	0.75	1.14	1.37	1.12
	30	a	315	1423	1624	294	95	812	166	334	642	96	74	65	343	451	1188	1368
		b	1.10	2.11	0.90	1.86	0.63	0.46	0.56	0.96	1.23	0.98	1.47	1.59	0.72	1.15	1.22	0.97

Samples: 1 (mentha vridis) 2 (mentha longifolia) 3 (organum majorana) 4 (cymbopogon winterianus)

Table (5) Contd:

Temp. °C	Time/ min	RSD %	Acetic acid				Ascorbic acid				Succinic acid				Lactic acid			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
80	5	a	340	1462	1687	314	102	867	183	358	649	115	84	72	370	482	1213	1422
		b	0.96	1.85	0.79	1.12	0.52	0.41	0.47	0.85	0.96	1.13	1.36	1.41	0.69	0.73	1.11	0.86
	10	a	395	1517	1722	345	114	907	210	379	651	168	97	83	412	509	1241	1476
		b	0.91	1.78	0.73	0.99	0.48	0.37	0.42	0.79	0.96	1.04	1.27	1.30	0.64	0.86	0.94	0.84
	15	a	434	1582	1785	377	125	972	234	403	658	194	121	103	445	541	1261	1512
		b	0.87	1.66	0.68	0.92	0.39	0.32	0.36	0.64	0.87	0.84	1.20	1.26	0.73	0.72	0.82	0.80
	20	a	440	1613	1817	392	139	1050	253	425	665	202	149	125	489	572	1279	1552
		b	0.79	1.53	0.64	0.86	0.34	0.28	0.30	0.52	0.87	0.81	1.10	1.18	0.77	0.49	0.72	0.77
25	a	447	1668	1864	406	147	1121	277	465	671	223	189	151	515	602	1294	1577	
	b	0.65	1.46	0.61	0.81	0.27	0.23	0.23	0.47	0.66	0.71	0.96	1.12	0.62	0.50	0.64	0.63	
30	a	450	1713	1913	427	152	1188	304	493	676	254	221	186	547	622	1321	1634	
	b	0.58	1.37	0.60	0.73	0.23	0.19	0.21	0.42	0.61	0.66	0.85	0.88	0.60	0.42	0.60	0.50	
90	5	A	465	1776	1954	443	163	1232	344	541	689	276	248	199	573	634	1356	1662
		B	0.47	1.17	0.56	0.64	0.17	0.14	0.15	0.36	0.56	0.52	0.80	0.83	0.75	0.36	0.44	0.42
	10	A	470	1811	1994	451	169	1299	365	567	697	295	265	224	584	661	1385	1723
		b	0.45	1.1	0.51	0.57	0.15	0.13	0.13	0.30	0.55	0.41	0.72	0.76	0.70	0.32	0.40	0.37
	15	a	475	1884	2013	460	173	1366	384	591	703	322	284	246	595	685	1423	1738
		b	0.36	0.71	0.47	0.50	0.14	0.11	0.12	0.21	0.43	0.32	0.72	0.73	0.72	0.30	0.35	0.35
	20	a	481	1974	2095	472	181	1423	403	617	711	342	321	266	610	721	1453	1817
		b	0.29	0.52	0.42	0.46	0.12	0.11	0.11	0.16	0.33	0.24	0.43	0.64	0.73	0.31	0.32	0.32
25	a	483	1986	2133	478	184	1479	435	647	716	365	351	287	620	754	1496	1846	
	b	0.25	0.32	0.37	0.41	0.11	0.10	0.10	0.13	0.30	0.24	0.25	0.43	0.70	0.31	0.30	0.28	
30	a	484	1997	2185	482	190	1520	450	698	720	385	362	328	650	795	1513	1986	
	b	0.20	0.24	0.31	0.37	0.11	0.10	0.10	0.12	0.16	0.19	0.21	0.32	0.73	0.32	0.29	0.27	

Table (5) Contd:

Temp. °C	Time min.	RSD %	Formic acid				Citric acid				Tartaric acid				Oxalic acid			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
60	5	a	57	ND	131	34	841	686	721	768	98	ND	205	234	112	73	86	92
		b	1.69	ND	1.54	1.92	2.28	2.41	2.32	2.23	2.26	ND	2.53	2.85	1.31	1.91	1.75	1.66
	10	a	101	ND	153	52	910	750	874	899	143	ND	269	289	176	86	104	136
		b	1.67	ND	1.55	1.89	2.20	2.29	2.25	2.20	2.22	ND	2.47	2.76	1.28	1.88	1.68	1.62
	15	a	131	ND	167	87	965	893	924	945	185	ND	322	345	201	102	138	158
		b	1.65	ND	1.53	1.79	2.11	2.21	2.22	1.79	2.18	ND	2.41	2.70	1.23	1.84	1.56	1.51
	20	a	152	ND	195	108	1025	955	988	996	216	ND	366	396	232	144	182	212
		b	1.63	ND	1.51	1.77	1.97	2.11	2.12	1.74	2.13	ND	2.35	2.66	1.20	1.80	1.48	1.45
25	a	182	ND	222	136	1149	1101	1107	1128	265	ND	419	442	295	175	252	267	
	b	1.63	ND	1.47	1.76	1.67	1.87	2.05	1.67	2.06	ND	2.30	2.61	1.19	1.67	1.42	1.37	
30	a	189	ND	244	157	1201	1134	1154	1166	294	ND	456	485	341	204	288	311	
	b	1.62	ND	1.44	1.75	1.54	1.85	1.74	1.60	1.92	ND	2.23	2.57	1.11	1.56	1.36	1.21	
5	a	203	ND	287	183	1230	1202	1209	1226	322	ND	505	543	368	245	322	341	
	b	1.58	ND	1.45	1.70	1.48	1.73	1.64	1.52	1.86	ND	2.17	2.33	0.89	1.32	1.27	0.99	
10	a	215	ND	314	198	1302	1275	1289	1295	362	ND	541	578	401	275	375	389	
	b	1.55	ND	1.43	1.66	1.45	1.70	1.53	1.45	1.83	ND	2.13	2.30	0.86	1.21	1.16	0.84	
15	a	237	ND	342	204	1355	1312	1343	1356	401	ND	581	636	435	303	412	422	
	b	1.53	ND	1.42	1.63	1.41	1.66	1.48	1.37	1.82	ND	2.11	2.29	0.76	1.13	1.08	0.79	
20	a	258	ND	356	232	1417	1371	1401	1415	434	ND	632	687	488	341	443	455	
	b	1.52	ND	1.46	1.60	1.36	1.42	1.42	1.31	1.80	ND	1.93	2.25	0.71	0.88	0.96	0.77	
25	a	299	ND	361	253	1471	1403	1438	1457	486	ND	664	721	517	385	471	482	
	b	1.51	ND	1.40	1.58	1.32	1.38	1.45	1.26	1.79	ND	1.90	2.20	0.68	0.78	0.77	0.77	
30	a	318	ND	379	264	1575	1535	1564	1570	525	ND	682	765	561	413	518	530	
	b	1.50	ND	1.39	1.58	1.30	1.32	1.34	1.21	1.77	ND	1.84	2.13	0.61	0.74	0.62	0.75	

Table (5) Condi:

Temp., C°	Time/ min	V _r and RSD %	Formic acid				Citric acid				Tartaric acid				Oxalic acid			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
80	5	a	347	ND	403	295	1635	1570	1618	1626	584	ND	738	812	604	434	574	586
		b	1.49	ND	1.37	1.54	1.28	1.26	1.21	1.10	1.72	ND	1.78	1.96	0.59	0.70	0.59	0.68
	10	a	404	ND	421	331	1745	1619	1625	1666	613	ND	787	849	665	499	602	629
		b	1.48	ND	1.35	1.51	1.20	1.25	1.12	0.89	1.70	ND	1.72	1.84	0.60	0.62	0.55	0.53
	15	a	438	ND	435	345	1811	1651	1717	1785	657	ND	821	883	726	544	643	679
		b	1.45	ND	1.35	1.47	1.21	1.20	0.92	0.86	1.68	ND	1.67	1.80	0.53	0.54	0.53	0.51
	20	a	443	ND	462	364	1837	1682	1742	1804	723	ND	858	935	776	617	668	718
		b	1.43	ND	1.32	1.47	1.27	1.17	0.83	0.77	1.62	ND	1.62	1.74	0.49	0.50	0.51	0.50
	25	a	451	ND	491	388	1873	1709	1777	1832	774	ND	911	982	811	668	705	739
		b	1.42	ND	1.29	1.44	1.23	1.17	0.74	0.65	1.60	ND	1.58	1.63	0.40	0.43	0.47	0.48
	30	a	457	ND	523	409	1897	1745	1811	1856	809	ND	937	1033	886	709	748	768
		b	1.40	ND	1.27	1.40	1.15	1.15	0.63	0.54	1.59	ND	1.54	1.57	0.40	0.38	0.44	0.41
90	5	A	470	ND	572	428	1913	1771	1878	1887	843	ND	988	1068	917	731	779	818
		B	1.39	ND	1.25	1.42	1.11	0.86	0.52	0.43	1.53	ND	1.49	1.51	0.32	0.31	0.37	0.36
	10	A	475	ND	583	445	1955	1802	1903	1928	895	ND	1025	1092	984	802	816	841
		b	1.38	ND	1.22	1.40	0.74	0.71	0.49	0.36	1.50	ND	1.48	1.49	0.26	0.27	0.30	0.29
	15	a	481	ND	597	463	1985	1843	1932	1943	923	ND	1096	1134	1003	855	935	965
		b	1.27	ND	1.20	1.37	0.63	0.53	0.31	0.27	1.49	ND	1.47	1.48	0.23	0.26	0.28	0.27
	20	a	493	ND	609	479	2019	1879	1969	1983	978	ND	1113	1152	1074	921	985	1013
		b	1.25	ND	1.17	1.35	0.32	0.32	0.27	0.19	1.47	ND	1.46	1.47	0.19	0.21	0.23	0.20
	25	a	498	ND	623	486	2120	1923	2109	2111	1042	ND	1198	1218	1185	954	1003	1058
		b	1.20	ND	1.16	1.32	0.18	0.12	0.13	0.11	1.44	ND	1.45	1.43	0.13	0.20	0.17	0.15
	30	a	504	ND	641	493	2336	1974	2256	2288	1130	ND	1244	1258	1235	973	1025	1096
		b	1.19	ND	1.15	1.30	0.10	0.12	0.10	0.10	1.42	ND	1.43	1.40	0.10	0.19	0.15	0.11

Table . 6 . The stability of medicinal samples at 90 °C and 30 min.

Time (h)	Mean RS ± SD	Acetic acid				Ascorbic acid				Succinic acid				Lactic acid			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
24	a	484	1997	2185	482	190	1520	450	698	720	385	362	328	650	795	1513	1986
	b	0.20	0.24	0.31	0.37	0.11	0.10	0.10	0.12	0.16	0.19	0.21	0.32	0.73	0.32	0.29	0.27
48	a	305	1993	2182	295	113	1518	448	425	416	382	360	165	349	791	1510	1023
	b	0.22	0.24	0.31	0.40	0.15	0.10	0.10	0.17	0.18	0.19	0.21	0.37	0.75	0.32	0.29	0.33
72	a	245	1128	2180	216	75	634	445	265	224	145	358	89	126	327	1499	735
	b	0.24	0.27	0.31	0.42	0.18	0.17	0.10	0.21	0.24	0.23	0.21	0.41	0.81	0.37	0.29	0.36
96	a	155	817	1345	124	29	168	126	114	52	74	147	36	33	133	254	73
	b	0.24	0.29	0.35	0.45	0.26	0.23	0.16	0.26	0.31	0.27	0.24	0.41	0.48	0.42	0.32	0.39
120	a	32	518	821	42	ND	ND	ND	ND	17	28	63	21	ND	ND	ND	ND
	b	0.25	0.29	0.37	0.47	ND	ND	ND	ND	0.35	0.32	0.25	0.44	ND	ND	ND	ND
144	a	ND	103	319	12	ND	ND	ND	ND	ND	11	19	9	ND	ND	ND	ND
	b	ND	0.30	0.40	0.52	ND	ND	ND	ND	ND	0.35	0.31	0.47	ND	ND	ND	ND
168	a	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	b	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Samples: 1 (mentha vridis) 2 (mentha longifolia) 3 (origanum majorana) 4 (cymbopogon winterianus)

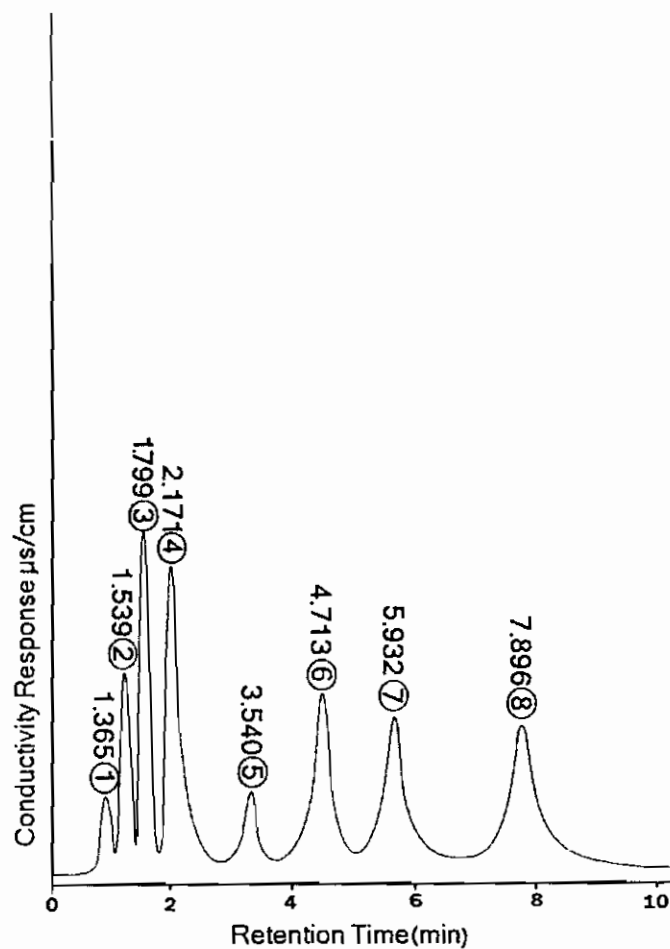


Fig . 5 . Typical resolution for a mixture of organic acids. column , shim – pack IC A1, temperature 40°C , eluent mixture of 2.45 mM phthalic acids and 2.35 mM tris (hydroxymethyl) aminomethane (pH) 3.40 ; flow rate 1.5ml/min .
peaks :1 = acetic acid ; 2 = ascorbic acid ; 3 = succinic acid ;
4 = lactic acid ; 5 = formic acid ; 6 = citric acid ;7 = tartaric acid
8 = oxalic acid

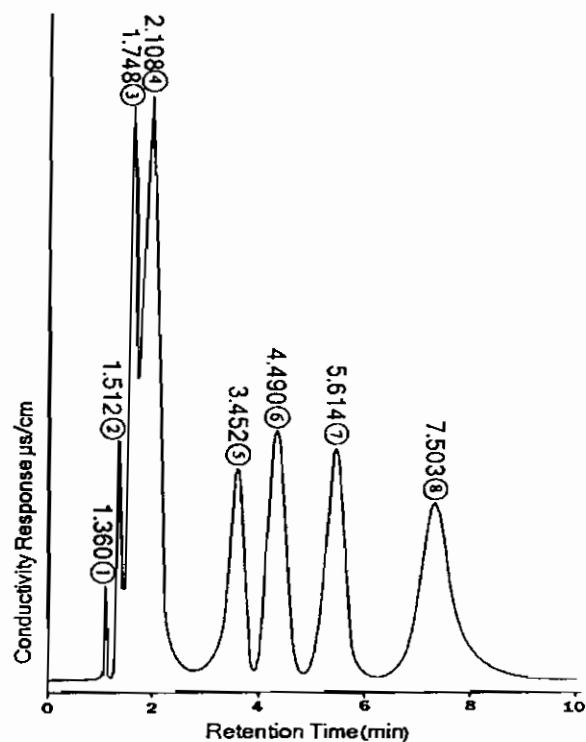


Fig . 6 . Typical resolution for a mixture of organic acids. column , shim – pack IC A1, temperature 40 C , eluent mixture of 2.45 mM phthalic acids and 2.35 mM tris (hydroxymethyl) aminomethane + 0.2mM N{[(ethylamino) thioxomethyl] hydrazinocarbonylmethyl}trimethyl ammonium chloride (pH 3.63) ; flow rate 1.5ml/min .
 peaks :1= acetic acid ; 2 = ascorbic acid ; 3 = succinic acid ;
 4= lactic acid ; 5 = formic acid ; 6 = citric acid ; 7 = tartaric acid
 8 = oxalic acid .

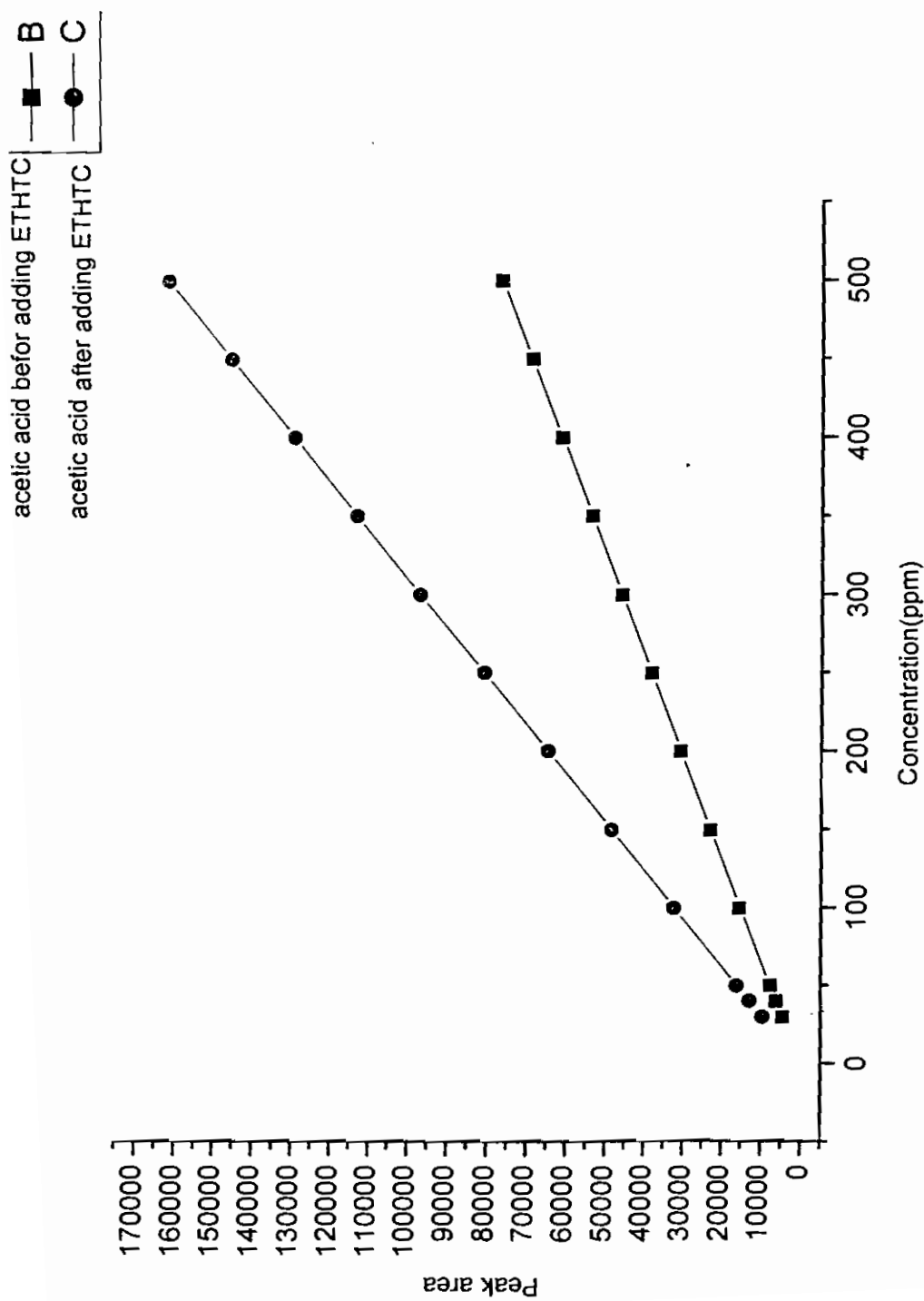


Fig.7. The relation between concentration and peak area (sensitivity) for acetic acid

CONCLUSION

An IC method with non suppressed conductivity detection for analyzing organic acids in some medicinal plants was developed and optimized. The optimal conditions for separating organic acids by using 2.45mM phthalic acid, 2.35mM tris (hydroxymethyl) aminomethane, 40 °C, flow rate 1.5ml/min. and pH 3.40 were found to be favorable. In order to increase the sensitivity the above eluent was used in addition to 0.2mM of ETHTC at pH 3.63. This new eluent gives more favorable and excellent results in comparison to that mentioned above in absence of ETHTC and that reported in literature. On using this ligand(ETHTC) a typical analysis was completed in less than 8 mins.

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الملخص العربي

تم استخدام التحليل الكروماتوجرافي المستمر لتعيين بعض الأحماض العضوية حيث تم التعرف على ثمانية أحماض عضوية وفصلها وتقديرها باستخدام عمود تبادل أيوني وتهدف الطريقة الى التعيين المتزامن وكذلك التقدير الكمي لعدد من الأحماض العضوية والذي يتيح استخدام هذه الطريقة في التحاليل الروتينية. ولهذه الطريقة عدة مميزات .. فزمن التحليل قصير - وحدود استخدام الطريقة ملائم حتى لتركيزات منخفضة كما أنها مستوفية للشروط المطلوبة في التحاليل الكيميائية كالدقة ، الحساسية - حدود التعيين الدقيق وغيرها.