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Identification of Vitamins and antioxidant in Carrot by HPLC

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Abstract

Vitamins and antioxidant were important Component for carrot .HPLC analysis proved that in carrot contains Soluble Vitamins (184.74 mg/ml Vita C,. 3394.08mg/ml Vita.B3,147.675 Vita.B2,12.918Vita.B6,21.903Vita.B1) and (34.481mg/ml Vita.A,652,488 Vita .E)and many types of antioxidant (56.005 mg/ml Carotine,580.380 Carotine,299.890 mg/ml lutein)

Key word: carrot ,antioxidant

INTRODUCTION

Carrot is a good source of vitamins (A,B,C) and contains folic acid and trice element (K, Na ,Mg ,P , S, MN ,Fe ,Cu) and Zn (1).vegetables are sources of health-promoting active in neutralization of reactive oxygen species(2)among them carrot has nutritional value and high concentration of bioactive constituents(3).Carrot is important root vegetables rich in carotenoids and dietary fibers with functional components including phenolic compound(4) and their antioxidant capacity have been determined(5).the presence of phenolic compounds in Carrot contributes to their sensory qualities ,like colours(6,7.8). There is two types of antioxidant lipophilic (carotenoids) and hydrophilic (phenolic compounds)characters (9,10,11).The presence of phytochemicals and vitamins in Carrot has been recently considered of crucial nutritional Importance in the prevention of chronic diseases ,such as cancer , cardiovascular diseases and diabetes (12,13,14)

MATERIAL AND METHOD

Plant material

The plant Carrot was collected from Tikrit city ,salahaldin .the root were washed with distilled water ,sample preparation is similar to the preparation of medicinal herbs. Fresh carrots were sliced by BRAUN blender (Germany) and dried at +40 c

Methods

Analysis of soluble vitamins in carrot

The main compound were separated on FLC (Fast liquid chromatographic)column under the optimum condition
Mobile phase: (A)0.01M potassium phosphate pH 2.6:5Mm octane Sulphonate (ion-pair) (B) acetonitrile , A:B (9:1,V/V) detection UV set at 270nm,
Flow rate 1.5ml /min .six stander solutions25mg/ml were used

Analysis of vit A and E in carrot

Mobile phase: n- hexane :isopropanol , (99:1.0, v/v)
Flow rate 1.2 ml/min. two stander solution 25mg/ml were used

Analysis of antioxidant

Column:C-18(50*20mm)
Mobile phase acetic acid ,methanol
Flow rate 1ml/min .four stander solution 25mg/ml were used. All the chromatographic analysis done by HPLC(Shimadzu ,JAPAN) .The concentration of identified compound was calculated to the equation(15,16)

Conc. Of sample(mg/ml)=Area of sample/Area of stand. *C*D
C=Conc.of standard solution
D=Dilution factor

RESULTS AND DISCUSSION

The HPLC analysis of soluble vitamins in the carrot showed five peaks fig.(1) with different
Rt (1.958,3.137,4.193,4.957,7.008) min. and area for each peak were (47453,85273,33927,3641,5122) showed in table(1)

Table1:Retention Times and Area under curves for soluble vitamins

Rt.(min)	AREA	IDENTIFIED COMPOUND	CONC µ g
1.958	47453	Vita.C	184.74
3.137	85273	Niacin,Vita.B**3**	3394.08
4.193	33927	Riboflavin,B**2**	147.675
4.957	3641	Pyridoxine,B**6**	12.918
7.008	5122	Thimine,B**1**	21.903

The chromatogram of the six standard vitamins(Vita.C,Vita.B3,Vita.B2,Vita.B6,Folic acid,VitaB1) Fig(2).The Rt of the six standard peaks were(2.03,3.17,4.10,5.00,5.93,6.97).table(2)

Table2:Retention time and area under curves for standards Vitamins

STANDARD	Rt.(min)	AREA
Vita.C	2.03	64215
Niacin,Vita.B**3**	3.17	62810
Riboflavin,B**2**	4.10	57435
Pyridoxine,B**6**	5.00	70460
Thimine,B**1**	6.97	58462
Folic acid	5.93	64190

Results shown in fig.(1)compared with chromatograms shown in fig(2) refears to that Carrot contained (184.74Vita C,3394.08B3,147.675B2,12.918B6,21.903B1).
HPLC analysis of vitaA and E showed 2 peaks fig.(3) with different Rt (1.84,3.848) min. and the area for each peak were (114945,188898) Table (3).

Table3:Retention Times and Area under curves for vitamins A and E

Rt.(min)	AREA	IDENTIFIED COMPOUND	CONC µ g
1.84	14945	Vita.A	34.481
3.848	188898	Vita.E	6529488

The chromatogram of the two standard **vitamins A and E** shown in the fig(4). The Rt of the two standard peaks were (1.86,3.85)

Table4:Retention Times and Area under curves for standard vitamins A and E

Rt.(min)	AREA	standard
1.86	83384	Vita.A
3.85	72376	Vita.E

Results in fig.(3) and its Rt value in table (3) compared with chromatograms of two standard vitamins A and E indicate that carrot contain(34.481 652,488).
HPLC analysis of antioxidant in the carrot showed 3 peaks fig.(5) with different Rt (3.34 , 4.32 5.573) min. and the area for each peak were (15000,166323,100689) Table (5).

Table5:Retention Times and Area under curves for antioxidant

Rt.(min)	AREA	IDENTIFIED COMPOUND	CONC µ g
3.34	15000	-carotine	56.005
4.32	166323	carotine-	580.380
5.573	100689	lutein	299.890

The chromatogram of the four standard antioxidant (-carotine , -carotine , lutein ,zeaxanthin) were shown in the fig(6). The Rt of the four standard peaks were(1.85,3.23,4.38,5.58)Table(6).

Table6:Retention Times and Area under curves for standard antioxidant

Rt.(min)	AREA	standard antioxidant
3.23	66958	-carotine
4.38	71644	carotine-
5.58	83938	lutein
1.85	76845	zeaxanthin

Results in fig.(5) and its Rt value in table (5) compared with chromatograms of four standard antioxidant indicate that carrot contain(56.005 , 580.380 ,299.890). Results are correspond with previous studies of [17,18,19] which indicates the presence of many types of vitamins and antioxidant in carrot.

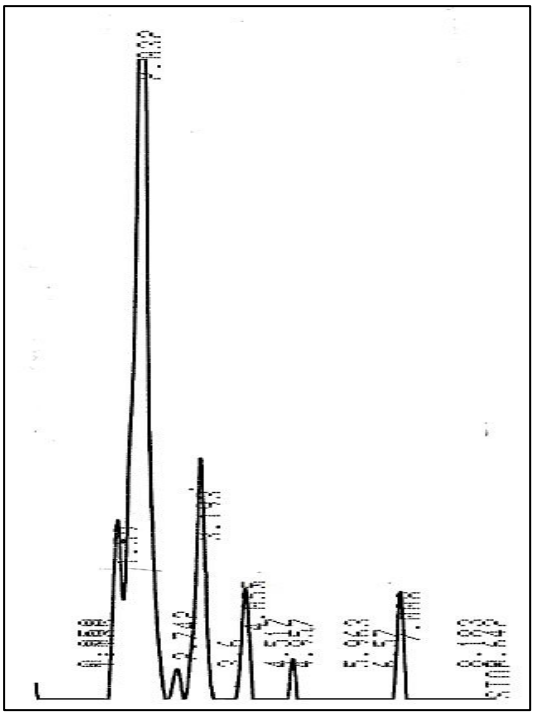

FIG 1 HPLC OF SOLUBLE VITAMINS IN CARROT

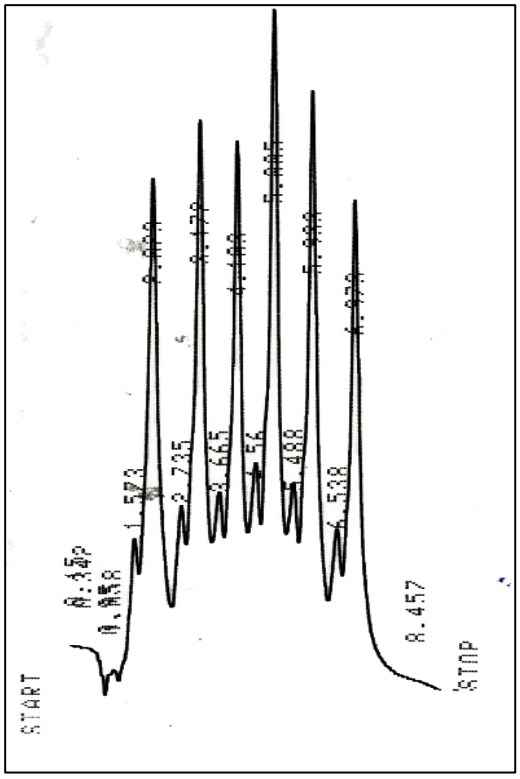

FIG 2 HPLC OF STANDARD SOLUBLE VITAMINS IN CARROT

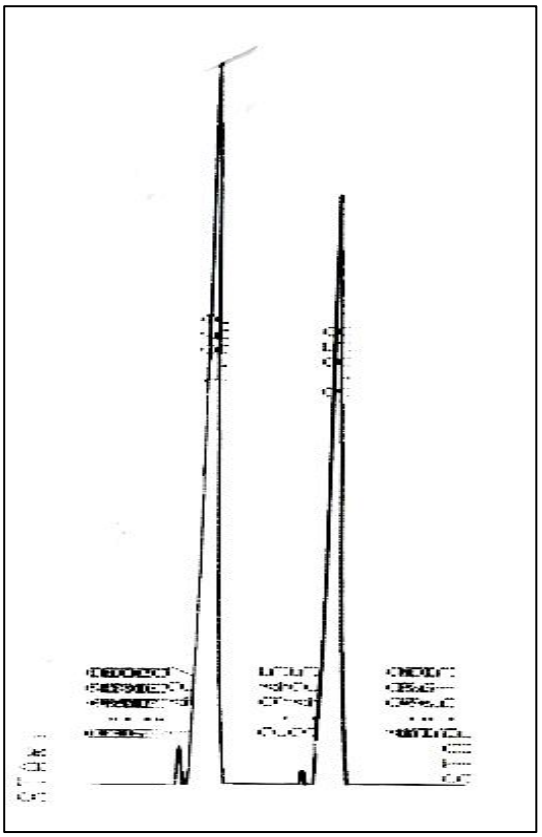

FIG 3 HPLC OF VITAMINS A and E IN CARROT

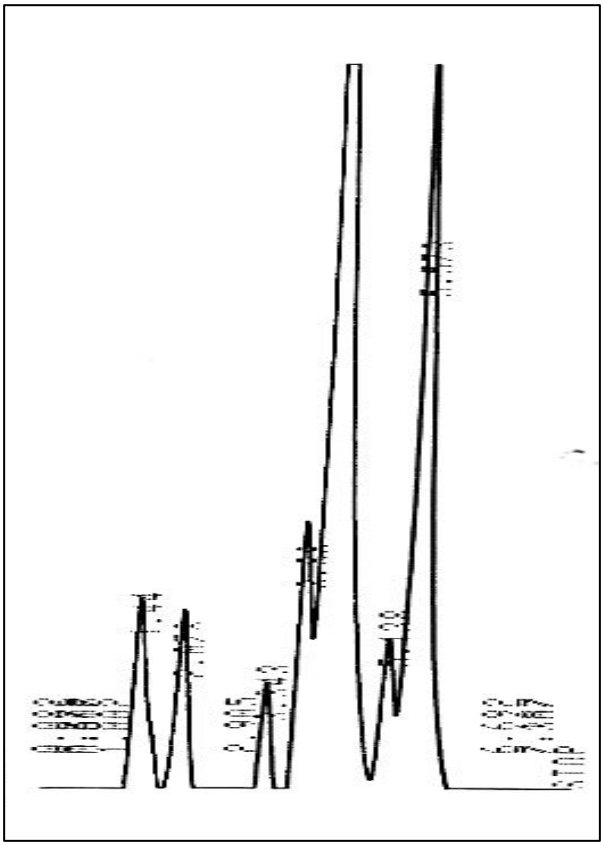

FIG 5 HPLC OF ANTIOXIDANT IN CARROT

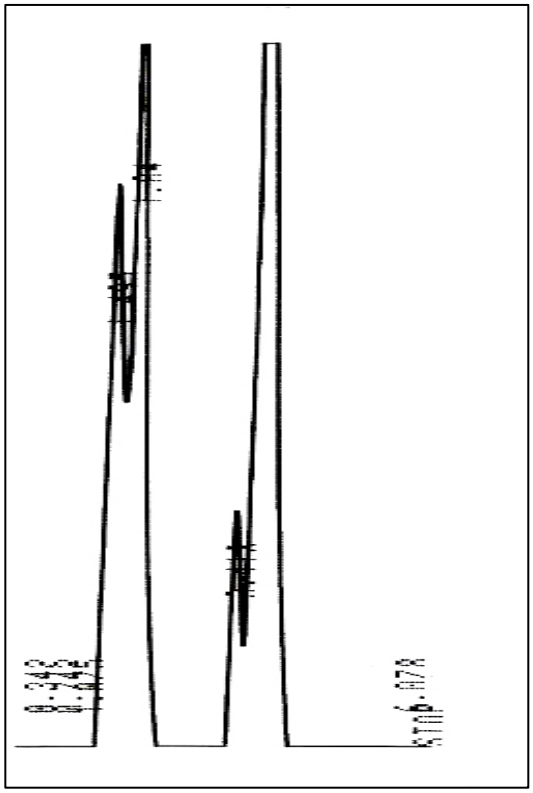

FIG 4 HPLC OF STANDARD VITAMINS A and E IN CARROT

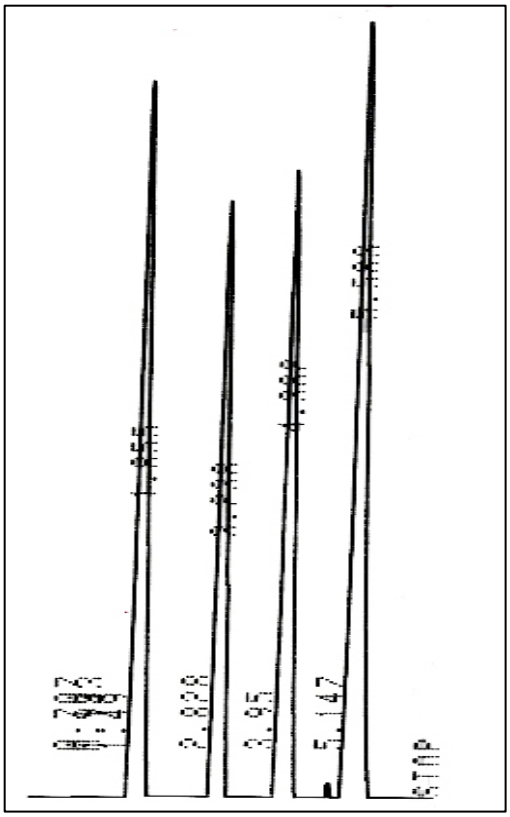

FIG 6 HPLC OF STANDARD ANTIOXIDANT IN CARROT

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