Determination Vit C in Food Samples using High Performance Liquid Chromatography

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Abstract

Vit C has $C_6H_8O_6$ chemical formula ,white crystal , water soluble , nutrition value and antioxidant, has an important factor to skin different type of tissues and bones . Quantity required to individual from this vitamin depend on: age, gender and healthy status .

Many different analytical methods has been done to determine quantity in substances containing [vit C, such as: (titration , spectrophotometer, electrochemical and chromatographic) methods .

Various analytical chromatographic conditions were tested in this search by using HPLC-RP (UV-Vis) , we have reached to the following separation conditions:

1- Sorbent C18 .
2- Moble phase (MeOH: H_2O) (97:3).
3- ø = 1 ml/ min .
4- λ_{max} = 254 nm .

By the proposed method , we achieved a sharp symmetric peak during (t_R = 4.6 min) , and a liner equation S =f (C) was applied in the range of (0.2-1.2) mg / ml, according to this concentration and liner equation we proceeded determining the quantity in each nutrition sample of the following fruits from our local market

Kiwi > Strawberry >Orange > Pineapple> Mango >Grape > Lemone >Apple
137.0 > 87.0> 81.3 >36.0> 28.0> 24.0> 18.2 >13.1(mg/100gr)
and RSD=(0.115-1.145)% .

Keyword: vit C , RP- HPLC , food sample, fruit sample .

1-Introduction:

Vit C has $C_6H_8O_6$ chemical formula ,[white crystal , water soluble(30 mg /100ml) , nutrition value and antioxidant , has an important factor to skin , different type of tissues and bones . There are two sources of vit C : food(vegetable, and fruit) , and nutritional [supplements (vitamins) [1] . the amount should be taken form vit C depend on : age , sex, and health situation [2-4] .

there are many analytical methods have been reported [5-7] for determination vitamin C in its samples , titration method is the common [8,9] , metal ion reduction[10] the ideal example of it : is using vit C to redox Fe^{2+}[11] .The enzymatic method use enzyme conversions vit C to chemical derivation which determine by spectrophotometer methods [12-14] . As a result of chemical analysis developing and use the methods to determination vitamins including vit C such as : spectrophotometer methods by all types [15,16] , electrochemical methods [17] , and chromatographic methods[18] .

In this search we fix on chromatographic methods because it's our goal , [19] used HPLC to determination vit C , the linear range was([1.25 – 100) μg /10^8 . [20] they used HPLC[, UV detector to determination vit C in food samples using redox by TCEP , the detection limit was (0.1 / 100gr) ,by recovery (93- 105)% , several redox agents used [21] as DTT,and BAL to determination vit C by(HPLC-UV) using a column C18 , the determination of vit C in fruit samples [22] by HPLC with a column C18 , λ_{max} = 254 nm .at [23] they determined water soluble vitamins include vit C in pharmaceutical samples (multi vitamins syrup) by HPLC method on discovery C18 by isocratic mode , mixture of ([water- fat][soluble vitamins were studied [24] in pharmaceutical samples , the(HPLC-UV) method was applied [25] on a column C18 to analyses canned fruit in the liner range (0.1-2.5) mg/ ml, recovery (94-101)% ,HPLC method was used for measurement of the concentration of vit C in in green pepper and broccoli [26] , the effect of processing , packaging and storage on the levels of vitamin were determined .

2-Result and discussion :

2- 1- Preparation of stander VIT C has been done ,by 10 mg /100ml , a chromatographic scan was did to be sure about the amount of t_R .

2-2 -Several mobile phase have been studied to be sure about the optimum mobile phase , table(1).

Table (1) :Study of several mobile phases on determination vit C by specific stationary phase(C18) .

NO	Mobile phase	Flow (ml/ min)	Wavelength (nm)
1	ACN +CH$_2$Cl$_2$+MeOH (60+20+20)%	1	254
2	MeOH + CH$_3$COONH$_4$	1	270
3	H$_2$O (PH=2.2 by H$_2$SO$_4$)	0.4	254
4	MeOH	1	254
5	MeOH +H$_2$O+ CH$_3$COOH (28+69+3)%	1.5	275
6	MeOH +H2O (97+3)%	1	254

ACN : Acetonitrill , MeOH: Methanol

The optimum chromatographic conditions we have achieved were :

1- Sorbent C18 .

2- Moble phase (MeOH: H$_2$O) (97:3).

3-   ø = 1 ml/ min .

4- λ_{max} = 254 nm .

By using above conditions ,the peak of vit C show in figure(1).
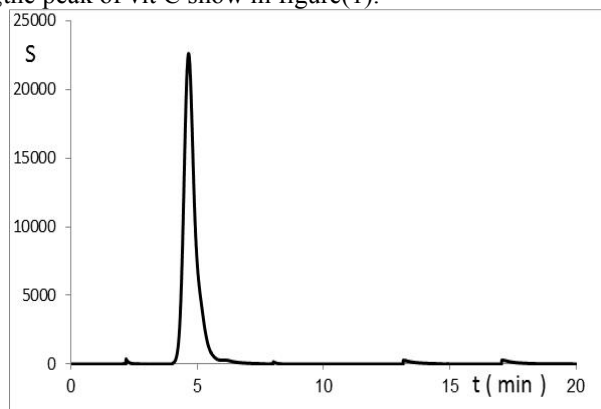

Figure (1) : Chromatographic peak of vit C , stationary phase C18

Moble phase (MeOH: H$_2$O) (97:3) , ø = 1 ml/ min . λ_{max} = 254 nm .

So using above Chromatographic conditions we achieved a sharp and symmetric peak at t_R= 4.6 min .

2-3 Study of slandered solutions, S= f(C) :

Five vit C slandered were prepared and injected on C18 column and the analysis carried out by elution with (MeOH:H$_2$O) , λ_{max} = 254 nm , figure (2). From figure 2 we observed :

a – the relation between (s) peak surface ,and Chromatographic peaks   of vit C concentration is linear .

b – Chromatographic peak   height was heigh , and it's surface was large , for that we tried to less vit C concentration between the range (0.2- 1.2) mg/ ml , figure (3)
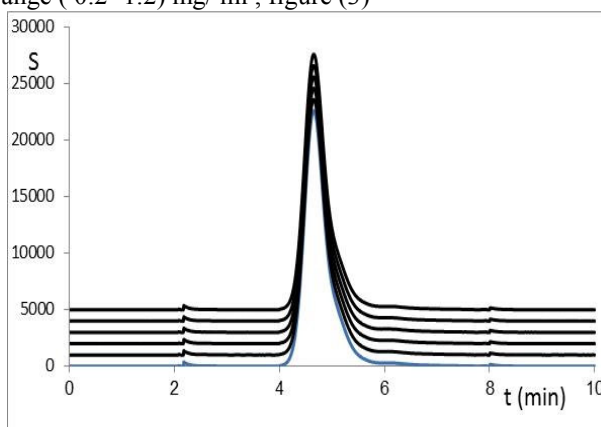

Figure (2) : Chromatographic peaks for vit C (standard solutions), stationary phase C18

Moble phase (MeOH: H$_2$O) (97:3) , ø = 1 ml/ min . λ_{max} = 254 nm .
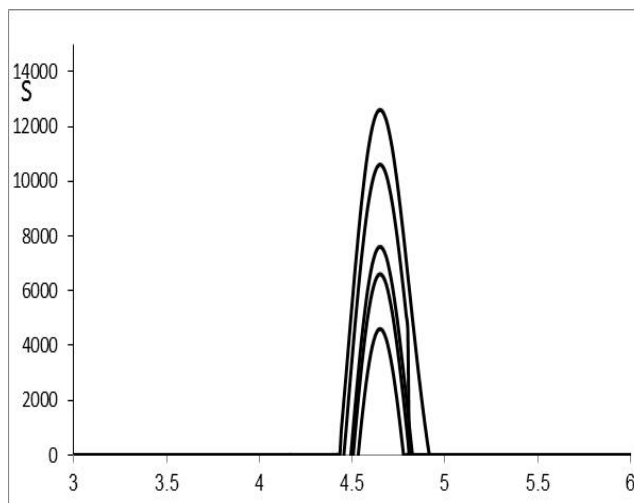
Figure (3) : Chromatographic peaks for vit C (standard solutions).
$C_{vit\,C}$ (mg/ ml) : 0.2 - 0.4 - 0.6 - 0.8 - 1.0 - 1.2 .
stationary phase C18 , Moble phase (MeOH: H_2O) (97:3) , ø =1 ml/ min . λ_{max} = 254 nm .
The liner relation between peak surface and the vit C concentration , we achieved in the wide range of
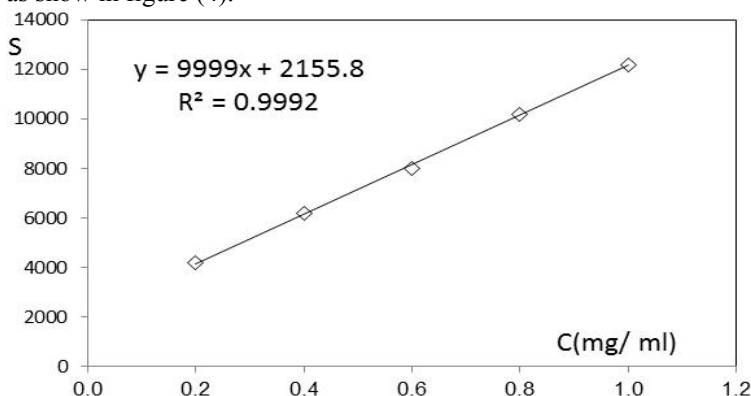(0.2- 1.2) mg/ ml , as show in figure (4).

Figure (4) : the linear range between surface peaks and vit C concentration
$C_{vit\,C}$ (mg/ ml) : 0.2 - 0.4 - 0.6 - 0.8 - 1.0 - 1.2 .
stationary phase C18 , Moble phase (MeOH: H_2O) (97:3) , ø =1 ml/ min , λ_{max} = 254 nm.

2-4-preparation of experimental vit C samples :
To be sure about the accuracy and precision of our proposed chromatographic method , the proposed method
was applied on experimental vit C samples for that 3 slandered solutions   were prepared their concentrations
include in the linear rang which we obtained above , and each concentration was repeated 3 times , than we
have done some statistic study ,table (2) .
Table(2) : determination experimental vit C samples using HPLC-RP , stationary phase C18
Mobile phase (MeOH: H_2O) (97:3) , ø = 1 ml/ min . λ_{max} = 254 nm
(n=3 , α=0.95)

Taken concentration mg /ml	Found concentration $\overline{X} \mp \Delta X$ mg /ml	RSD%	Recovery %
0.300	0.3082±0.028	0.0413	102.7
0.500	0.4969± 0.016	1.3600	99.3
0.700	0.7054±0.0167	0.9560	100.7

2-5- Natural samples :
The proposed method was applied in natural samples of local fruit:
(Kiwi , Strawberry ,Orange, Pineapple , Mango , Grape , Lemone and Apple) as follows :
1   50 gr from each sample mixed in mixer until a homogeneous solution has been achieved

2   Added to that solution (MeOH:H_2O) , centrifuged for 10 min .

3   isolated by two steppes: first with ashless paper , second by special filter for HPLC .

4   Diluted to 100 ml by water for HPLC [these solution is : mother sample solution]

    Each mother sample solution was diluted according to vit C concentration by recording analytical signal (peak vit C surface) at same time[retention time for vit C (t_R)] , calculation the vit C concentration in each sample was applied parallel by stander solution at same time , result in table(3) .

Table(3) : determination of vit C in Natural samples using HPLC-RP , stationary phase C18

Mobile phase (MeOH: H_2O) (97:3) , ø =[1 ml/ min . λ_{max} = 254 nm

(n=3 , α=0.95)

Sample	Found concentration $\overline{X} \mp \Delta X$ mg/100 gr	RSD%
Lemon	18.2 $\mp$ 0.652	1.453
Orange	81.3 $\mp$ 0.392	0.194
Apple	13.1 $\mp$ 0.420	1.320
Grape	24.0 $\mp$ 0.430	0.721
Strawberry	87.0 $\mp$ 0.340	0.160
Kiwi	137.0 $\mp$ 0.280	0.115
Pineapple	36.0 $\mp$ 0.620	0.690
Mango	28.0 $\mp$ 0.370	0.530

    from the table we concluded : a rapid and sensitive high performed liquid chromatography proposed method was carried out , according to it we can determined vit C in the wide range of samples , the method has a repeatedly and accuracy , we can observed from the lower RSD ; and a high precision which can observed from the recovery , Accuracy is the degree of agreement between test results and true values. The precision of a method is the degree of agreement among individual test results when an analysis is applied repeatedly to multiple samplings. Precision is measured by injecting a series of standards and then calculating the relative standard deviation of retention times and areas or peak heights. Precision may be measured at three levels: repeatability, intermediate precision, and reproducibility.. Repeatability off low rates, gradient formation, and injection volumes can affect precision, as can response stability of the detector, aging of the column, and temperature stability of the column oven. The equipment should be inspected on a regular basis using the test methods recommended by the supplier to ensure reliability, high performance, and good analytical results the proposed method doesn't consume high price solvents ; possibility usage in shorter time (t_R = 4.6 min), from all method characteristics we can applied the proposed method in the relevance laboratory according chromatographic conditions as :

1-   Sorbent C18 .

2-   Moble phase (MeOH: H_2O) (97:3).

3-   ø = 1 ml/ min .

4- λ_{max} = 254 nm .

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