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# **Comparison of vitamin C content between fruits sourced from an Iraqi market via HPLC quantification**

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

((قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ  
وَالَّذِينَ لَا يَعْلَمُونَ إِنَّمَا يَتَذَكَّرُ أُولُو  
الْأَلْبَابِ))

صدق الله العظيم

## شكر وتقدير

لابد لنا ونحن نخطو خطواتنا الأخيرة في حياة الجامعة من وقفه تعود الى أعوام قضايها في رحاب الجامعة مع أساتذتنا الكرام الذين بذلوا جهودا كبيرة في الوصول الى هذه المرحلة فواجب علينا شكرهم ووداعهم

ونحن نخطو خطواتنا الأولى في غمار الحياة ونخص بالجزيل الشكر والعرفان الى كل من أشعل في درب عملنا والى من وقف على المنابر واعطى من حصيلة فكره لينيير دربنا

الى اساتذتي الكرام في الكاية نتوجه بالشكر الجزيل الى الأستاذ(حيدر عبد الكريم الوافي) الذي تفضل علينا بالأشراف على هذا البحث ومتابعته المستمرة وجزاه الله عنا كل خير وله كل التقدير والاحترام.

# Comparison of vitamin C content between fruits sourced from an Iraqi market via HPLC quantification

*Key/ Vitamin C, HPLC, peak area, fruit*

## Abstract

This study was undertaken to quantitative estimate of the Vitamin C amount in different available Iraqi market fruits from different countries to find the best fruit has highest content of the vitamin according to percentage of HPLC peak area for each fruit. The findings clarified that the Turkey pomegranate has the largest amount of the vitamin followed by Syrian red Cindy then Syrian Strawberry among these all fruits. While, the lowest amount of the Vitamin C was in the Iranian pear followed by plumb and yellow apple for the same country. In Addition to that, Mango, red Apple, Kiwi, Orange, and green Grape have close amounts of the vitamin. Also, Iranian yellow Cindy and Pakistani Tangerine have about same the content of the Vitamin.

## 1-Introduction

One of the important vitamins to survive and thrive of the human life is vitamin C (ascorbic acid).<sup>1</sup> Therefore, many studies have directed to investigate the levels of vitamin C in blood according to the gender and race, their results were considerable difference between male (lower level) and female (higher level) although the supplemented diet for all was rich with vitamin C.<sup>(2-5)</sup>

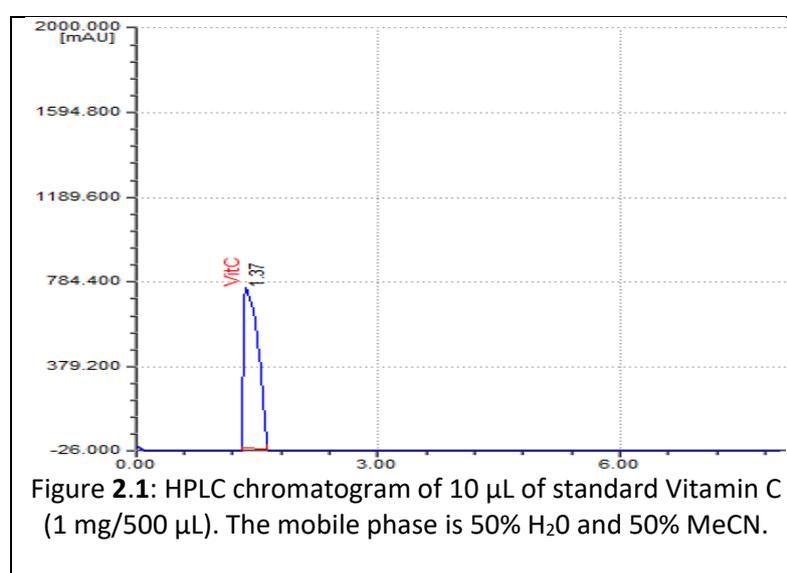
The positive effects of the Vitamin C on the health of human body have attractive the attention of the medicinal chemists, one of these effects is the relationship between the vitamin and its influence to increase the Iron level in blood human.<sup>(6-10)</sup> Also, many studies illustrated that the rich diets with fruits and vegetables decreased risk of developing cancer and cardiovascular disease (CVD) which are considered the main reasons to increase the rates of the mortality.<sup>11</sup> In addition to that, more than one study showed that the immune system is activated by high dose of vitamin C.<sup>(12,13)</sup> Moreover, it has been hypothesised that the high dose of the vitamin C has ability to resistance against the COVID-19 virus.<sup>(14-16)</sup> Furthermore, it has been found that the decreased levels of the vitamin C in the blood has relationship with diabetes, chronic hypertensions and viral induced sepsis.<sup>(17-20)</sup> Therefore, this research is trying

to find the best fruit has high content of the vitamin C consequently, more natural treatment to enhance the immune system and resistance against many diseases.

## 2-Results and discussion

### 2.1 HPLC analysis results for the standard of Vitamin C

Figure 2.1 shows HPLC chromatogram of standard Vitamin C. The high intensity sharp single peak without any overlap at the retention time 1.37 min. with less than one min. (start to end of the peak) was considered to be the Standard Vitamin C. According to this result, the used standard is high purified compound and ready for using in the recent research.



### 2.2 HPLC analysis Results for used fruits

Subsequent to an investigation (quantitative analysis according to the peak area) of the Vitamin C in different fruits by HPLC as in Table 2.1 (average of three values of three peaks areas) and Figure 2.2 (Pi chart of percentages for the average of three peaks areas for Vitamin C to each fruit). There was clear difference in the amount of Vitamin C between the fruits for instance, the peak area of the Vitamin was the highest (3657612) in Turkey pomegranate, while it was the lowest (354247) in the Iranian Pear as in Table 2.1 (entry 8 and 9) and Figure 2.2, See Figure A.8 and A.9 in the Appendix A. In addition to that, the low peak area of the Vitamin in some fruits was very close to other for example, Iranian yellow Apple and Iranian Plumb as in Table 2.1 (entry 7 and 10) and Figure 2.2, See Figure A.7 and A.10 in the Appendix A. Also, the high peak area of the Vitamin in the Syrian Strawberry was close to the peak area in the Iraqi Naranji as in the same mentioned Table 2,1(entry 11 and 18) and Figure 2.2, See Figure A.11 and A.14 in the appendix A.

Furthermore, as a comparison in the same fruit from the same country but are different in the colour, the red Apple (6) has more peak area than green and Yellow Apple (5 and 7 in the Table 2.1 and Figure 2.2).

**Table2.1:** Averages of Areas for HPLC peaks of vitamin C Amounts in different fruits

<b>No</b>	<b>Name of fruit</b>	<b>Peak area (average)</b>
1	Pakistani Tangerine	815629
2	Malaysian Banana	882347
3	Syrian Peach	1181794
4	Egypt Mango	1516123
5	Iranian Green Apple	1167605
6	Iranian Red Apple	1373206
7	Iranian yellow Apple	541273
8	Turkish pomegranate	3657612
9	Iranian Pear	354247
10	Iranian plumb	461044
11	Syrian Strawberry	2095508
12	Iranian yellow Cindy	765613
13	Syrian red Cindy	2966940
14	Turkish Lemon	2211051
15	Iranian Kiwi	1509165
16	Iraqi Orange	1527519
17	Turkish Grape (green)	1402082
18	Iraqi Naranji	2011353

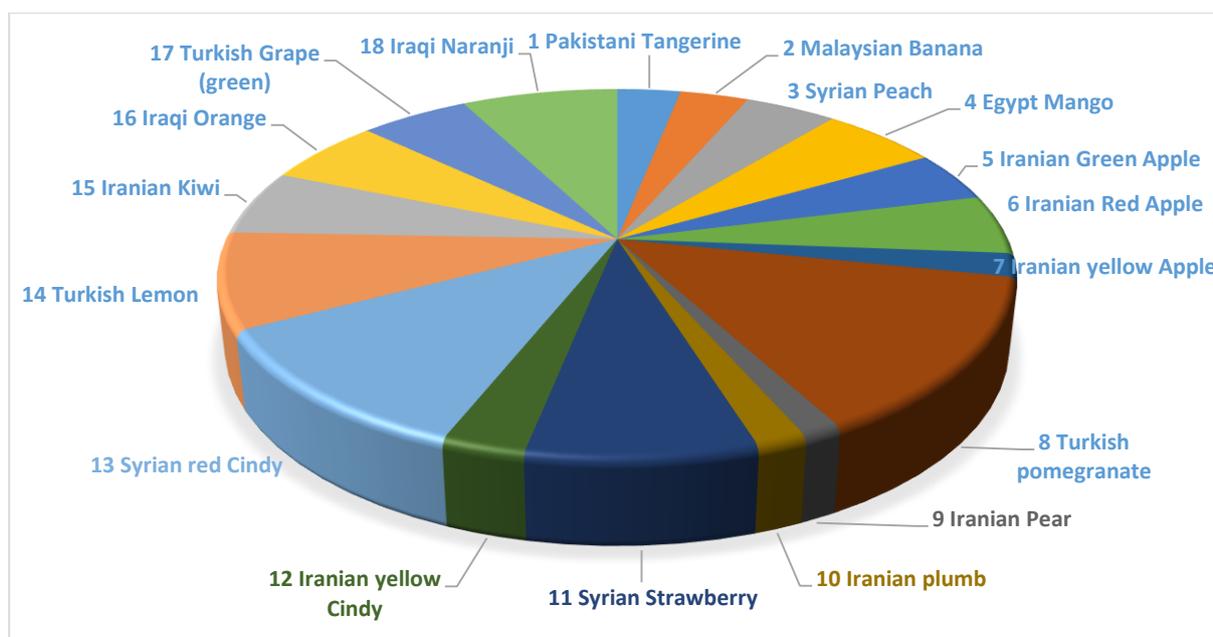


Figure 2.2: Pi chart of Areas of HPLC peaks for Amount of Vitamin C as a percentage in different fruits

### 3-Conclusion

There are some fruits contain high level of the vitamin C as Turkey pomegranate and Syrian red Cindy while, some fruits contain low level of the vitamin C as Iranian Pear. In addition to that, several fruits include about equal low content of the vitamin C as Plumb and yellow Apple from the same country (Iran). furthermore, it was found that Iranian red Apple has more content of the Vitamin C than the same fruit but with different coulure (green and yellow).

### 4-Methods

#### 4.1 Analytical methods

##### 4.1.1 RP-HPLC

HPLC analysis was performed on Dionix isocratic instrument with a Merck (Germany) Chromolith xRP-C18E, 4.6 × 10 mm column, a 5 µL injection and a flow rate of 1 cm<sup>3</sup> min<sup>-1</sup>. The detector wavelength was set at 265 nm. The temperature was 25 °C. Unless stated otherwise, solvent A was H<sub>2</sub>O (50%) and solvent B was MeCN (50%).

#### 4.2 Preparation of standard vitamin C

In 1.5 mL Eppendorf tube, 1 mg of L+-Ascorbic acid (antibiotieous S.P.A, BP 616-27016V AL DE Reuil, France) was dissolved in 400 µL of water then vortexed for 30 seconds until getting clear solution without any precipitated Vitamin. This dissolved Vitamin C was stored in the fridge as a standard solution, ready to check by HPLC.

### 4.3 preparation and analysis of fruit sample

All the different fruits were bought from the local Iraqi market then transport to the fridge of the laboratory directly. 1 mL of the concentrated juice (100%) was centrifuged (6000 rpm) for 3 minutes after its drawing from peace of the fruit by syringe (3 mL). 5  $\mu$ L from the clearance layer of the centrifuged juice was loaded into the injector of the HPLC by the Hamilton syringe (10  $\mu$ L) then was injected to start the run. Table (3.1) indicates the method that has been set up to analysis of each sample (Standard and fruits samples).

Table 4.1: HPLC method for standard vitamin C and fruits samples, which has retention time  $1.37 \pm 0.65$  min.

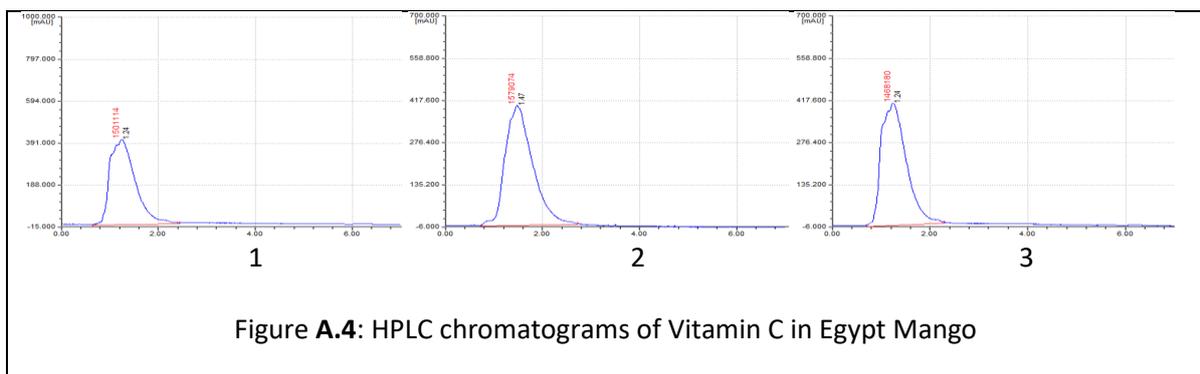
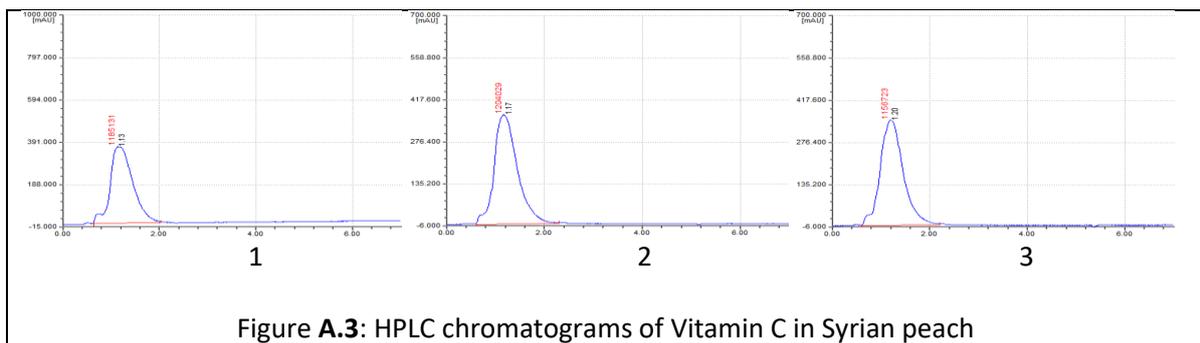
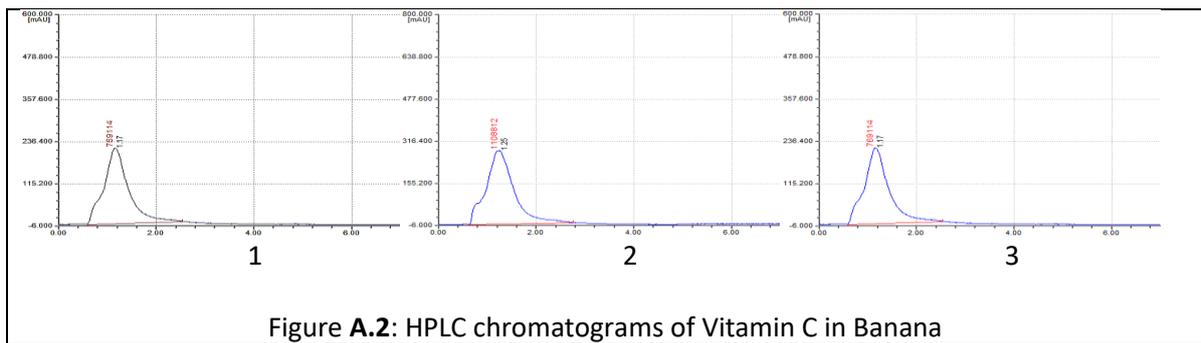
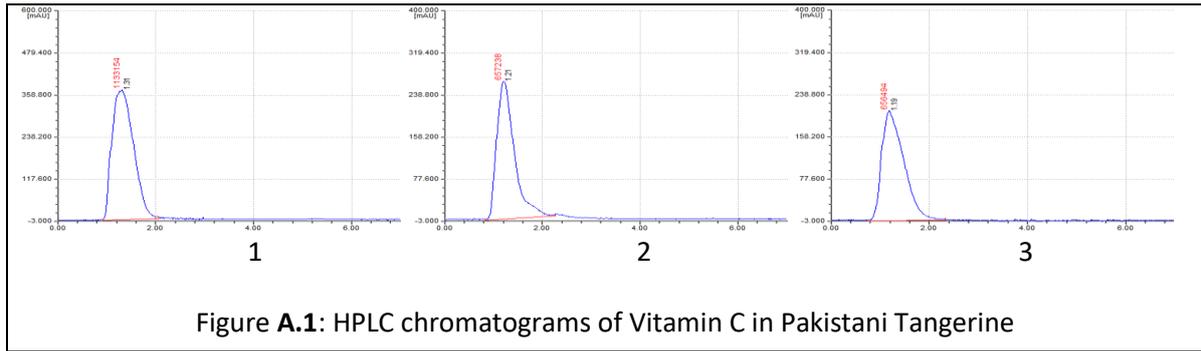
Time (min)	% water	% acetonitrile	Flow rate (mL/min)
0	50	50	1
5	50	50	1
10	50	50	1

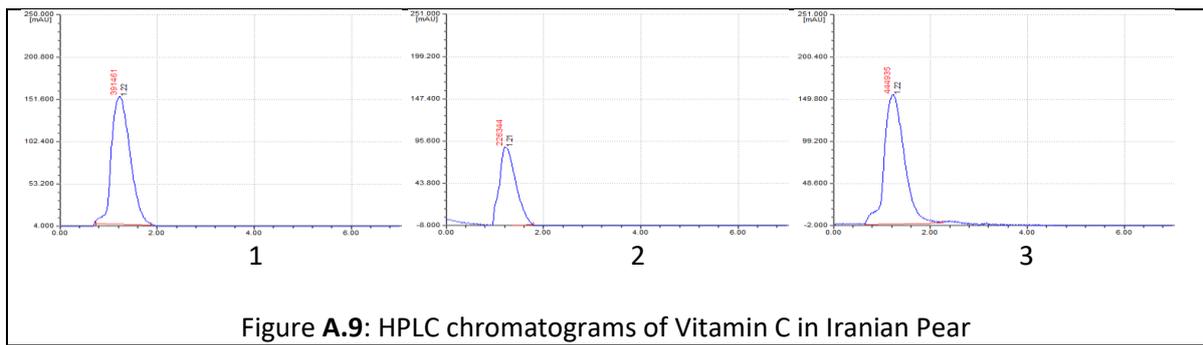
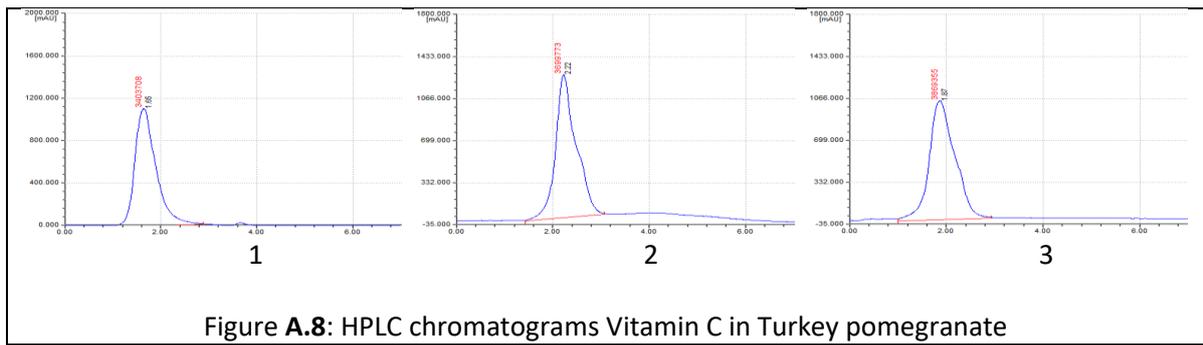
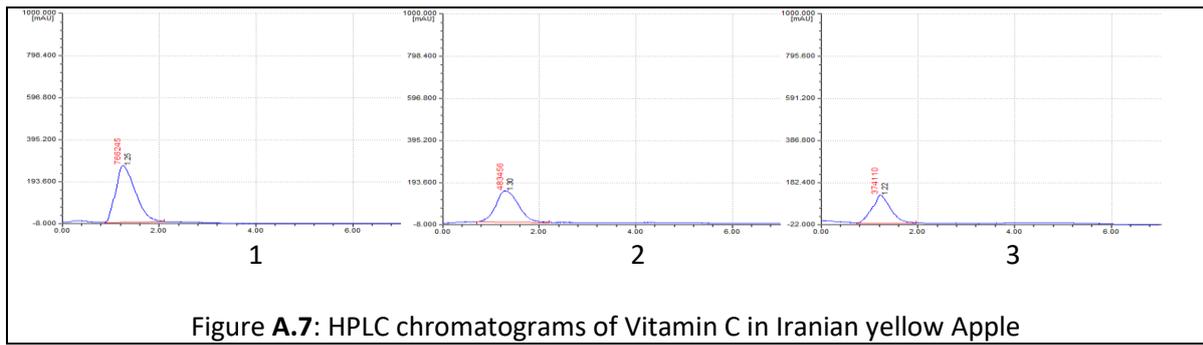
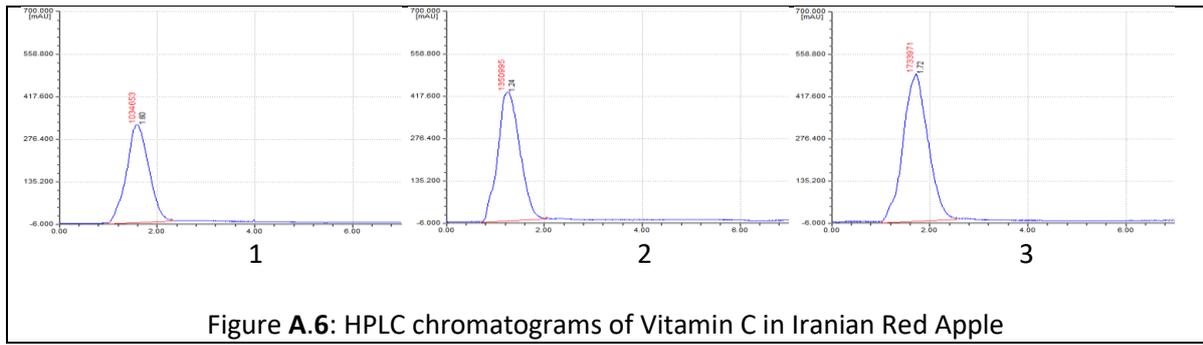
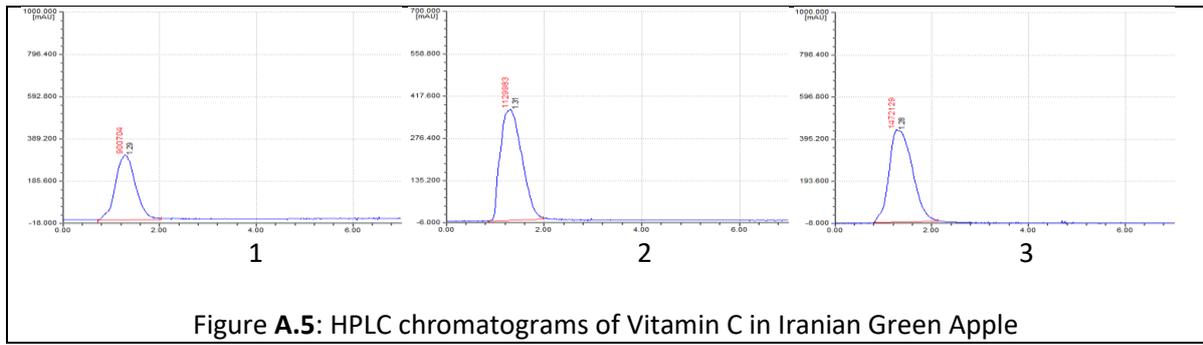
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## 6-Appendix A





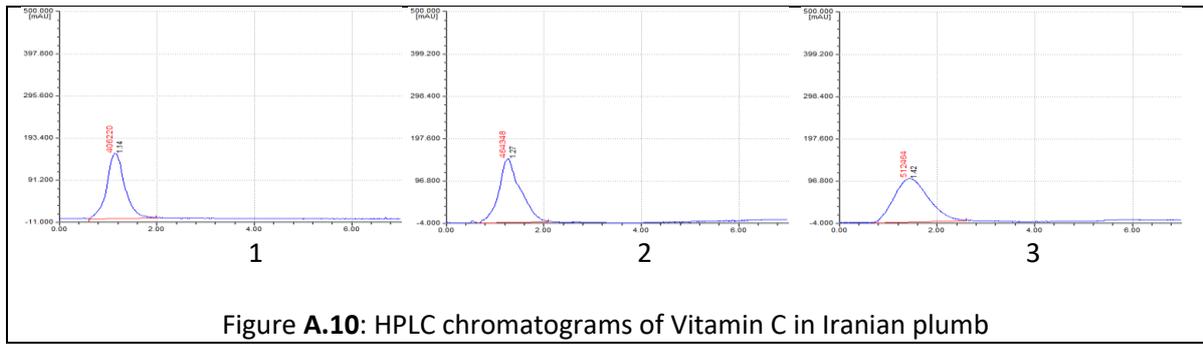


Figure A.10: HPLC chromatograms of Vitamin C in Iranian plumb

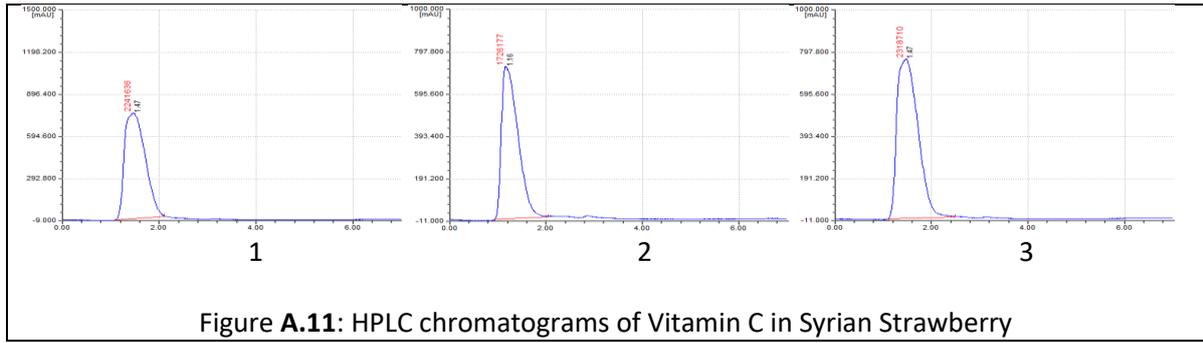


Figure A.11: HPLC chromatograms of Vitamin C in Syrian Strawberry

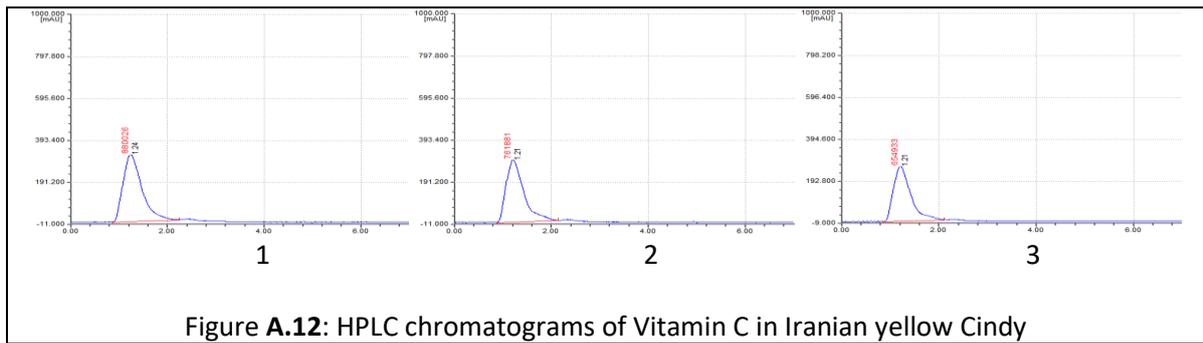


Figure A.12: HPLC chromatograms of Vitamin C in Iranian yellow Cindy

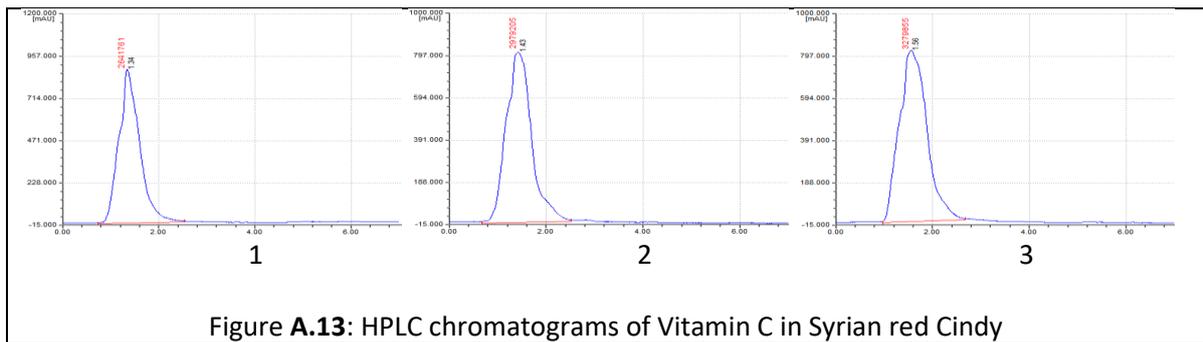


Figure A.13: HPLC chromatograms of Vitamin C in Syrian red Cindy

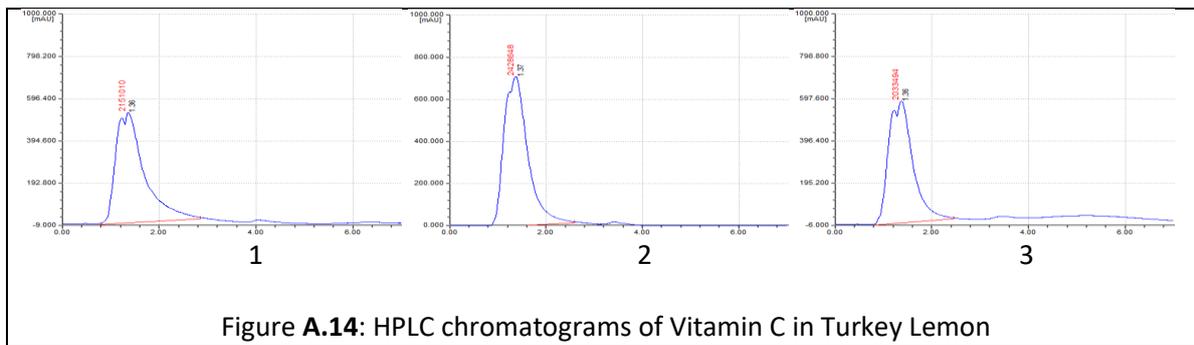


Figure A.14: HPLC chromatograms of Vitamin C in Turkey Lemon

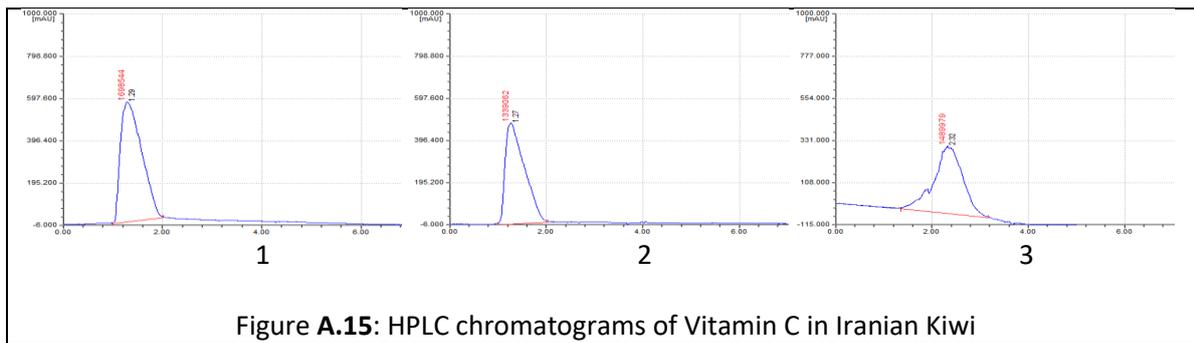


Figure A.15: HPLC chromatograms of Vitamin C in Iranian Kiwi

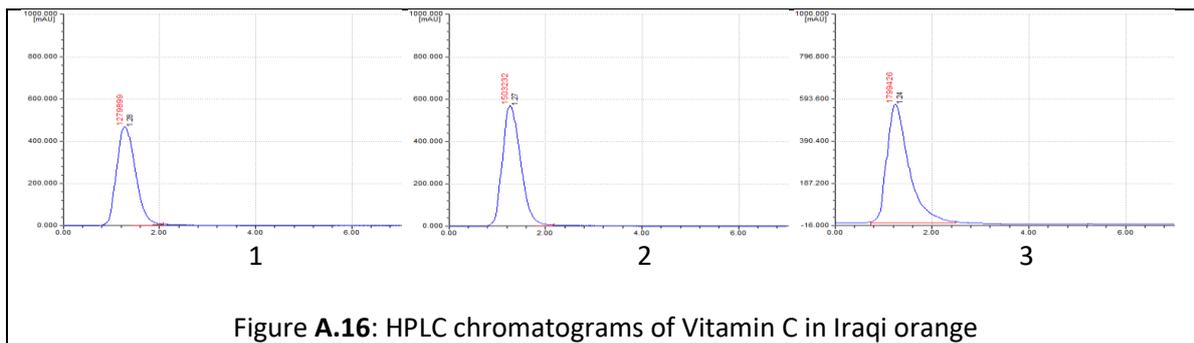


Figure A.16: HPLC chromatograms of Vitamin C in Iraqi orange

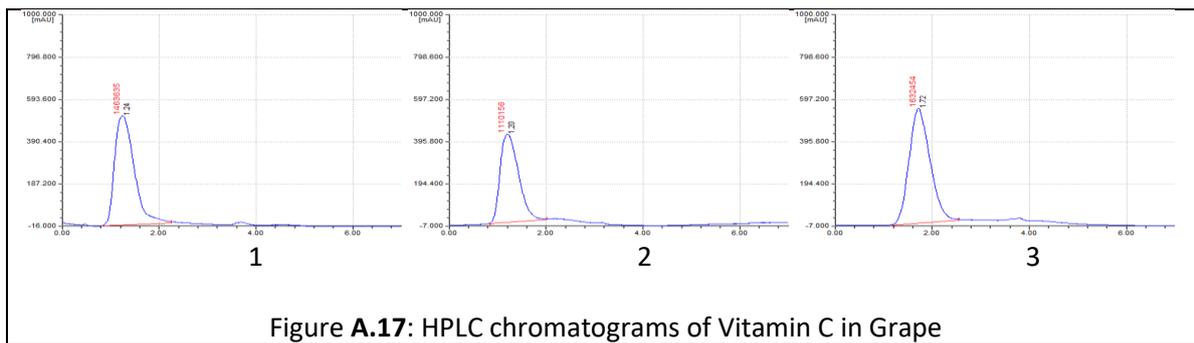


Figure A.17: HPLC chromatograms of Vitamin C in Grape

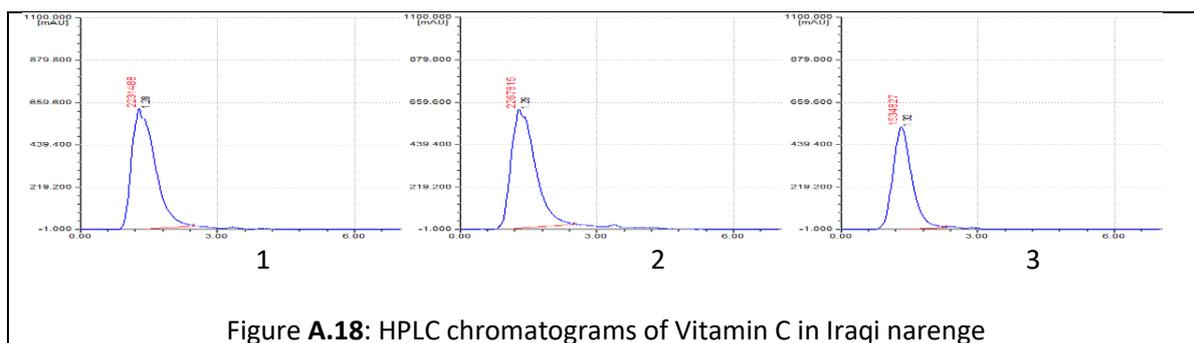


Figure A.18: HPLC chromatograms of Vitamin C in Iraqi nareng

**Table A.1:** Areas of HPLC peaks of vitamin C Amounts in different fruits (three runs)

<b>No</b>	<b>Name of fruit</b>	<b>Peak area (three runs)</b>
<b>1</b>	Pakistani Tangerine	<b>1-1133154 2-657238 3-656494</b>
<b>2</b>	Malaysian Banana	<b>1-769114 2-1108812 3-769114</b>
<b>3</b>	Syrian Peach	<b>1-1185131 2-1204029 3-1156223</b>
<b>4</b>	Egypt Mango	<b>1-1501114 2-1579074 3-1468180</b>
<b>5</b>	Iranian Green Apple	<b>1-900704 2-1129983 3-1472129</b>
<b>6</b>	Iranian Red Apple	<b>1-1034653 2-1350995 3-1733971</b>
<b>7</b>	Iranian yellow Apple	<b>1-766245 2-483465 3-374110</b>
<b>8</b>	Turkish pomegranate	<b>1-3403708 2-3699773 3-3869355</b>
<b>9</b>	Iranian Pear	<b>1-391461 2-226344 3-444935</b>
<b>10</b>	Iranian plumb	<b>1-406220 2-464348 3-512564</b>
<b>11</b>	Syrian Strawberry	<b>1-2241636 2-1726177 3-2318710</b>
<b>12</b>	Iranian yellow Cindy	<b>1-880026 2-761881 3-654933</b>
<b>13</b>	Syrian red Cindy	<b>1-2641761 2-2979205 3-3279855</b>
<b>14</b>	Turkish Lemon	<b>1-2151010 2-2448648 3-2033494</b>
<b>15</b>	Iranian Kiwi	<b>1-1698455 2-1339062 3-1489979</b>
<b>16</b>	Iraqi Orange	<b>1-1279899 1-1503232 3-1799426</b>
<b>17</b>	Turkish Grape (green)	<b>1-1463635 2-1110156 3-1632454</b>
<b>18</b>	Iraqi Naranji	<b>1-2231418 2-2267815 3-1534827</b>