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Comparative evaluation of UV-HPLC methods and reducing agents to determine vitamin C in fruits

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Abstract

Vitamin C is one of the most important antioxidant supplied by fruits and vegetables. Therefore a reliable and easy method is needed for its determination. In this work, two UV-HPLC methods for the determination of ascorbic acid were validated and compared in strawberries, tomatoes and apples. In addition, two different reducing agents [DL-1,4-dithiotreitol (DTT) or 2,3-dimercapto-1-propanol (BAL)] were tried for differentiate dehydroascorbic acid and determine vitamin C. Reliability resulted satisfactory for the UV-HPLC methods in each fruit. UV-HPLC methods resulted linear up to 5 mg/100 g and the least detection and quantification limits were <0.18 mg/100 g and <0.61 mg/100 g, respectively. Precision, as relative standard deviation, ranged from 0.6% to 3.9% and the recovery between 93.6% and 104.4%. Although, the UV-HPLC methods resulted useful for the routine analysis of AA and vitamin C in fruits, the best reliability was achieved when using a C18 column and DTT as reducing agent. Moreover, it may be the UV-HPLC method of choice because it is the easiest and cheapest to perform.
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Keywords: Ascorbic acid; Vitamin C; Fruits; Reliability; Validation; Comparison

1. Introduction

The term vitamin C is used as the generic descriptor for all compounds exhibiting the biological activity of ascorbic acid. It includes esters of ascorbic acid, synthetic forms such as 6-deoxy-L-ascorbic acid and oxidized compounds (Davey et al., 2000). However, vitamin C in fruits is assumed to be the sum of the content of ascorbic acid (AA) plus dehydroascorbic acid (DHAA) (Combs, 1998). These two substances are readily oxidized, especially when exposed to elevated temperatures, some divalent cations (e.g. copper and iron), oxygen, alkaline pH, light, or degradative enzymes (Gregory, 1996). While the oxidation of AA to DHAA is reversible, DHAA can undergo irreversible hydrolysis to diketogulonic acid, which is not biologically active as vitamin C (Russell, 2004). Vitamin oxidation and loss during processing and cooking is of great concern for nutritionists, processors and consumers. Vitamin C is used as an index of the health-related quality of fruits, since, as compared to other beneficial compounds, it is more sensitive to degradation by processing and storage.

Due to the labile nature of vitamin C, preparation procedures are designed to avoid loss of vitamin. Metaphosphoric acid is the most common solvent used which inhibits L-ascorbic oxidase and metal catalysis, and, in addition, precipitates proteins (Eitenmiller & Lande, 1999). On the other hand, in order to determine vitamin C content, DHAA should be reduce to AA in samples. Usually, the DHAA content in samples can be calculated by the difference between the vitamin C (after DHAA reduction) and the ascorbic acid concentrations (previous to reduction) (Fernández-Muiño, Sancho-Ortiz, & Valls-García, 2002). Various thiol-containing compounds such as dithiotreitol (DTT) and dimercaptopropanol (BAL)

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can be used as a reducing agent. However, Diop, Franck, Grimm, and Hasselmann (1988) observed an incomplete reduction of DHAA to AA depending on the reducing agent used.

Various methods have been employed for the analysis of vitamin C in food, including electrochemical (Calokerinos & Hadjiioannou, 1983), spectrophotometric (Liu, Chin, Kiser, & Bigler, 1982), spectrofluorimetric (Sánchez-Mata, Cámara-Hurtado, Díez-Marqués, & Torija-Isasa, 2000) and chromatographic methods. However, high-performance liquid chromatographical (HPLC) methods have some advantages regarding specificity, sensitivity or easy operation (Gökmen & Acar, 1996). Reversed-phase (Furusawa, 2001), bonded-phase NH_2 (Arakawa, Otsuka, Kurata, & Inaka, 1981), ion-exchange (Nelis, De Leenheer, Merchie, Lavens, & Sorgeloos, 1997) or ion-pair reversed columns (Madigan, McMurrough, & Smyth, 1996) have been the most commonly employed columns for vitamin C analysis. Regarding the way of detection, AA can be easily detected by UV at wavelengths between 245 nm and 254 nm. Although, UV detectors are usually included in HPLC systems and are simpler and faster than others, few UV-HPLC methods have been validated to be used for vitamin C determination in foods. Most of these methods have been validated in beer, wine and fruit beverages. However, fruits are different from those fermented products and more complex matrices than beverages and so naturally occurring compounds could affect the detection or interfere in the identification and quantification of AA. Davey et al. (2000) reported that considerable caution should be taken when using methods that have been developed for the analysis of specific plant tissue in the assay of other different matrixes. On the other hand, according to the available literature, vitamin C concentration varied greatly among the type of fruit and cultivars. The content of vitamin C in fruits ranged from 200 to 210 mg/100 g for blackcurrant to 2–0 mg/100 g for apple (Davey et al., 2000; Russell, 2000).

In general, fruits tend to be a good source of vitamin C; however, fruits such as pears, plums and apples, contain only a very modest concentration of this vitamin. Consequently, obtaining an adequate method is needed for measuring the concentration of vitamin C in specific fruits.

The aim of this work was to evaluate the feasibility of using different UV-HPLC methods for determining vitamin C in fruits with different concentration of vitamin C. Strawberry (high vitamin C concentration), tomato (medium concentration) and apples (low concentration) were chosen. Two reducing agents and two UV-HPLC methods were tried. Moreover, AA was determined before and after reduction to calculate the DHAA in the sample. The reliability of the methods was evaluated in terms of linearity, sensitivity, precision and recovery. A comparative study was carried out between reducing agents as well as UV-HPLC methods.

2. Materials and methods

2.1. Reagents

Metafosforic acid, DL-1,4-dithiotreitol (DTT) and 2,3-dimercapto-1-propanol (BAL) were purchased from Acros Organics (NJ, USA); ascorbic acid, acetonitrile, potassium dihydrogen phosphate and sulphuric acid were obtained from Scharlau Chemie, SA (Barcelona, Spain).

2.2. Sample preparation

2.2.1. Ascorbic acid

Strawberries, tomatoes and apples were bought from a local supermarket at commercial maturity and storaged at 4(±1) °C before analysis. The extraction was based on a procedure proposed by Brubacher, Müller-Mulot, and Southgate (1985). A portion of 25 g of fruit was added to 25 ml of 4.5% metaphosphoric solution. The mixture was homogenized and centrifuged at 22,100*g* for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No. 1. Then, 10 ml of the vacuum-filtered sample were passed through a Millipore 0.45 μm membrane and thus were ready to be injected in the HPLC system.

2.2.2. Vitamin C

To quantify the total concentration of vitamin C, two different reductors were tried. A solution of DTT (20 mg/mL) was prepared and an aliquot of 0.2 ml was added to 1 ml of the vacuum-filtered sample, obtained in AA analysis, following the method proposed by Sánchez-Mata et al. (2000). An aliquot of 2 μl of the other reductor (BAL) was combined with each ml of the vacuum-filtered sample based on a method proposed by Soliva-Fortuny and Martín-Belloso (2003). The mixtures were kept in the darkness for 2 h. Then they were passed through a Millipore 0.45 μm membrane and injected into the HPLC system.

The DHAA was calculated as the difference between the vitamin C (after reduction) and AA (without reduction) (Sánchez-Mata et al., 2000).

2.3. Chromatographic conditions

The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 245 nm. Samples were introduced onto the column through a manual injector equipped with a sample loop (20 μl). The flow rate was fixed at 1.0 ml/min at room temperature. Two different chromatographic conditions were tried: (a) A reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm × 250 mm) was used as stationary phase. The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH 2.6 (Sánchez-Mata et al., 2000). (b) A NH_2-Spherisorb S5 Column (250 × 4.6 mm, 5 μm) was employed. The eluent was 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and aceto-

nitrile in a ratio 60:40 under isocratic conditions (Soliva-Fortuny & Martín-Belloso, 2003).

2.4. Validation

The reliability of HPLC-methods was validated through their linearity, sensitivity, precision and recovery.

2.4.1. Linearity

Once verified the normal distribution of the results, linearity was evaluated through the relationship between the concentration of acid ascorbic (independent variable) and the absorbance obtained thought the HPLC-UV detector (dependent variable). Then an analysis of variance of the regression and a residual plot were carried out. The experimental Fisher value (F_{cal}) was compared to its tabulated value (F_{tab}) for 1 and $n-2$ degrees of freedom (Steel & Torrie, 1980). The determination coefficient (r^2) was calculated by means of the least-squares analysis. Three calibration lines were carried out for each chromatographic condition and reducing agent. Moreover, every calibration line was done through three replicates of each concentration of ascorbic acid (0.5, 1, 1.5, 3 and 5 mg/100 g) to know the extent of the total variability of the response that could be explained by the linear regression model.

2.4.2. Sensitivity

The detection limit (DL) and quantification limit (QL) were calculated from the calibration lines that defined linearity, using the Long and Winefordner criterion (Long & Winefordner, 1983) as expressed in Eqs. (1) and (2).

$$\mathrm{DL} = \frac{3 \times S}{a} \quad (1)$$

$$\mathrm{QL} = \frac{10 \times S}{a} \quad (2)$$

where a is the slope of the calibration line and S is the standard error of the intercepted point.

2.4.3. Precision

The precision of the method indicates the degree of dispersion within a series of determinations on the same sample. Six measurements were performed for each tried UV-HPLC method and reducing agent in strawberries, tomatoes and apples giving a total of 144 samples. The relative standard deviations (RSD_{exp}) were calculated dividing the standard deviation by the mean of the concentration, and the adequacy of the (RSD_{exp}) to the Horwitz criterion (Horwitz, 1982) was evaluated.

2.4.4. Recovery

Recovery was tested by the standard addition procedure at two levels for each method on strawberries, tomatoes and apples. The concentrations of AA added to the sample were: 30 and 60 mg/100 g in strawberry, 10 and 20 mg/100 g for tomatoes and 1.5 and 3 mg/100 g for apples. In each addition level, six determinations were carried out for each UV-HPLC method, reducing agent and fruit (216 samples), and the recovery (%) was calculated in every case. The homogeneity of variances between levels of addition was verified by a Cochran test (Steel & Torrie, 1980). The mean recoveries of each level were compared using a Student's t-test, the experimental value (t_{exp}) was compared to the tabulated value (t_{tab}) for $(n-1)$ degrees of freedom (Steel & Torrie, 1980). Therefore, an average value of both levels could be considered when t_{exp} was lower than t_{tab}.

2.5. Comparison of the methods

A comparison procedure was carried out to find significant differences among the mean values obtained throughout the UV-HPLC methods, with or without addition of reducing agent. The least significant difference test was employed to determine differences among means at a 5% significance level. Moreover the principle of Bland and Altman (1986) was used to compare UV-HPLC methods and kind of reducing agent. The statistical treatments were performed with Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md).

A comparative study was carried out in terms of linearity from three calibration lines with their respective r-value, sensitivity by DL and QL and precision through the RSD values. To carry out the comparison test on recovery terms, all the values of recovery of each set of analysis were considered in each case.

3. Results and discussion

3.1. Validation of the methods

HPLC-methods methods were validated through their linearity, sensitivity, precision and recovery, with and without reducing agent added.

3.1.1. Linearity

Absorbance responses of AA, with and without reducing agent addition, were significantly linear up to 5 mg/100 g according to the determination coefficient (r^2) shown in Table 1. In addition, the residuals are randomly distributed around the line with zero mean (Fig. 1). Therefore the regression model represents the data correctly for all HPLC-UV methods, with or without reducing agent addition. There is a good relationship between the concentration of AA and the area obtained throughout both UV-HPLC methods, C18 column with mobile phase of sulphuric acid (0.01%) adjusted to pH 2.6 and NH_2 column with 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile (60:40) as a mobile phase. The coefficients of determination (r^2) were higher than 99.36% in every method and reducing agent used. On the other hand, similar slopes of the calibration lines were observed between UV-HPLC methods without reducing agent addition. However, slopes were lower when using a NH_2 column than a

Table 1
Linearity and sensitivity for the evaluated UV-HPLC methods to determine ascorbic acid

Column	Reducing agent	Calibration line[a]	r^2 (%)	Standard error[b]	DL[c] (mg/100 g)	QL[c] (mg/100 g)
–C18	–	$y = 651431x - 15425$	99.44	36,983	0.17	0.57
	DTT	$y = 619745x + 48537$	99.36	37,702	0.18	0.61
	BAL	$y = 649402x - 414$	99.60	31,373	0.14	0.48
$–NH_2$	–	$y = 616385x + 44301$	99.80	20,882	0.10	0.34
	DTT	$y = 524769x + 13532$	99.71	21,455	0.12	0.41
	BAL	$y = 544883x \pm 25944$	99.87	14,838	0.08	0.27

DTT = 1,4-dithiotreitol; BAL = 2,3-dimercapto-1-propanol; DL = detection limit; QL = quantification limit.
[a] y = slope·$x \pm$ intercepted point ($n = 9$).
[b] Standard error of the intercept point of the calibration line.
[c] No significant differences were found among UV-HPLC methods and reducing agent.

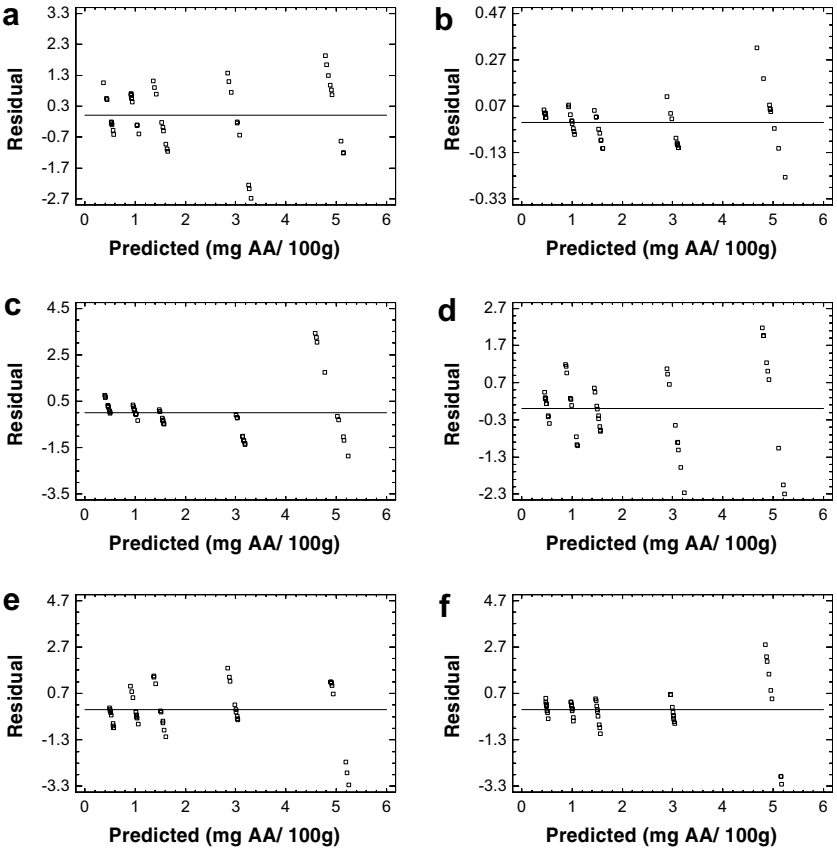

Fig. 1. Residual plots of the regression model for the evaluated UV-HPLC methods to determine ascorbic acid: (a) through C18 column and without reducing agent, (b) through NH_2 column without reducing agent, (c) through C18 column and DTT as reducing agent, (d) through C18 column and BAL as a reducing agent, (e) through NH_2 column and DTT as a reducing agent and (f) through a NH_2 column and BAL as a reducing agent.

C18 column. The consequence of these different slopes may affect the sensitivity of the methods.

3.1.2. Detection (DL) and quantification (QL) limits

DL can be defined as the minimum concentration capable of giving a chromatographic signal three times higher than background noise. The QL is the lowest amount of analyte in the sample which can be quantitatively determined with precision and accuracy. Lower standard errors of the intercepted point were achieved throughout a NH_2 column than a C18 column with or without reducing agent addition (Table 1). In addition, the higher the standard error of the intercepted point the lower the sensitivity was. The DL and QL obtained for AA through a C18 column without reducing agent were 0.17 and 0.57 mg/100 g, respectively, while 0.10 and 0.34 mg/100 g were the corresponding limits using a NH_2 column (Table 1). On the other hand, DL and QL were lower than 0.18 and 0.61 mg/100 g, respectively, when AA was determined with reducing agent irrespective of the UV-HPLC method. The QL values achieved through the tried UV-HPLC methods, with or without reducing agent addition, were lower than

the content of vitamin C present in fruits, thus they can be considered sensible enough for general determination of vitamin C in fruits.

3.1.3. Precision

The relative standard deviations (RSD) achieved for each UV-HPLC method and reducing agent was less than 5% for AA and vitamin C determination (Table 2). According to the Horwitz criterion (Horwitz, 1982), all RSD obtained were satisfactory, thus all UV-HPLC methods irrespective of the reducing agent tried may be considered precise for AA and vitamin C determination. The RSD values range from 0.6% to 3.9% in AA analysis. The lowest value of RSD for vitamin C determination was obtained in strawberries (0.8%) using BAL as a reducing agent and the UV-HPLC system with a C18 column, whereas the results obtained throughout a NH_2 column and DTT led the maximal value of RSD (3.9%) in apples. The precision values obtained in the present work were also in the range recommended by the Association of Official Analytical Chemists for substances around 10 mg/L (AOAC, 1998). Sánchez-Mata et al. (2000) proposed the calculation of DHAA by difference between the content of vitamin C and AA. These authors calculated, with good results, the content of DHAA in green beans. However, neither the methods with a C18 column nor those using a NH_2 column gave precise results for DHAA in the studied fruits because of their high RSD values (Table 2). On the other hand, as can be observed in Table 2, the concentration of AA and vitamin C in strawberries, tomatoes and apples obtained

Table 2
Precision of the assayed UV-HPLC methods for the determination of ascorbic acid (AA), dehydroascorbic acid (DHAA) and vitamin C (VitC) in strawberry (STR), tomatoes (TOM) and apples (APP)

Column	Reducing agent	Substance	Fruit	Concentration[a] (mg/100 g)	RSD_{exp} (%)[c]	RSD Horwitz[b]
−C18	DTT	AA	STR	57.2 ± 0.4	0.7[a]	6.153
			TOM	21.7 ± 0.2	0.9[a]	7.119
			APP	1.78 ± 0.02	1.1[a]	10.373
		DHAA	STR	2.2 ± 0.6	27.3	10.048
			TOM	1.8 ± 0.3	16.7	10.356
			APP	0.07 ± 0.06	85.7	16.882
		VitC	STR	59.1 ± 0.9	1.5[a]	6.122
			TOM	23.6 ± 0.2	0.9[a]	7.030
			APP	1.78 ± 0.06	3.4[a]	10.373
	BAL	AA	STR	52.7 ± 0.3	0.6[a]	6.229
			TOM	18.0 ± 0.4	2.2[a]	7.323
			APP	3.6 ± 0.1	2.8[a]	9.330
		DHAA	STR	7.8 ± 0.9	11.5	8.305
			TOM	2.8 ± 0.5	17.9	9.689
			APP	0.48± 0.08	16.7	12.635
		VitC	STR	60.6 ± 0.5	0.8[a]	6.100
			TOM	20.9 ± 0.4	1.9[a]	7.160
			APP	4.11 ± 0.05	1.2[a]	9.146
$-NH_2$	DTT	AA	STR	53.0 ± 1.9	3.6[b]	6.224
			TOM	19.0 ± 0.4	2.1[b]	7.263
			APP	2.7 ± 0.1	3.7[b]	9.743
		DHAA	STR	10.3 ± 1.8	17.5	7.964
			TOM	9.6 ± 1.0	10.4	8.049
			APP	2.4 ± 0.3	12.5	9.917
		VitC	STR	63.4 ± 0.8	1.3[b]	6.058
			TOM	28.6 ± 0.4	1.4[b]	6.830
			APP	5.1 ± 0.2	3.9[b]	8.853
	BAL	AA	STR	54.5 ± 2.1	3.8[b]	6.198
			TOM	22.7 ± 0.7	3.1[b]	7.071
			APP	3.1 ± 0.1	3.2[b]	9.542
		DHAA	STR	7.8 ± 2.4	30.8	8.305
			TOM	5.1 ± 1.3	25.5	8.853
			APP	0.7 ± 0.2	28.6	11.938
		VitC	STR	62.3 ± 1.0	1.6[b]	6.074
			TOM	27.8 ± 1.0	3.6[b]	6.859
			APP	3.8 ± 0.1	2.6[b]	9.254

DTT = 1,4-dithiotreitol BAL = 2,3-dimercapto-1-propanol.
Values of RSD of DHAA were not analyzed because of the high RSD shown.
[a] Mean ± standard deviation ($n = 6$).
[b] Acceptable RSD value based on the Horwitz criterion.
[c] Values in the same column with different letters are significant different ($p < 0.05$).

in this work are in the range of those published in the literature which varied from 40–90 mg/100 g in strawberries, 20–30 mg/100 g for tomatoes and 2–10 mg/100 g for apple (Davey et al., 2000; Russell, 2000) and confirm the different content of vitamin C depending on the type of fruit. Breene (1994) reported that variability of vitamin C within the type of fruit might be attributed to environmental and cultural practices. Vitamin C content varied considerably among cultivars, ripeness and growing condition. Harvest maturity, soil fertilization, irrigation, light intensity and day/night temperatures could also affect vitamin C content in fruits (Davey et al., 2000).

3.1.4. Recovery

Mean recovery percentages ranged from 93.6% to 104.4% (Table 3). All the variances of the recovery obtained for UV-HPLC methods, with or without reducing agent addition, were homogeneous through the Cochran test. The Student test showed that the recovery of AA did not depend on the addition of this compound in each fruit, and thus, the final recovery was the average of the results obtained in both levels of addition for each fruit. Moreover, recovery was similar to the theoretical 100% for each assay, so all recovery values were satisfactory according to Student's *t*-test.

3.1.5. Comparison of the methods

UV-HPLC methods were significantly linear up to 5 mg/100 g and sensitive enough to determine AA using or not reducing agent (Table 1 and Fig. 1). However, lower slopes and standard errors of the intercepted point were observed using a NH_2 column than a C18 column. As a result, the sensitivity of the methods was different. DL and QL were lower when a NH_2 column and the mobile phase was 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile than those methods using a C18 column with the mobile phase of 0.01% solution of sulphuric acid adjusted to pH 2.6. Through this UV-HPLC method, minimal values of DL and QL of 0.08 and 0.27 mg/100 g were found when the determinations of AA were performed with BAL as a reducing agent. On the contrary, DL and QL were <0.18 and <0.61 mg/100 g, respectively, when the analysis was done throughout a C18 column irrespective of the reducing agent used. However, the differences were not significant for linearity and sensitivity among UV-HPLC methods with or without addition of reducing agent. On the contrary, precision results depended significantly on the UV-HPLC method. As can be seen in Fig. 2a, there was lack of agreement between UV-HPLC methods in strawberry samples determination. In the same way and complementary, the ANOVA test indicated that the obtained RSD throughout a C18 column was significantly better than that achieved by a NH_2 column with or without reducing agent addition (Table 2). However, Bland and Altman (1986) and ANOVA test indicated that both reducing agents might be used to determine vitamin C in fruits irrespective of the content of this vitamin in the fruits (Fig. 2b, Table 2). In contrast, neither method with NH_2 nor with C18 column is precise for DHAA irrespective of the reducing agent

Table 3
Recovery of the assayed UV-HPLC methods to determine ascorbic acid (AA) and vitamin (Vit C) in strawberry (STR), tomatoes (TOM) and apples (APP)

Column	Reducing agent	Substance	Fruit	Recovery (%)[a]		Mean[c] recovery	100-Student test[b]
				Level I	Level II		
−C18	–	AA	STR	100.7 ± 1.9	101.9 ± 3.1	$101.3^{aA\alpha}$	1.802
			TOM	95.7 ± 2.3	96.3 ± 4.6	$96.0^{aA\alpha}$	3.925
			APP	107.9 ± 2.5	100.5± 5.7	$104.2^{aB\alpha}$	2.561
	DTT	VitC	STR	104.5 ± 0.9	101.3 ± 2.1	$102.9^{aA\alpha}$	4.235
			TOM	100.1 ± 2.9	97.6 ± 3.4	$98.8^{aA\alpha}$	1.202
			APP	106.4 ± 1.6	101.2 ± 2.8	$103.8^{aB\alpha}$	3.795
	BAL	VitC	STR	107.8 ± 1.9	100.9 ± 4.7	$104.3^{aA\beta}$	3.174
			TOM	96.0 ± 7.2	95.4 ± 5.9	$95.7^{aA\beta}$	3.506
			APP	107.0 ± 3.7	101.8 ± 2.8	$104.4^{aB\beta}$	3.681
$-NH_2$	–	AA	STR	91.0 ± 4.4	96.1 ± 8.9	$93.6^{bA\alpha}$	3.098
			TOM	96.0 ± 3.6	95.4 ± 5.1	$95.7^{bA\alpha}$	3.492
			APP	91.2 ± 5.1	100.8 ± 11.3	$96.0^{bB\alpha}$	1.427
	DTT	VitC	STR	91.6 ± 5.6	98. 9 ± 5.5	$95.2^{bA\alpha}$	2.511
			TOM	104.0 ± 3.5	100.3 ± 7.5	$102.1^{bA\alpha}$	0.411
			APP	97.1 ± 9.9	99.5 ± 8.6	$98.3^{bB\alpha}$	0.662
	BAL	VitC	STR	105.7 ± 1.8	96.8 ± 1.5	$101.2^{bA\beta}$	1.298
			TOM	104.0 ± 3.5	98.7 ± 2.3	$101.3^{bA\beta}$	1.179
			APP	105.3 ± 1.0	96.1 ± 1.8	$100.7^{bB\beta}$	0.495

DTT = 1,4-dithiotreitol BAL = 2,3-dimercapto-1-propanol.

[a] Recovery mean ± standard deviation ($n = 6$ in each level).

[b] Test to determinate differences among the mean recovery obtained and the theoretical 100% $t_{tab(11,0.001)} = 4.437$.

[c] Different values of lower case letter stand for significant differences between columns. Different values of capital letter in the same column correspond to significant differences among fruits. Different values of symbol in the same column correspond to significant differences between reducing agents.

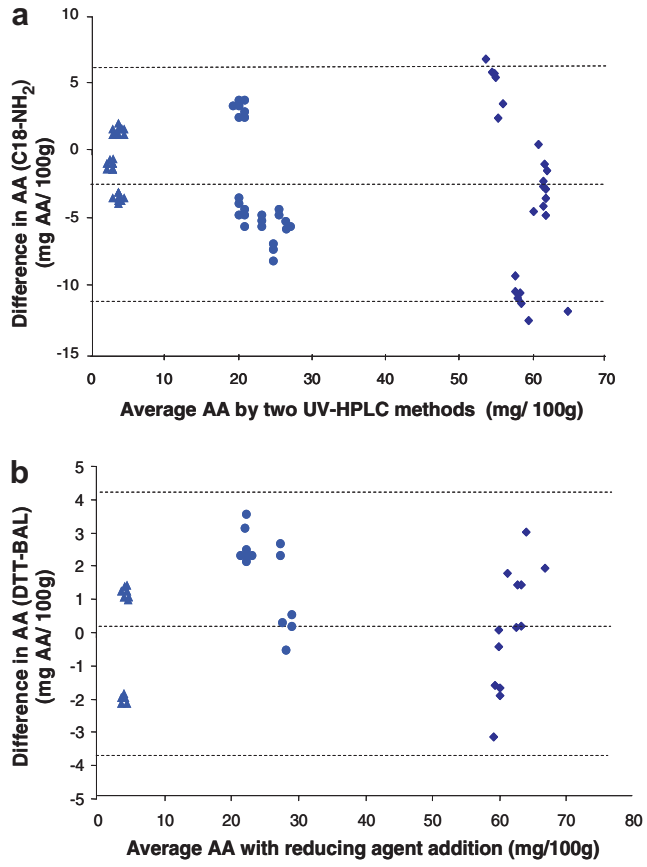

Fig. 2. Comparison of two UV-HPLC methods: (a) a C18 column with a mobile phase of sulphuric acid (0.01%) adjusted to pH 2.6 and a NH_2 column with 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile (60:40) as a mobile phase and two different reducing agent, (b) DTT (DL-1,4-dithiotreitol) and BAL (2,3-dimercapto-1-propanol) to determine ascorbic acid content (AA) in strawberry (♦), tomato (●) and apple (▲) according to the Bland and Altman test. In both cases the detection took place at 245 nm.

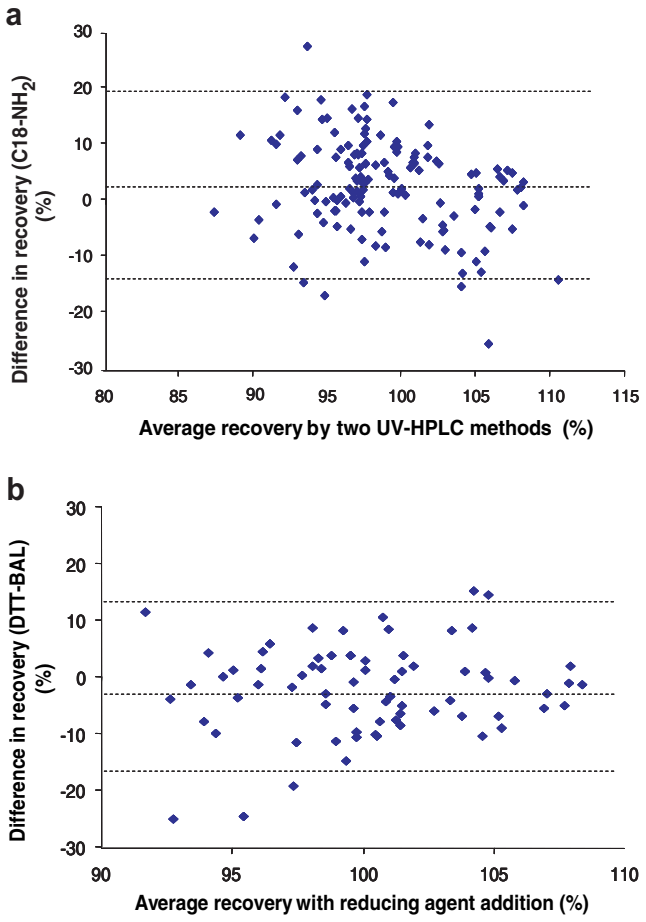

Fig. 3. Comparison of the recovery obtained with two UV-HPLC methods: (a) a C18 column with a mobile phase of sulphuric acid (0.01%) adjusted to pH 2.6 and the other with a NH_2 column with 10 mM potassium dihydrogen phosphate buffer adjusted to pH 3.5 and acetonitrile (60:40) as a mobile phase and two different reducing agent and (b) DTT (DL-1,4-dithiotreitol) and BAL (2,3-dimercapto-1-propanol) in strawberry, tomato and apple according to the Bland and Altman test. In both cases the detection took place at 245 nm.

added (Table 2). Ball (1997) reported that errors in DHAA content can be observed if the concentration of this compound in the sample is very low in comparison to the content of AA. Some authors (Fernández-Muiño et al., 2002; Wills, Wimalasiri, & Greenfield, 1984) have reported that DHAA is present at low levels in fresh fruit; consequently, the high RSD values found in this study for DHAA may be due to its low concentration in the sample. Furthermore high RSD values might be a consequence of an incomplete reduction of DHAA to AA. Diop et al. (1988) observed a DHAA reduction of 55% using homocysteine as a reducing agent. In contrast, Deutsch and Santhosh-Kumar (1996) observed that using sulfhydryl compounds in the reduction of DHAA to AA, other substances different to DHAA can be transformed to AA. On the other hand, differences in recoveries among UV-HPLC methods and reducing agents were observed (Fig. 3). The HPLC system constituted by a C18 column with mobile phase of sulphuric acid (0.01%) adjusted to pH 2.6 led to better recoveries of AA and vitamin C, according the LSD test (Table 3). In addition, recoveries closer to 100% were observed using DTT when reducing agent was added (Table 3). Significantly better recoveries were achieved in strawberries and tomatoes than in apples (Table 3). Vitamin C content added to apples was between 10 and 20 times lower than those used in tomatoes and strawberries, consequently, differences in recovery results for apples might be due to the low concentration of vitamin C in this fruits.

4. Conclusions

Reliability has been satisfactory for all the evaluated UV-HPLC methods, reducing agents and fruits. In every case, suitable linearity, sensitivity, precision and accuracy through recovery for AA and vitamin C analysis in strawberries, tomatoes and apples were obtained. Both UV-HPLC methods and reducing agent studied are useful for determining the content of AA and vitamin C in strawberries, tomatoes and apples. However, the determination of AA through

the method where a C18 column is used, results more adequate in terms of precision and recovery. Furthermore, it is easier and cheaper to carry out than the UV-HPLC method with a NH_2 column. So, the UV-HPLC method with C18 column may be chosen for routine analysis. On the other hand, significantly better recoveries values were reached using DTT as reducing agent, thus DTT may be selected for the analysis of vitamin C.

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