Vitamin C: Present State of Methodology; Content Variability in Food Samples

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Introduction

High performance liquid chromatographic (HPLC) procedures are now available for the quantitation of vitamin C in foods and other biological matrices. These procedures are superior to the classical "wet-chemistry" methods in that they are relatively free of interferences and are much less tedious to perform on an on-going basis.

The new labeling laws, to be implemented in the coming year, have introduced a new wrinkle into the equation. Vitamin C content will be required on labels for food products. For many products this presents no problems but, increasingly, the food industry has been using isoascorbic (erythorbic) acid (IAA) as an antioxidant in foods. Isoascorbic acid is an epimer of ascorbic acid (AA) but has little or no vitamin C activity. Since they are epimers, many of their chemical and physical properties are the same and, consequently, it is difficult and sometimes impossible to distinguish between these compounds with the classical methods of analysis. The recent HPLC methods, however, can easily distinguish between these compounds and their oxidation products, dehydroascorbic (DHAA) and dehydroisoascorbic acid (DHIAA) which have the same vitamin activity as the original acids. HPLC procedures have an added advantage in that they can be easily automated.

It is our intent to discuss the most recent HPLC methods which have been tested on foods and mention what we believe to be their weaknesses and strengths and to suggest what methodologies could be used so as to satisfy the new labeling requirements. In addition, we will address the problem of the vitamin C content variability in food samples as this does present a problem to the analyst with regard to identifying a representative sample for a given food product.

HPLC Methods for Food Matrices

The most promising methods for the determination of the vitamin C content of foods are shown in Table I. There are two groups -- the first using ODS columns and the second using PLRP-S columns (Polymer Laboratories, Amherst, MA). With one exception, the methods can distinguish between AA and IAA.

In methods that measure AA and IAA directly, either electrochemical detection (EC) or ultraviolet absorbance (UV) is used for quantitation. Since many foods

contain from 10-20% of their vitamin C activity in the form of DHAA (Vanderslice and Higgs, 1991), it is desirable to reduce DHAA to AA before analyzing for total vitamin C content. As can be seen from the table, cysteine and homocysteine have been used for reduction. It has been found, for homocysteine at least, that this increased the levels of vitamin C determined (Lloyd et al., 1988). In two of the methods (Kitada et al., 1989; Schüep and Keck, 1990), no reduction step was used; such a step would have to be incorporated in those procedures to make the methods more generally applicable.

Two of the methods listed (Dodson et al., 1992; Vanderslice and Higgs, 1990 a,b) oxidize the acids to the dehydro forms and then use orthophenylene diamine (OPD) to form a fluorescent derivative for quantitation purposes. Dodson et al. (1992) use the activated charcoal method of Deutsch and Weeks (1965) for pre-column oxidation while Vanderslice and Higgs (1990 a,b) use post-column oxidation with mercuric chloride. Vanderslice and Higgs' (1990 a,b) method does distinguish between AA, IAA, DHAA and DHIAA while Dodson's (1992) procedures determine total vitamin C. Both Dodson's (1992) and Vanderslice and Higgs' (1990 a,b) procedures have been successfully applied to a large variety of samples while the other procedures have been applied mainly to cured and smoked meats, some beverages and fruit juices. This suggests that further tests should be run on the latter methods to ensure that no interferences occur in different food matrices. The reaction of OPD with DHAA is a fairly specific reaction and no interferences have been reported with the use of this reagent by either Dodson (1992) or Vanderslice and Higgs (1990 a,b).

The Vanderslice and Higgs (1990 a,b) method allows for the direct determination of DHAA in food samples. The other methods could presumably measure the DHAA control by performing two analyses on each sample, one before and one after reduction, and obtain DHAA by difference. For most regulatory purposes, this is not necessary as it is only total vitamin C that is of interest.

Finally, a word about columns. Our laboratory has had extensive experience with both the ODS and the PLRP-S columns. We have found that the PLRP-S columns give better separations of IAA and AA, are much more rugged than the ODS columns and can be used for thousands of sample runs without loss of efficiency. The PLRP-S resin is a polyvinyl-divenyl benzene resin with no active side chains which perhaps explains its durability.

Suggested Methods

When IAA is known not to be present in samples, the method of Dodson et al. (1992) is perhaps the simplest and will probably be the first to be certified. It has been used on a variety of samples, is not subject to interferences and is now in the

process of round-robin trials. An external standard method of calibration is necessary in this method.

When the presence of IAA is suspected, one of the other methods has to be used. Our preference is for that of Vanderslice and Higgs (1990 a,b). It has been used on the greatest variety of samples, has shown no interferences and an internal standard calibration can be used in most cases. If AA and IAA are both present in samples (unlikely), an external standard must be used for calibration purposes. However, if either one is absent, the absent species can be used as the internal standard. This use of an internal standard does have certain advantages. Since IAA and AA have the same half-cell potential toward oxidation to their dehydro forms, any appearance of the dehydro form of the internal standard indicates that some oxidation has occurred during analysis and appropriate precautions can be taken. The reproducibility of the analytical method is 1% which increases to 3% due to extraction procedures. Quantitation is possible at the 3 ng level.

The other methods listed in Table I can be used, but further tests must be conducted on a variety of food samples and on the ruggedness of the method. As mentioned earlier, PLRP-S column is the preferred one in our laboratory because of its long life. The Vanderslice and Higgs (1990 a,b) method does have one problem. It uses $HgCl_2$ to oxidize AA to DHAA and we have been recently informed that it is going to be almost impossible to dispose of mercury wastes.

The method will require minor modification. The most obvious procedure would be to oxidize prior to injection with activated charcoal as Dodson et al. (1992) do. One then could determine total AA and total IAA with no loss in sensitivity. Another obvious approach would be to oxidize AA to DHAA in line electrochemically. This should be possible since this oxidation does occur with a dropping mercury electrode. Another approach would be to use N-biomosuccinamile for oxidation as we had done earlier but abandoned for $HgCl_2$ oxidation for convenience.

Sample Variability

It has been known for some time that a large variation in the vitamin C content of foods from natural sources is observed. In Table II, we show the range of values of vitamin C in some fruits and vegetables. The entries in the first column in the table were calculated from the data given in Handbook 8, while the second column gives ranges observed in our laboratory. With such variations, how does the analyst choose a representative sample? We do not have a completely satisfactory answer at the moment, but general guidelines have been outlined by Holden et al. (1991) for the case of selenium in white bread. The guidelines have been followed by Russell et al. (1992) to develop a nationwide sampling procedure for riboflavin. Similar approaches could be used for vitamin C.

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Table I. Recent HPLC Methods for the Determination of Vitamin C in Foods.

Reference	Species	Extraction[*]	Mobile Phase	Detector	Samples	Comments
ODS Columns (C-18)						
Kutnink & Omaye ion-pairing Sauberlich et al. (1991)	AA, IAA meats, beverage	MPA	Acetate, of samples,	EC	cured & smoked	limited variety (1987) cysteine reduction step
Kitada et al. (1989)	AA, IAA	MPA	Phosphate ion-pairing	EC	sausage, beverage, pickled relish	limited variety of samples, no reduction
Schüep and Keck (1990)	AA, IAA	MPA	Acetate ion-pairing	UV	cured & smoked meats	limited variety of samples, no reduction step
Dodson et al. (1992)	Total AA	MPA/AC	H_2O/methanol	Fluor	large variety	precolumn activated charcoal oxidation, and OPD reaction
PLRP-S Columns (5μm, 100Å)						
Lloyd et al. (1988) oxidation	AA, IAA	Mobile phase	Phosphate	UV	fruit juices	limited variety of samples, homo- cysteine
Vanderslice & Higgs (1990 a,b)	AA, IAA DHAA, DHIAA	MPA/AC/ EDTA	Phosphate	Fluor.	large variety	post-column OPD reaction, HgCl oxidation

[*] Abbreviations: MPA = metaphosphoric acid; AC = acetic acid; EDTA = ethylene diamine tetra-acetic acid.

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Table II. Range of Vitamin C Values (mg/100g) in Some Fruits and Vegetables.

SAMPLE	HANDBOOK 8[*]	OUR STUDY
APPLE JUICE	0 - 2	< 1
BANANAS	7 - 12	12 - 19
BEANS, SNAP, RAW	11 - 22	12 - 18
BROCCOLI, RAW	77 - 109	97 - 163
CABBAGE, RAW	31 - 64	42 - 83
CUCUMBER	0 - 10	13 - 14
GRAPEFRUIT, RED	28 - 48	21 - 31
LETTUCE	1 - 7	5 - 6
ORANGES	40 - 74	52 - 78
POTATOES, IDAHO	0 - 30	11 - 13
RED SKINNED		27
SPINACH, FRESH	6 - 50	25 - 70
TOMATOES	9 - 26	14 - 19

*   Range obtained from mean value $\pm$ twice the standard deviation found
     from standard error and number of samples tabulated in handbook.

References

Composition of Foods: Raw, Processed, Prepared. Revisions of Agricultural Handbook No. 8-9 and 8-11, U.S. Department of Agriculture, Science and Education Administration, Agricultural Research Service, 1986, 1984.

Deutsch, M. J. and Weeks, C. E. (1965). Microfluorometric assay for vitamin C. J.A.O.A. C. 48, 1248-56.

Dodson, K. Y., Young, E. R., and Soliman, A. M. (1992). Analysis of total vitamin C in various food matrices by HPLC and fluorescence detection. J.A.O.A.C. (accepted).

Holden, J. M., Gebhardt, S., Davis, C. S., and Luire, D. G. (1991). A nationwide study of the selenium contents and variability in white bread. J. Food Comp. Anal. 4, 183-195.

Kitada, Y., Tamase, K., Sasaki, M., and Yamazoe. (1989). Determination of L-ascorbic acid and erythorbic acid in foods by high performance liquid chromatography with amperometric detection. Nippon Shokuhin Kogyo Gakkaishi 36, 592-596.

Kutnink, M. A. and Omaye, S. T. (1987). Determination of ascorbic acid, erythorbic acid, and uric acid in cured meats by high performance liquid chromatography. J. Food Sci. 52, 53-56.

Lloyd, L. L., Warner, F. P., Kennedy, J. F., and White, C. A. (1988). In suppression reversed phase high performance liquid chromatography method for the separation of L-ascorbic acid in fresh juice. J. Chromotogr. 437, 447-452.

Russell, L. F., Douglass, L. W., and Vanderslice, J. T. (1992). Preparation of sampling plans for B_2 vitamers in fast food hamburgers. J. Food Comp. Anal. 5, in press.

Sauberlich, H. E., Ward, S. M., Tamara, T., and Freeberg, L. E. (1991). Influence of dietary intakes of erythorbic acid on plasma vitamin C analyses. Am. J. Clin. Nutr. 54, 1319S-22S.

Schüep, W. and Keck, E. (1990). Measurement of ascorbic acid and erythorbic acid in processed meat by HPLC. Z. Lebenem. Unters Lozsch 191, 290-292.

Vanderslice, J. T. and Higgs, D. J. (1990 a). Separation of ascorbic acid, isoascorbic acid, dehydroascorbic acid and dehydroisoascorbic acid in food and animal tissue. J. Micronutrient Anal. 7, 67-70.

Vanderslice, J. T., Higgs, D. J., Hayes, J. M., and Block, G. (1990 b). Ascorbic acid and dehydroascorbic acid content of foods-as-eaten. J. Food Comp. Anal. 3, 105-118.