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Note

Ultramicromethod for the measurement of ascorbic acid in plasma and white blood cells by high-performance liquid chromatography with electrochemical detection

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Ascorbic acid content in plasma, serum and leucocytes was determined using high-performance liquid chromatography (HPLC) with electrochemical detection [1—3]. The method is highly sensitive and can be used to accurately detect as low as 10 ng of ascorbic acid in as little as 5 μl of serum or plasma.

EXPERIMENTAL

The materials, equipment and instrumentation were as described by Pachla and Kissinger [2, 3]. All HPLC data were obtained using commercially available components and an amperometric detector (Bioanalytical Systems, West Lafayette, IN, U.S.A.).

Preparation of plasma samples

Each 10 μl of plasma was deproteinized with 30 μl of cold 6% trichloroacetic acid; after being shaken vigorously the deproteinized sample was allowed to stand for 10 min in ice to ensure complete deproteinization. Then 50 μl of cold 50 m*M* perchloric acid were added to the sample which was agitated again. At this time, the samples were centrifuged at 1520 *g* for 15 min at 4°C. The acidified supernatant was removed from the denatured protein for analysis. Aliquots of 2—20 μl were injected into the chromatographic column.

Preparation of white blood cell samples

To isolate the white blood cells from whole blood, the Ficoll—Hypaque method [4, 5] was used. The purified leucocytes were suspended in physiological saline. A small aliquot of the leucocyte suspension was removed in order to

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carry out the white cell count. Another measured aliquot was centrifuged at 1520 *g* at 4°C for 15 min and the supernatant discarded. The pellet was taken up in trichloroacetic acid, homogenized, diluted with 50 m*M* perchloric acid and analyzed by HPLC.

RESULTS

The ascorbic acid content of plasma samples from seven human subjects was measured by the HPLC—electrochemical detection method and by the colorimetric method using 2,4-dinitrophenylhydrazine [6—8]. The results are listed in Table I. Comparison of the two sets of data shows that results obtained by the two methods are highly consistent. However, the ascorbic acid content obtained by the colorimetric method is systematically higher than that obtained by the present method. This suggests that the electrochemical method is probably more selective than the colorimetric method.

TABLE I

ASCORBIC ACID IN HUMAN PLASMA

Human plasma*	Ascorbic acid (mg/100 ml plasma)	
	Present method	Colorimetric method
1	1.30	1.47
2	1.10	1.18
3	1.03	1.20
4	1.89	2.05
5	1.03	1.38
6	0.62	0.67
7	1.15	1.25

*Blood samples were obtained from American Red Cross Blood Services, Central California Region, Palo Alto, CA, U.S.A.

The ascorbic acid content of leucocytes and that of the buffy coat from human subjects have been measured. The ascorbic acid concentration is expressed as μg per 10^8 white blood cells (WBC). Results listed in Table II are shown to compare well with literature values.

Plasma samples with ascorbic acid concentration ranging from 0.2—3 mg per 100 ml plasma have been satisfactorily analyzed in our laboratory using a variation of the procedure described in the Experimental Section. Furthermore, the detector system can easily detect one-hundred-fold differences by varying sample size, recorder span and detector sensitivity. Our results also show that the ascorbic acid level in a plasma sample of 2 μl can be estimated with this technique.

The precision of this assay method was checked by multiple analyses on a single plasma sample. Typically, the standard deviation for five measurements is calculated to be better than 4%. When 200 ng of ascorbic acid were added to 20 μl of plasma, 99% of the vitamin was recovered. We have also analyzed a series of 6 micro samples (10 μl each) and have found excellent agreement com-

TABLE II

COMPARISON OF ASCORBIC ACID CONTENT OF HUMAN WHITE BLOOD CELLS AND BUFFY COAT OBTAINED BY THE HPLC METHOD WITH LITERATURE VALUES

Subject	Ascorbic acid (μg/10^8 WBC)*			
	Leucocyte		Buffy coat	
	Present method	Lit. values	Present method	Lit. values
1	18.7			
2	21.4			
3	17.5			
4	25.9			
5	18.0			
6	25.2			
7	25.0			
8	21.8			
9	22.2			
10	16.8		35.7	
11	30.5		57.4	
12	19.6		43.8	
13	20.2		41.5	
Range	16.8—30.5	11—21 [9] 12—27 [10]** 8—38 [11]**	35.7—57.4	21—53 [10] 21—57 [9] 16—76 [11]

*White cell counts were performed by Diagnostic Laboratory Services, Clinical Pathology Laboratory, Stanford University, Stanford, CA, U.S.A.

**Calculated from values of buffy coat ascorbate content; a conversion factor of 2 was used [9].

pared to results obtained from larger samples (6 samples, 1 ml) from the same subject (ascorbic acid content 1.32 ± 0.05 and 1.40 ± 0.04 mg/100 ml plasma ± S.D., respectively).

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