Spectrophotometric Determination of Ascorbic Acid in Pharmaceuticals by Background Correction and Flow Injection

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Background correction has been shown to be an effective and indispensable modification in the spectrophotometric determination of ascorbic acid. The decomposition of ascorbic acid in pharmaceutical samples was carried out by incubation with sodium hydroxide to give products that were insensitive to ultraviolet light. The rapid oxidation in air of ascorbic acid, especially in dilute solutions, was avoided by the use of the flow injection principle for spectrophotometric determination and by employing a carrier stream of an anti-oxidizing nature consisting of 6 μg ml^{-1} of 2-mercaptoethanol in 0.25% sulphuric acid. The optimized method with a single channel manifold made use of a carrier stream flow rate of 1.1 ml min^{-1}, an injection volume of 50 μl, a delay coil of 50 cm (0.5 mm i.d.) and detection at 245 nm. The throughput was at least 180 injections h^{-1}. The proposed flow injection method yielded results for the analysis of 0–20 μg ml^{-1} of ascorbic acid that were 99–102% (relative standard deviation 0.6% or better) in agreement with those produced by comparable methods involving titration with iodine, chloranil or 2,6-dichlorophenolindophenol [4-(2,6-dichloro-4-hydroxyphenylimino)cyclohexa-2,5-dienone], and high-performance liquid chromatography. When the agreement was not good (as low as 14% with respect to the method being compared), this was traced to the presence of substances which are known to interfere in one or other of the methods of comparison.

Keywords: *Ascorbic acid (vitamin C) determination; pharmaceuticals; spectrophotometry; background correction; flow injection*

Owing to the wide use of ascorbic acid (vitamin C) in canned fruits, vegetables, animal foods and drugs, many analytical techniques are available for its determination in different matrices and at different levels.[1]

The reducing property of ascorbic acid, which is due to the presence of the ene-1,2-diol system, forms the basis of many analytical methods.[2–10] However, the value of the oxidimetric methods is limited because of partial reaction of most oxidimetric reagents with cysteine, glutathione and iron(II), which are often present in biological fluids and plant extracts, or with sulphite which is commonly added to soft drinks as a preservative. Methods have been proposed for the chemical masking of interfering substances.[5,6,11,12] Another technique involves the formation of an osazone [bis(2,4-dinitrophenylhydrazone)] derivative of ascorbic acid.[13,14] Nevertheless, in spite of overcoming most interferences this procedure does have several disadvantages, it is complex, time consuming (over 180 min per analysis), and subject to several interferences. Recently, modifications have been suggested to improve this method.[15]

Chemical methods for the removal of interferences in the determination of ascorbic acid vary widely because of the diverse nature of interferences that occur with natural and synthetic samples including pharmaceuticals. Ascorbic acid absorbs strongly at 245 nm in acidic media and at 267 nm in neutral solutions, a property which makes it suitable for determination by spectrophotometry. However, this absorbance corresponds to any ascorbic acid present and to absorbance by the sample matrix. A correction can be made by measuring the absorbance of the matrix on a second aliquot of sample solution in which ascorbic acid is pre-decomposed to produce materials that are insensitive to ultraviolet (UV) light; the difference in absorbance observed is related to the amount of ascorbic acid present.

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Experimental

Apparatus

Instrumentation for high-performance liquid chromatography (HPLC) was used for the construction of a single channel flow injection (FI) manifold (Fig. 1). This consisted of a Shimadzu LC-5A reciprocating pump, an SIL-1A manual six-valve loop injector, an SPD-2A variable wavelength UV detector (range 195–350 nm) with a Z-type flow-through quartz cell (8 μl volume, 10 × 10 mm i.d.), and a Shimadzu C-R2AX integrator fitted with a printer–plotter (1 mV full scale). The flow rate of the carrier stream was 1.1 ml min^{-1}. Polytetrafluoroethylene (PTFE) tubing (0.5 mm i.d.) was used for transportation of stream effluents, the joints being made with HPLC high-pressure connectors (0.5 mm i.d.). An injection volume of 50 μl (loop made of 0.5 mm i.d. tubing) was used throughout except in preliminary experimentation. The delay coil was 50 cm long. The detector was set to 245 nm and a back-pressure tubing (25 cm in length, 0.25 mm i.d.) was placed after it. The peak maximum appeared 9.5 s after injection of the sample. The measurement of peak height was used for quantification.

Reagent

The carrier solution was 0.25% sulphuric acid containing 6 μg ml^{-1} of 2-mercaptoethanol.

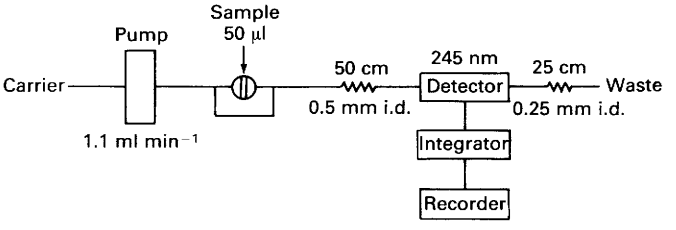

Fig. 1 Flow injection manifold for the determination of ascorbic acid

Samples

Ascorbic acid was of guaranteed-reagent grade from Sarabhai M. Chemicals (Baroda, India), with a purity of 99.7% as determined by titration with 2,6-dichlorophenol-indophenol [4-2,6-dichloro-4-hydroxyphenylimino)cyclohexa-2,5-dien-one][2] and 99.8% as determined by titration with chloranil.[6] A standard solution (1000 μg ml^{-1}) was prepared by dissolving 100.00 mg of ascorbic acid in 100 ml of de-ionized water in a calibrated flask. Calibration standards were prepared by sequential dilution of the stock solution with the carrier solution.

All drug samples tested were fresh and purchased from the local pharmacy. A known number of vitamin C tablets or the contents of a known number of capsules were weighed and ground into a fine powder. An accurately weighed portion containing about 100 mg of ascorbic acid was shaken with about 25 ml of water and filtered through Whatman No. 41 filter-paper. The insoluble mass was washed with three successive 5 ml portions of water, and the filtrate plus washings were diluted to volume in a 100 ml calibrated flask. A known volume was further diluted with the carrier solution for analysis. The stock solution was used for analysis by the comparison methods. The contents of a known number of vitamin C injections were mixed together and a suitable aliquot of the mixture was sampled.

All other chemicals used were high-purity materials.

Background Correction

A known aliquot of ascorbic acid test solution containing 0–20 μg ml^{-1} of vitamin C is mixed with 1 ml of 1 mol dm^{-3} sodium hydroxide and kept at room temperature (25 °C) for 10 min. Then, 200 μl of 1 mol dm^{-3} sulphuric acid is added and the contents are made up to the mark in a 10 ml calibrated flask with the carrier solution. A 50 μl aliquot is injected into the FI manifold.

A second aliquot of ascorbic acid test solution is diluted to 10 ml in a calibrated flask with the carrier solution and a 50 μl portion is injected. The difference beween the peak heights of untreated and treated test solutions is a measure of ascorbic acid content. A calibration graph was constructed using ascorbic acid standard solutions.

Results and Discussion

Optimization of Flow Injection System

Owing to rapid oxidation in air, ascorbic acid deteriorates quickly in the dilute solutions, the dissolved oxygen being sufficient to completely oxidize 1–20 μg ml^{-1} of ascorbic acid within 20 min. Thus, after dissolution of vitamin C samples, the solutions must be handled very quickly, which is possible using the flow injection technique.

In order to avoid the oxidation in air of ascorbic acid, various suggestions have been made which involve the use of acid conditions, typically, the addition of formic acid is recommended,[16] or addition of ethylenediaminetetraacetic acid (EDTA) and hydrogen sulphite,[17] or thiourea.[6] Formic acid alone was observed not to be an efficient stabilizer. The use of other anti-oxidants with the proposed method resulted in a large reagent blank. Thus, a suitable stabilizer which was transparent to UV radiation, expecially at 245 nm, was required. 2-Mercaptoethanol was found to have this property which prompted its use as an anti-oxidant in the carrier solution. The recommended carrier solution was 0.25% sulphuric acid containing 6 μg ml^{-1} of 2-mercaptoethanol.

In order to optimize the flow injection conditions, other parameters such as the length of the delay coil, flow rate, injection volume and sampling frequency were evaluated. Maintaining a carrier flow rate of 1 ml min^{-1} and an injection volume of 50 μl, ascorbic acid solution was injected into the FI manifold while the length of the coil was varied. It was observed that with an increase in the length of the coil the peak height decreased significantly (Fig. 2). A coil length of 50 cm was selected for subsequent studies. The effect of the flow rate of the carrier solution on the peak height, upon the injection of 50 μl of ascorbic acid into the FI manifold that had a delay coil 50 cm in length, is summarized in Fig. 3. Optimum peak height was obtained when a flow rate of 1.1 ml min^{-1} was used. The peak height increased with an increase in the volume of sample injected (Fig. 4). A 50 μl volume was selected for further studies because although larger volumes gave higher peak heights, peak broadening occurred and consequently the sampling frequency decreased sharply. In the optimized flow injection manifold the value of the dispersion coefficient, D,[18] as determined by injection of a standard solution of potassium dichromate, was found to be 2. With an optimum injection

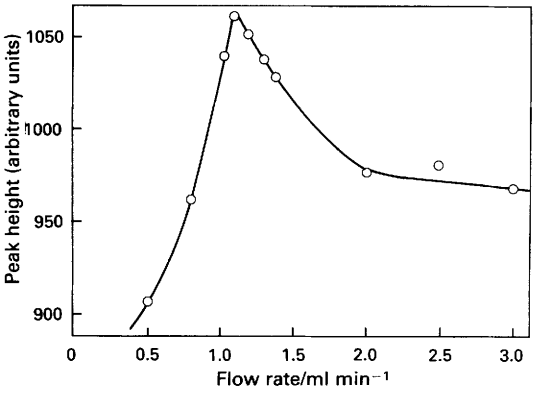

Fig. 3 Effect of flow rate of the carrier stream on the peak height of the signal for ascorbic acid. Delay-coil length, 50 cm; and injected sample volume, 50 μl

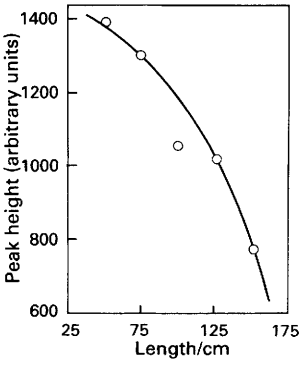

Fig. 2 Effect of delay-coil length (0.5 mm i.d.) on the peak height of the signal for ascorbic acid. Flow rate of the carrier stream, 1 ml min^{-1}; and volume of injected sample, 50 μl

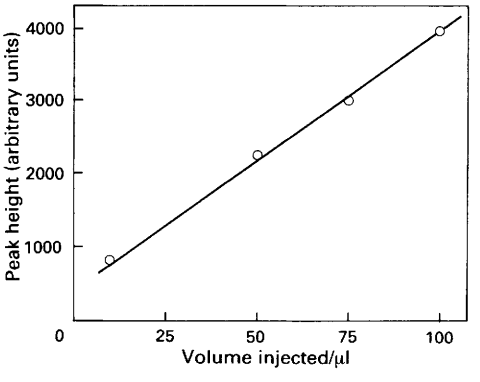

Fig. 4 Effect of injected sample volume on the peak height of the signal for ascorbic acid. Coil length, 50 cm; and flow rate of carrier stream, 1.1 ml min^{-1}

rate of 180 injections h^{-1} the peaks returned to the baseline without any cross-contamination.

Sample Matrix Interference

The sample matrix in pharmaceutical formulations is very complex owing to the presence of a variety of active substances and a large number of additives which act as supporting materials, binders or stabilizers. Certain flavour enhancing substances are also present in chewable vitamin C preparations. Any additives that absorb at 245 nm can affect the proposed method of assay.

Various methods are described in the literature for producing a sample blank, such as enzymic methods,[19,20] thermal decomposition,[21] catalytic decomposition[21–23] and UV irradiation.[24] As ascorbic acid is notoriously unstable at high pH,[21,25] adjustment of the sample pH to the alkaline range was studied as a prospective blank correction method. However, additives in pharmaceuticals should be stable to alkaline treatment otherwise the results could be vitiated. Thus, a screening test was carried out, using the FI method, on a large variety of additives. About 40 compounds including active drugs, sweeteners, acids, colouring agents, preservatives, mineral salts, amino acids and vitamins among others were investigated (Table 1). The efficacy of the alkaline treatment method for the decomposition of ascorbic acid in pure solutions is shown in Fig. 5.

Maleic acid, benzoic acid, salicylic acid, salicylamide, acetaminophen, acetylsalicylic acid, caffeine, most colouring agents and vitamins such as thiamine hydrochloride and riboflavin showed appreciable absorption at 245 nm. Iron when present as iron(II) fumarate caused no optical interference, ostensibly owing to its insolubility in aqueous media. Tannic acid, saccharin, caramel and polyhydroxy aromatics are unstable in alkaline media whereas most other additives are fairly stable.

In an effort to investigate any interaction between the analyte and matrix, recovery tests were performed on the synthetic samples prepared in the laboratory (Table 2). The mean recovery observed was 100.5% and the relative standard deviation (RSD) was in the range 0.5–1.2%.

Iron(II) is an important constituent of ascorbic acid formulations. When present as an ion it causes strong interference in the present FI method. However, this problem was avoided by removing the ion as iron(II) sulphide. An aliquot of sample solution was shaken with 5 ml of sodium sulphide solution containing 100 μg ml^{-1} of sulphide for 5 min and then filtered. The filtrate was diluted with the carrier solution and analysed using the FI method. Large amounts of sodium sulphide can be tolerated in the proposed method.

Table 1 Matrix interference in the determination of ascorbic acid by flow injection. The results are the average of three determinations

Additive type	Added as	Concentration of additive*/μg g^{-1}
Sweetener	Glucose	2.5×10^4
	Sucrose	3.0×10^4
	Fructose	1.1×10^4
	Lactose	5.2×10^3
	Mannitol	6.1×10^3
Acid	Citric	1.5×10^2
	Succinic	3.1×10^3
	Oxalic	1.9×10^2
	Tartaric	1.0×10^2
	Maleic	10
	Malic	2.9×10^2
	Benzoic	1
	Salicylic	1
Colouring matter	Amaranth	2
	Tartrazine	2
	Caramel	8
	Ponceau 4R	1.5
Amino acid	Cysteine	5.1×10^2
	Glycine	2.8×10^3
	Serine	5.0×10^3
	Alanine	3.8×10^3
	Tryptophan	2
	Tyrosine	5
	Histidine	10
Antioxidant	Sodium hydrogen sulphite (metabisulphite)	2.1×10^2
	Thiomalic acid	20
	2-Mercaptoethanol	50
	Hydroquinone	2
Mineral salt	$Na_2HPO_4 \cdot 12H_2O$	1.4×10^2
	Sodium sulphide	2.0×10^2
	Potassium nitrate	2.0×10^2
	Calcium chloride	6.3×10^2
	Sodium chloride	2.7×10^3
	Potassium chloride	6.2×10^3
	Ammonium iron(II) sulphate	1.2
	Copper(II) sulphate	70
Vitamin	Thiamine hydrochloride	1
	Riboflavin	1
	Pyridoxine hydrochloride	2
Active drug	Acetaminophen	1
	Caffeine	3
	Salicylamide	1
	Acetylsalicylic acid	1

* Concentration of additive producing a peak height equivalent to 1 μg g^{-1} of ascorbic acid.

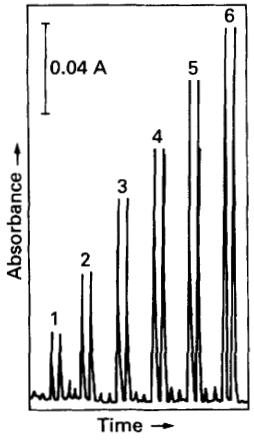

Fig. 5 Flow injection peaks for duplicate 50 μl injections of ascorbic acid standards obtained after treatment with sodium hydroxide, for background correction (un-numbered peaks), and without treatment with sodium hydroxide (numbered peaks). Values on peaks correspond to the concentration of ascorbic acid (μg ml^{-1}) in untreated samples and in preceding treated samples. Injection throughput, 180 samples per hour

Table 2 Determination of ascorbic acid by background correction

Ascorbic acid taken/ μg ml^{-1}	Substance added*	Ascorbic acid found†/ μg ml^{-1}	RSD (%)
1.04	Acetaminophen (5)	1.05	0.5
2.34	Acetaminophen (8)	2.31	0.5
5.12	Caffeine (10)	5.15	0.6
8.03	Caffeine (20)	8.00	0.6
10.9	Salicylamide (5)	10.8	0.5
14.0	Salicylamide (10)	14.2	0.6
16.8	Thiamine hydrochloride (5)	16.5	0.5
18.2	Thiamine hydrochloride (10)	18.4	0.6
20.1	Acetylsalicylic acid (4)	20.4	0.6
21.5	Acetylsalicylic acid (10)	22.1	0.6

* Values in parentheses are amounts added in μg ml^{-1}.
† The results are the average of five determinations.

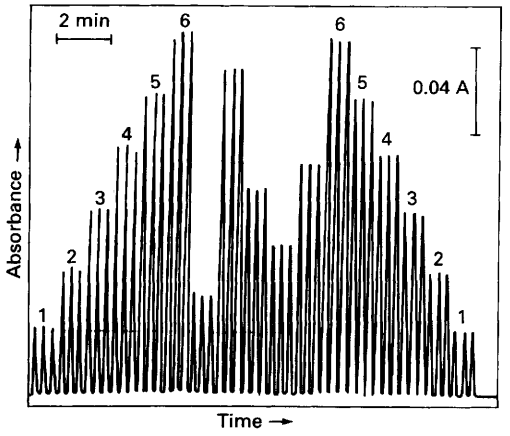

Fig. 6 Recorder output for the analysis (in triplicate) of ascorbic acid. The peaks correspond to the concentration of ascorbic acid (μg ml^{-1}) in six standards followed by five unknown samples and then the same six standards

Table 3 Comparison of results for the formulations prepared in the laboratory. The results, given in mg of ascorbic acid, are the average of five determinations

Formulation	Nominal	Proposed method	RSD (%)	Comparison method	Difference* (%)
No. 1†	100	101.3	0.4	99.8 (IOD)‡	+2.5
				99.3 (CA)§	+2.0
				100.5 (IND)¶	+0.8
				101.8 (HPLC)‖	+0.8
No. 2**	100	101.1	0.5	99.7 (IOD)	+1.4
				101.0 (CA)	+0.1
				101.6 (IND)	−0.5
No. 3††	150	150.2	0.5	152.8 (IOD)	−1.7
				150.7 (CA)	−0.3
				152.0 (HPLC)	−1.2
No. 4‡‡	200	205.6	0.6	236.3 (IOD)	−14.9
				205.8 (CA)	−0.1
				233.1 (IND)	−13.4
				204.0 (HPLC)	+0.8
No. 5§§	500	506.0	0.6	504.8 (IOD)	+0.2
				506.0 (CA)	0.0
				507.1 (IND)	−0.2
No. 6¶¶	100	98.1	0.6	98.5 (IOD)	−0.4
				102.5 (CA)	−4.5
				103.2 (IND)	−5.2
No. 7‖‖	500	507.0	0.5	503.8 (IOD)	−0.3
				504.2 (CA)	−0.4
No. 8***	100	99.5	0.5	98.9 (IOD)	+0.6
				103.6 (IND)	−4.1

* Proposed method − comparison method.
† Also contains acetaminophen (50 mg), and glucose (50 mg).
‡ IOD = iodine titration (reference 3).
§ CA = chloranil titration (reference 6).
¶ Ind = 2,6-dichlorophenol-indophenol (reference 2).
‖ HPLC = high-performance liquid chromatography (reference 26).
** Also contains acetylsalicylic acid (50 mg), starch (50 mg), and glucose (50 mg).
†† Also contains lactose (50 mg), and salicylamide (60 mg).
‡‡ Also contains methionine (40 mg), and potassium hydrogen sulphite (metabisulphite) 10 mg.
§§ Also contains thiamine hydrochloride (100 mg), and fumaric acid (50 mg).
¶¶ Also contains iron(II) sulphate (20 mg).
‖‖ Also contains Indigo Carmine (2 mg).
*** Also contains ammonium iron(II) sulphate (50 mg).

Validation of the Method

Typical recorder peaks for the analysis of standards and solutions containing unknown amounts of ascorbic acid are shown in Fig. 6. The dynamic linear range was 0–20 μg ml^{-1}

Table 4 Comparison of results for the commercial formulations. The results, given as mg of ascorbic acid, are the average of five determinations

Formulation	Nominal	Present method	RSD (%)	Comparison method	Difference* (%)
Tablet—					
Celin†	500	504.0	0.5	504.6 (IOD)‡	−0.1
				505.5 (CA)§	−0.3
				504.6 (HPLC)¶	−0.1
Chewcee‖	500	502.1	0.4	500.0 (IOD)	+0.4
				497.2 (CA)	+1.0
				501.3 (IND)**	+0.2
Chromostat††	150	165.5	0.5	169.0 (IODD)	−2.1
				165.9 (CA)	−0.4
				162.6 (IND)	+1.7
				164.7 (HPLC)	+0.5
Rutin‡‡	100	99.6	0.6	98.2 (CA)	+1.4
				102.4 (IND)	−2.8
Becozym§§	150	148.0	0.5	145.8 (IOD)	+1.5
Capsule—					
Livogen¶¶	75	77.2	0.4	79.3 (IOD)	−2.7
				78.0 (CA)	−1.0
Beplex‖‖	150	142.9	0.5	142.6 (IOD)	+0.2
				143.5 (CA)	−0.4
Fesovit***	50	48.0	0.6	48.6 (IOD)	−1.2
				53.1 (IND)	−10.6
Syrup—					
Becadex†††	75	86.1	0.5	89.6 (IOD)	−4.1
				85.5 (CA)	+0.7
Vidalin-M‡‡‡	50	53.6	0.6	54.0 (IOD)	−0.7
				53.6 (CA)	0.0
Injection—					
Redoxon§§§	500	501.0	0.5	498.0 (IOD)	+0.6
				502.3 (IND)	−0.3
				501.6 (HPLC)	−0.1

* Proposed method − comparison method.
† Also contains Sunset Yellow.
‡ IOD - iodine titration (reference 3).
§ CA = chloranil titration (reference 6).
¶ HPLC = high-performance liquid chromatography (reference 26).
‖ Also contains Erythrosin, and Sunset Yellow.
‡‡ Also contains adrenochrome monosemicarbazone (5 mg), menophthone sodium hydrogen sulphite (10 mg), rutin (50 mg), dibasic calcium phosphate (132 mg), and calciferol (300 U).
‡‡ Also contains rutin (100 mg), acetaminadione (20 mg), adrenochrome monosemicarbazone (1 mg), and dibasic calcium phosphate (150 mg).
§§ Also contains riboflavin (10 mg), nicotinamide (50 mg), pyridoxine hydrochloride (3 mg), calcium pantothenate (16.3 mg), and cyanocobalamin (10 μg).
¶¶ Also contains iron(II) fumarate (150 mg), folic acid (150 μg), vitamin B_{12} (10 μg), vitamin B_1 (5 mg), vitamin B_2 (5 mg), vitamin B_6 (1.5 mg), calcium pantothenate (5 mg), nicotinamide (45 μg), and dried yeast (25 mg).
‖‖ Also contains thiamine mononitrate (10 mg), riboflavin (10 mg), nicotinamide (45 mg), nicotinic acid (15 mg), pyridoxine hydrochloride (25 mg), folic acid (1.5 mg), vitamin B_2 (15 mg), and Amaranth.
*** Also contains iron(II) sulphate (150 mg), thiamine mononitrate (2 mg), nicotinamide (15 mg), pyridoxine hydrochloride (1 mg), panthothenic acid (calcium salt) (2.5 mg) and Amaranth.
††† Also contains vitamin A (2500 U), vitamin D_3 (200 U), vitamin B_7 (2.5 mg), vitamin B_2 (2.5 mg), nicotinamide (25 mg), and vitamin B_{12} (2.5 μg).
‡‡‡ Also contains vitamin A (3000 U), calciferol (400 U), thiamine hydrochloride (1.5 mg), riboflavin (1.2 mg), cyanocobalamin (3 μg), nicotinamide (10 mg), iron [as iron(II) gluconate] (3 mg), potassium iodide (98 mg), calcium lactate (158 mg), calcium hypophosphite (82 mg), hypophosphorous acid (27.5) mg), magnesium gluconate (55 mg), panthenol (5 mg), zinc gluconate (3.9 mg), and flavoured syrups bases.
§§§ Also contains methyl paraben (0.08% m/v), and propyl paraben (0.01% m/v).

Table 5 Recovery of ascorbic acid from spiked solutions of formulations samples. The results are given as μg ml^{-1} of ascorbic acid

Found	Added	Recovered*	Recovery (%)
128	202	205	101.5
156	75	76	101.3
174	102	100	98.0
132	162	164	101.2
146	108	110	101.8

* The results are the average of five determinations.

[regression coefficient (r) = 0.9998] of ascorbic acid which suffices for application of the proposed method to the analysis of drugs. The precision was always less than 0.6% and the detection limit (concentration producing a peak height equal to three times the standard deviation of the most dilute standard solution) was 0.2 μg ml^{-1}. The upper range of determination can be extended up to 100 μg ml^{-1} of ascorbic acid but the efficacy of the background correction is then only poor.

Applications

The flow injection technique was used to determine ascorbic acid in formulations prepared in the laboratory (Table 3) and commercially available pharmaceuticals (Table 4). The results obtained with the present method were compared with those yielded by previously checked analytical techniques involving titration with 2,6-dichlorophenol-indophenol,[2] iodine[3] and chloranil,[6] and by HPLC.[26] Most of the existing methods for the determination of vitamin C are based on the use of redox reactions and are susceptible to possible interference from oxidizable and reducible substances or those which may undergo halogenation. Thus as compared to the indophenol and iodine titration methods, chloranil titration seems to offer more reliable results. High-performance liquid chromatography appears to tolerate most interferences, however, ascorbic acid elutes near the void volume and substances poorly retained on the column are potential causes of error.[27,28]

Overall, the FI method is rapid, sensitive and accurate, and enables the use of an anti-oxidant in the carrier solution. The background correction technique, involving decomposition of ascorbic acid with sodium hydroxide, is the method of choice and it works satisfactorily in the presence of a wide variety of the materials that are encountered with multi-component vitamin C formulations. This method was further evaluated by analysing solutions of a formulation spiked with known amounts of ascorbic acid; a mean recovery of 100.7% (range 98.0–101.8%) was obtained (Table 5).

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References

1 Cooke, R. J., and Moxon, R. E. D., in *Vitamin C*, eds. Counsell, J. N., and Horning, D. H., Applied Science Publishers, Barking, 1981, pp. 167–198.
2 Horwitz, W., *Official Methods of Analysis of the Association of Official Analytical Chemists*, ed. Horwitz, W., Association of Official Analytical Chemists, Washington, DC, 13th edn., 1980, p. 746.
3 Bassy, O. A., and King, C. C., *J. Biol. Chem.*, 1933, **103**, 687.
4 Hernandez-Mendez, J., Alonso Mateos, A., Alnendral Parra, M. J., and Garcia de Maria, C., *Anal. Chim. Acta*, 1986, **184**, 243.
5 Verma, K. K., and Gulati, A. K., *Anal. Chem.*, 1980, **52**, 2336.
6 Verma, K. K., Jain, A., and Rawat, R., *J. Assoc. Off. Anal. Chem.*, 1984, **67**, 262.
7 Girish Kumar, K., and Indrasenan, P., *Talanta*, 1990, **37**, 269.
8 Lázaro, F., Ríos, A., Luque de Castro, M. D., and Valcárcel, M., *Analyst*, 1986, **111**, 163.
9 Salinas, F., and Galeano Díaz, T., *Analyst*, 1988, **113**, 1657.
10 Abdalla, M. A., and Al-Swaidan, H. M., *Analyst*, 1989, **114**, 583.
11 Nazar, M., Ikram-ul-Haq, M. Y., and Ehteshamuddin, A. F. M., *Pak. J. Sci. Ind. Res.*, 1968, **11**, 38.
12 Sarwar, M., Aqbal, Z., and Zaidi, S., *Mikrochim. Acta*, 1975, 699.
13 Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, **147**, 399.
14 Roe, J. H., *J. Biol. Chem.*, 1961, **236**, 1611.
15 Dabrowski, K., and Hinterleitner, S., *Analyst*, 1989, **114**, 83.
16 Berka, A., Vulterin, J., and Zyka, J., *Newer Redox Titrants*, Pergamon, Oxford, 1965, p. 162.
17 Henshall, J. D., in *Vitamin C*, eds. Counsell, J. N., and Horning, D. H., Applied Science Publications, Barking, 1981, p. 130.
18 Růžička, J., and Hansen, E. H., *Flow Injection Analysis*, Wiley, New York, 1988, p. 23.
19 Tono, T., and Fujita, S., *Agric. Biol. Chem.*, 1981, **45**, 2947.
20 Tono, T., and Fujita, S., *Agric. Biol. Chem.*, 1982, **46**, 2953.
21 Fung, Y.-s., and Luk, S.-f., *Analyst*, 1985, **110**, 201.
22 Lau, O.-w., Luk, S.-f., and Wong, K.-s., *Analyst*, 1986, **111**, 665.
23 Lau, O.-w., Luk, S.-f., and Wong, K.-s., *Analyst*, 1987, **112**, 1023.
24 Moussa, A. F. A., *Pharmazie*, 1977, **32**, 724.
25 Fung, Y.-s., and Luk, S.-f., *Analyst*, 1985, **110**, 1439.
26 Nicolson, I. A., Macrae, R., and Richardson, D. P., *Analyst*, 1984, **109**, 267.
27 Behrens, W. A., and Madere, R., *Anal. Biochem.*, 1987, **165**, 102.
28 Hou, W., and Wang, E., *Talanta*, 1990, **37**, 841.

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