

Sequential Injection Spectrophotometric Determination of Ascorbic Acid in Pharmaceutical Preparations Using Potassium Permanganate

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Abstract: A reliable and sensitive indirect sequential injection method with spectrophotometric detection for the determination of ascorbic acid in various pharmaceutical preparations is proposed in this paper. The method is based on a redox reaction between ascorbic acid and permanganate in 0.1 mol L⁻¹ sulphuric acid medium. The fading permanganate color as a result of the proposed reaction, was measured at 548 nm. The method involved the injection of 250 µL of ascorbic acid sample or standard, into the holding coil containing potassium permanganate as carrier solution. In the next step the injected volume of standard or sample is dispensed through the detector. This simple two step procedure enables high sample throughput of 240 h⁻¹. The relative standard deviation was calculated to be 0.35 %. The obtained results by the proposed method were found to be in good agreement with the standard titrimetric method. The proposed method is simple and cost-effective and also can be an alternative to more sophisticated methods. The method is suitable for a quick and sensitive quantitative analysis of ascorbic acid in pharmaceutical preparations.

Keywords: Ascorbic acid, Vitamin C, Sequential injection analysis, Spectrophotometry.

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I. Introduction

Ascorbic acid (IUPAC name: (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one), commonly referred to as vitamin C, is an essential water soluble vitamin. Vitamin C is one of the most tested and described vitamins and the first synthetically obtained vitamin. Haworth and Hirst were the first to synthesize vitamin C.[1]

Vitamin C participates as a reducing agent in numerous biological processes. It is important for the synthesis of collagen and carnitine and for the metabolism of fatty acids, in addition to this, ascorbic acid is an important antioxidant.[2] As a factor of coenzyme hydroxylase and monooxygenase, it participates in the synthesis of collagen, carnitine and neurotransmitters.[3]

The lack of ascorbic acid results in decreased hydroxylation of proline and lysine, which affects the synthesis of collagen. Carnitine is essential for transport and transfer of fatty acids into mitochondria, which serve as a source of energy. [4]

Latest research show that vitamin C has an important role in the protection of an organism from oxidation stress, and acts as an antioxidant. However, ascorbate, chemical pharmacophore of vitamin C can react with radicals and oxidants in the organism, so it acts as a reduction agent and an antioxidant.[5,6] Vitamin C neutralises free radicals and hydrogen peroxide, and consequentially affects the activity of glutathione peroxidase and catalase.[7] Removal of free radicals and increased activity of aforementioned enzymes induces suppression of peroxidation in lipid and water region of the cell as a consequence.[8,9] Therefore, clinical and epidemiological research suggest that daily diet intake of ascorbic acid in amount of 100 mg is related to decreased frequency of mortality by cardiovascular illnesses, stroke and cancer.[10]

Plants and some animals synthesize their own vitamin C but due to lack of an enzyme gulonolactone oxidase, humans do not. Most plants and animals synthesize ascorbic acid from D-glucose or D-galactose. However, the human organism cannot synthesize vitamin C, due to lack of enzyme gulonolactone oxidase. Therefore, ascorbic needs to be replenished through diet or pills. Vitamin C is commonly present in fresh fruits

and vegetables. Pharmaceutical preparations such as tablets, capsules, cristal powder, effervescent tablets are artificial supplements of vitamin C in human diet[11].

It is important to note that natural and synthetic ascorbic acid are identical, and there are no known differences in their biological activity or bioavailability.[12]

Numerous analytical methods have been proposed and developed for quantitative determination of ascorbic acid in pharmaceutical formulations: titrimetry [13], fluorimetry [14-16], chemiluminescence [17, 18] and spectrophotometry [19-12]. However, according to literature data, due to significant physiological role of ascorbic acid, new methods for routine analysis of this vitamin in pharmaceutical and diet preparations are being developed.[23]

Herein a simple and fast sequential injection method is proposed for routine analysis of ascorbic acid. The method is appropriate for ascorbic acid determination in a number of pharmaceutical preparations such as vitamin C tablets.

II. Material And Methods

Reagents: Deionized water was used for preparation of all reagents. A stock L-ascorbic acid solution (1.0×10^{-2} mol L⁻¹) was freshly prepared from Sigma-Aldrich (St. Louis, Missouri, USA) by dissolving 0,1762 g of ascorbic acid in deionized water and diluted up to 100.0 mL. Working solutions of different concentrations were prepared by diluting the ascorbic acid with deionized water.

Stock sulphuric acid solution (0.1 mol L⁻¹) was prepared by adding the appropriate volume of reagent grade concentrated sulfuric acid (95–98 %, specific gravity 1.84 kg L⁻¹, Merck, UK) in a portion of deionized water and diluted up to 500.0 mL.

A standard potassium permanganate (1.0×10^{-3} mol L⁻¹) solution was prepared in a 100.0 mL standard flask by dissolving 0.0158 g of potassium permanganate, (Sigma-Aldrich St. Louis, Missouri, USA) in 0.1 mol L⁻¹ sulfuric acid. The prepared reagent solution was transferred and stored in a dark bottle.

The developed sequential injection method was applied for analysis of three commercially available pharmaceutical preparations: Plivit C tablets 500 mg (Pliva, Croatia) contain the following excipients: cornstarch, talc, microcrystalline cellulose and magnesium stearate; BIO-C 500, (Dietpharm, Croatia), each tablet contains 500 mg of L-ascorbic acid, 40 mg of a 30 % citrus-bioflavonoid complex, along with excipients, cornstarch, magnesium stearate, glycerol and microcrystalline cellulose; Vitamin C Alkaloid 500 mg tablet (Alkaloid, Macedonia) contains 500 mg of ascorbic acid, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, lactose and starch.

The solutions of the described Vitamin C pharmaceuticals were prepared by dissolving appropriate amounts of each pharmaceutical sample in a suitable volume of deionized water to match the ascorbic acid concentration to the experimental conditions of the proposed method. The validation experiment was conducted by the standard iodine titrimetric method recommended by the European Pharmacopoeia. [24]

Manifold for sequential injection spectrophotometry

A schematic diagram of the sequential injection manifold is shown in Figure 1. The manifold for sequential injection spectrophotometry comprises a bidirectional syringe-free stepper motor driven pump (M50 VICI Valco Instruments, Houston, Texas, USA), a microprocessor-driven controller (Micro Lynx 4, Intelligent Motion Systems, Inc.) and a 10-port selection valve (model C25-3180D, VICI Valco Instruments, Houston, Texas, USA) with a multiposition actuator control module (EMHCA-CE VICI, Valco, Houston, Texas, USA).

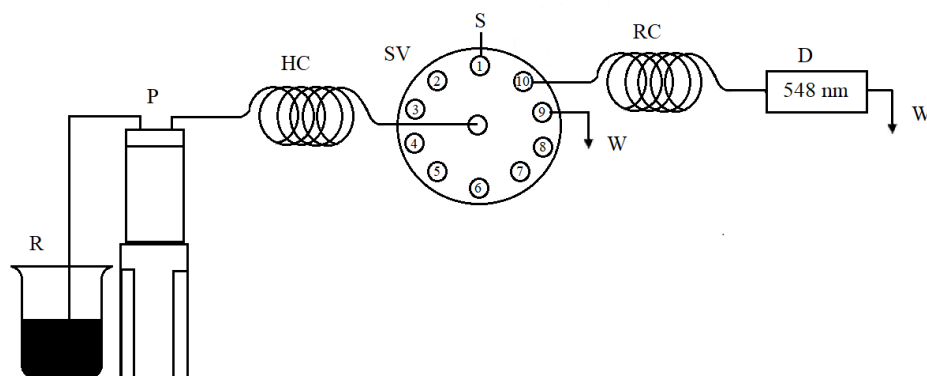


Figure 1. Schematic representation of the sequential injection manifold. R-reagent, P-pump, HC-holding coil, SV-selection valve, S-sample, RC-reaction coil, D-detector, W-waste.

PTFE tubing (0.8 mm i.d.) and 1/8" PTFE fittings were used to assemble different manifold parts. The liquid handling pump and the selection valve were connected to a computer via RS-232 communication protocols. M6-LHS software allows the control of a syringe-free pump and electrically actuated selection valve, by programming the application methods. The programmed method determines the operation of the liquid handling pump and selection valve. A double beam UV-Vis spectrophotometer (UV-1800 Shimadzu Kyoto, Japan) equipped with a flow through cell from Hellma (Jamaica, NY) with 80 µL internal volume and 10 mm optical path, connected to a second computer through serial communication ports, was used as a detection system. The detector response was recorded digitally using UVProbe software, version 2.43 and the acquired data imported to GraphPadPrism 5 for further statistical analysis and graphic presentation.

Sequential injection procedure

The sequence for the determination of ascorbic acid is shown in Table 1. The selection valve was set to position 10 and 1000 µL of carrier solution was dispensed by the pump at a flow rate of 5000 µL min⁻¹ through the holding coil, reaction coil and the flow cell. The same sequence was used to obtain a stable baseline. In the next step 250 µL of ascorbic acid was aspirated into the holding coil through port 1 at 3000 µL min⁻¹. The valve was then switched to position 9 and the content of the coil was dispensed to waste. This was done in order to eliminate air from the system.

The measuring procedure was as follows. The valve was set to position 1 and 250 µL of ascorbic acid was aspirated into the holding coil, the injected sample zone were then dispensed through position 10 through the flow cell at 5000 µL min⁻¹. The decrease in absorbance, or increase in transmittance of permanganate as a result of the proposed redox reaction was spectrophotometrically measured at 548 nm.

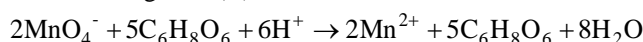
Table 1. Program sequence for determination of ascorbic acid.

Action		Volume (µL)	Flow rate (µL min ⁻¹)	Operation time (s)*	Description
Pump	Valve				
	Position 1				Set valve to position 1
Aspirate		250	5000	3	Aspirate sample to holding coil
	Position 10				Set valve to position 10
Dispense		1000	5000	12	Dispense holding coil content towards the flow cell

*Note: Volume and flow rate are assigned, operation time is calculated.

III. Results and discussion

The proposed method is based on a redox reaction in which ascorbic acid reduces the permanganate anion to manganese(II)-ion:



The decrease of recorded absorbance or increase of transmittance is proportional to the concentration of permanganate. The decrease in recorded absorbance due to the decrease of permanganate concentration was measured and was brought in linear correlation with the concentration of ascorbic acid.

Optimization of chemical parameters and parameters of the sequential injection system

Reagent concentration optimization was conducted by using potassium permanganate solutions of different concentrations as carrier solution in the range of 2.0×10^{-4} – 1.0×10^{-3} mol L⁻¹. The optimum reagent concentration was found to be 6.0×10^{-4} mol L⁻¹. Addition of ascorbic acid reduced potassium permanganate and caused a decrease in recorded absorbance or increase in transmittance.

The optimization of physical parameters was conducted by using the univariate method, where one randomly selected parameter is varied while the rest of the parameters are held at constant levels. The variables tested were: flow rate, sample volume, holding coil and reaction coil length.

The reaction between ascorbic acid and permanganate is rapid, resulting in an immediate reduction of potassium permanganate to manganese(II)-ion. At high flow rates the time available for the redox reaction is diminished, resulting in an inadequate decrease of permanganate concentration for spectrophotometric detection.

The flow rate was examined in the range between 1000 and 8000 µL min⁻¹. Higher flow rates cause a smaller decrease in absorbance, significantly decreasing sensitivity, while low flow rates cause higher dispersion of the injected sample zone, causing a smaller decrease in recorded absorbance. Further optimization procedure was carried out at an optimum flow rate of 5000 µL min⁻¹.

The effect of sample volume on recorded absorbance was studied in the range of 50 to 500 µL. The recorded absorbance decreased with increasing injected sample volume. The value of 250 µL of sample segment

was finally chosen for subsequent experiments as it gave good response for lower concentrations of ascorbic acid. Further increase of sample volume significantly prolonged return time thus reducing sample throughput.

In this sequential injection experiment the total volume of injected sample should not exceed the volume of the holding coil. During the sample volume optimization procedure a holding coil with a 1000 μL inner volume was used. Under the optimum injected volumes a holding coil of 500 μL was sufficient for further optimization.

Under the previously optimized parameters, the effect of the length of the reaction coils was examined in the range of 120–500 μL . Increasing the length of the reaction coil decreases the signal, confirming that the reaction is rapid and the decrease in signal is a consequence of physical dispersion. The maximum analytical signal was recorded at a reaction coil volume of 120 μL , which was chosen for further measurements.

Table 2. Optimization of parameters for the proposed method of ascorbic acid determination

Variable	Studied range	Optimum condition
Reagent concentration	2.0×10^{-4} – 1.0×10^{-3} mol L ⁻¹	6.0×10^{-4} mol L ⁻¹
Wavelength	400–800 nm	548 nm
Flow rate	1000–8000 μL	5000 μL
Analyte volume	50–500 μL	250 μL
Holding coil volume	500–1000 μL	500 μL
Reaction coil volume	120–500 μL	120 μL

Linearity precision and repeatability

Series of working solutions were prepared in the range 2.0×10^{-5} – 6.0×10^{-4} mol L⁻¹. The linearity of the method was confirmed by injecting series of solutions of ascorbic acid at levels of 2.0×10^{-5} , 4.0×10^{-5} , 6.0×10^{-5} , 8.0×10^{-5} , 1.0×10^{-4} , 2.0×10^{-4} , 4.0×10^{-4} , 6.0×10^{-4} mol L⁻¹.

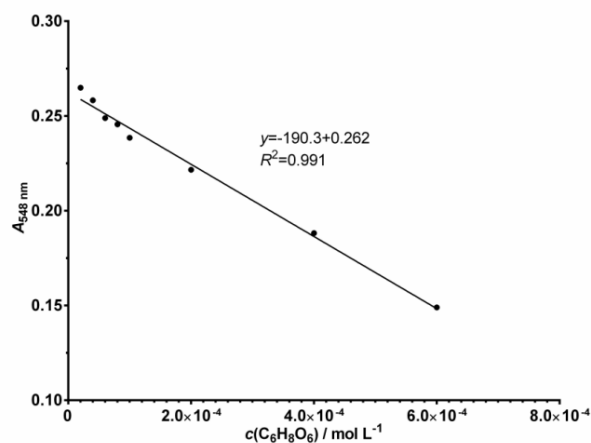


Figure 2. Calibration curve for the determination of ascorbic acid. Experimental conditions: holding coil volume 500 μL ; reaction coil 120 μL ; flow rate: 5000 $\mu\text{L min}^{-1}$; $c(\text{MnO}_4^-) = 6.0 \times 10^{-4}$ mol L⁻¹; $c(\text{ascorbic acid}) = 2.0 \times 10^{-5}$ – 6.0×10^{-4} mol L⁻¹. $V(\text{MnO}_4^-) = 1000$ μL ; $V(\text{ascorbic acid}) = 250$ μL .

A linear response of the analytical signal (recorded absorbance) versus concentration (as mol L⁻¹) was obtained, $y = -190.3x + 0.262$ with the correlation coefficient $R^2 = 0.991$.

The detection limit and the limit of determination were calculated to be 5.4×10^{-6} mol L⁻¹ and 1.8×10^{-5} mol L⁻¹ respectively. The elapsed time between sample injection, signal formation and return to baseline was 15 s. Under optimized conditions a sampling rate of 240 determinations per hour was obtained. The baseline remained stable throughout the 1 h run.

A carryover study was performed by preparing three different concentration solutions of ascorbic acid, 2.0×10^{-5} , 8.0×10^{-5} and 6.0×10^{-4} mol L⁻¹.

The order in which the standard solutions were injected into the holding coil was randomly selected and the signal was recorded three times for each solution. Obtained results show that there is no carryover, the signal return to baseline is complete.

The repeatability of the proposed method was assessed by carrying out 10 successive injections of a 8×10^{-5} mol L⁻¹ ascorbic acid solution on a single occasion. The obtained relative standard deviation of the measured signal was 0.35 %. This was considered to be satisfactory.

Analysis of samples

Three commercially available pharmaceutical preparations containing ascorbic acid were analyzed by the proposed method and the results were compared to the standard iodine titrimetric method recommended by the European Pharmacopea[24]. A very good relation with the results from titrimetric method was determined with analysis of pharmaceutical preparation with the researched method. The results are represented in Table 3.

Table 3. Comparison of the results of the proposed method and standard method obtained from real samples

Sample	Labeled amount (mg/tablet)	Proposed method (mg/tablet)	Titrimetry (mg/tablet)
Plivit C 500 tablets	500	497.4±0.3	525.1±0.6
BIO-C 500 tablets	500	494.6±0.5	514.1±0.3
Vitamin C 500 tablets	500	486.3±0.5	507.2±0.2

IV. Conclusion

The proposed sequential injection method is a simple and robust method for the determination of ascorbic acid in pharmaceutical products. An advantage over the previously reported methods is low reagent and sample consumption, which is one of the main advantages of the sequential injection technique. The proposed method is very fast, the analysis time for the standard method is 30 minutes for three samples, while the analysis time for three samples by the proposed method is 45 seconds. The analysis was carried out in the visible range at 548 nm. The obtained results by the proposed sequential injection spectrophotometric method show good agreement with results obtained by the standard titrimetric method. The spectrophotometric method is preferred because of its simplicity and lower time consumption. This pilot research has shown that the method in question is fast, sensitive, simple and could be applied in routine analyses of quality control of pharmaceutical preparations.

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