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Indirect method for spectrophotometric determination of ascorbic acid in pharmaceutical preparations with 2,4,6-tripyridyl-s-triazine by flow-injection analysis

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ABSTRACT

A flow-injection indirect spectrophotometric method for the determination of ascorbic acid (AA) in pharmaceutical preparations is proposed. The method is based on the reduction of iron(III) to iron(II) by the AA, and by the subsequent reaction of the produced iron(II) with 2,4,6-tripyridyl-s-triazine (TPTZ) in buffered medium (pH = 3.6) to form a coloured complex (λ_{max} = 593 nm). The three-line manifold with one reaction coil was used. The linear range of the method is from 0.08 to 10 μM of ascorbic acid, with the detection limit 24 nM of AA. The proposed method is simple, rapid (sampling rate of 180 samples per hour), sensitive and reproducible (RSD 0.8%, n = 100). The proposed method is very selective, because only the reducing substances with standard (formal) potentials lower than 0.6 V would have the thermodynamic predisposition to interfere in the proposed method. Tested reducing substances (thiol compounds) did not give serious errors when present at the same concentrations as the ascorbic acid. The proposed method can be applied for the determination of AA in pharmaceutical preparations, down to picomolar quantity.

1. Introduction

Ascorbic acid (AA) is an essential water-soluble vitamin in the human diet, participating in many different physiological and metabolic processes. In the pharmaceutical and food industries, the determination or monitoring of AA content is particularly important to assure the quality of their products. There is a need for fast, selective, automated and economic method for its determination, primarily in routine analyses.

Flow injection analysis (FIA) is an interesting alternative in AA determinations instead of conventional batch methods and chromatography with different detectors. The advantages afforded by the FIA are high sample frequency, low sample and reagents consumption, low contamination risks, significant reproducibility that provides high precision and enhanced selectivity as a result of the kinetic nature of the recorded analytical signal. Furthermore, FIA requires very limited laboratory bench space and necessary instrumentation.

The combination of the great biological value of ascorbic acid, with the abovementioned advantages of FIA, has led to the development of numerous flow injection methods for its determination. The reported FIA systems are mainly based on spectrophotometric and electrochemical detectors, but there are also several systems with chemiluminescence and fluorimetric detectors [1]. The flow injection systems with chemiluminescence or fluorimetric detectors offer methods with great sensitivity, but insufficient sample analysis frequency and/or narrow analytical range, which are critical requirements in the routine analysis [1]. Spectrophotometric and electrochemical flow injection methods have a great sample throughput, but electrochemical methods are not robust under continuous use for long periods in process control [2]. The flow injection methods with spectrophotometric detectors are the most effective and suitable for routine analysis due to its simplicity and low operational costs. The direct flow injection spectrophotometry in UV region [3,4] suffers from serious interferences as a result of many compounds, present in samples, that also absorb in UV region. Among the described indirect flow injection spectrophotometric procedures one of the most sensitive approaches to the determination of ascorbic acid is based on the coupled redox complexation reaction. The first step is the reduction of iron(III) by ascorbic acid, followed by the determination of produced iron(II). With the reduction of iron(III) to iron(II) there is an sensitivity amplification factor of two, leading to the increase of sensitivity of this indirect determination. The produced iron(II) is determined through the second step using suitable chromogenic reagents such as: 1,10-phenantroline [5–12], hexacyanoferrate(III) [13], ferrozine [14], 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) [2] and 2,2′-dipyridyl [15].

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In this work 2,4,6-tripyridyl-s-triazine (TPTZ) is proposed as a chromogenic reagent for the determination of AA. This reagent has been already used for the batch spectrophotometric determination of AA in serum and plasma [16,17], and AA in blood platelets, when combined with thin-layer chromatography [18]. The proposed flow injection spectrophotometric procedure for determination of AA in pharmaceutical preparations is simple, inexpensive, does not involve any pre-treatment procedure and has a high sample analysis frequency.

2. Material and methods

2.1. Reagents

All chemicals were of analytical-reagent grade, and all solutions were prepared in Milli-Q deionized water.

Ascorbic acid stock solution (1.0×10^{-2} M) was prepared daily by transferring 176.1 mg of AA (Merck, Darmstadt, Germany) into a 100.0 mL calibrated flask and diluted to the mark with 0.1 M acetic acid (pH = 2.88). In order to avoid the oxidation of ascorbic acid in the air, the addition of acetic acid is recommended. Acetic acid is effective stabilizer and AA oxidation is slower in it [12]. Working solutions of lower concentration were prepared immediately before use by dilution of stock solution with 0.1 M acetic acid.

A stock solution of iron(III) (1.0×10^{-2} M) was prepared by transferring 270.3 mg of iron(III) chloride hexahydrate (Kemika, Zagreb, Croatia) into a 100.0 mL calibrated flask and diluted to the mark with 0.05 M hydrochloric acid.

A stock solution of TPTZ (Merck, Germany), 1.0×10^{-2} M, was prepared by dissolving 312.3 mg in 2.0 mL of 6.0 M hydrochloric acid and diluted to 100.0 mL with deionized water. A stock solution of TPTZ was stored in a dark bottle at 4 °C.

Acetate buffer, pH 3.6, was prepared by mixing 934.8 mL of 0.5 M acetic acid with 65.1 mL of 0.5 M sodium acetate.

The following commercial pharmaceutical preparations have been analysed by the developed flow-injection spectrophotometric method: (a) Plivit C 500 tablets (containing ascorbic acid 500 mg, starch, magnesium stearate, talc and other excipient up to 0.6 g, manufactured by Pliva, Croatia); (b) Plivit C 50 tablets (containing ascorbic acid 50 mg, starch, magnesium stearate, talc, povidone and other excipient up to 0.08 g); (c) Plivit C 1000 dispersible tablets (containing ascorbic acid 1000 mg, citric acid, sodium hydrogen carbonate, mannitol, aspartame, colour β-carotene (E 160a) and other excipient up to 4.0 g); (d) Hair factors tablets (containing ascorbic acid 500 mg, L-cysteine 167 mg, calcium 15.7 mg, phosphor 12 mg, biotin 1 mg, inositol 334 mg, PABA 167 mg and excipient up to 1.8 g, manufactured by Twinlab, USA); (e) Aspirin plus C dispersible tablets (containing ascorbic acid 240 mg, acetylsalicylic acid 400 mg, sodium citrate, sodium hydrogen carbonate, sodium carbonate and other excipient up to 3.2 g, manufactured by Bayer, Germany); (f) Efferalgan plus C dispersible tablets (containing ascorbic acid 200 mg, paracetamol 330 mg, citric acid, sodium hydrogen carbonate, potassium hydrogen carbonate and other excipient up to 2.9 g, manufactured by Bristol-Myers Squibb, France); (g) Lekadol plus C granules (containing ascorbic acid 300 mg, paracetamol 500 mg, aspartame, colour quinoline yellow (E 104) and other excipient up to 5.0 g, manufactured by Lek, Slovenia); (h) Childlife liquid vitamin C (containing ascorbic acid 250 mg, fructose, potassium hydrogen carbonate, other excipient and purified water up to 5 mL (serving size), manufactured by BioZeal, USA).

The solutions of pharmaceutical preparations were prepared by dissolving suitable amounts of the commercial samples in 0.1 M acetic acid and diluted the resulting solution to adjust the concentration to that required by the experimental conditions adopted.

For the validation experiments a 0.5 M iodine solution was prepared and standardized according to the literature [19]. The iodine method is recommended by the European Pharmacopoeia as the standard method for determination of ascorbic acid in pharmaceutical preparations.

2.2. Apparatus

The flow injection system consisted of an Ismatec IPC eight-channel peristaltic pump (Ismatec, Zurich, Switzerland), a Rheodyne low pressure Teflon six port rotary valve, Model 5020 (Anachem, Luton, UK), flow through cell (10 mm optical path and 160 μL inner volume), polytetrafluoroethylene (PTFE) tubing (0.8 mm i.d.), connectors and Chemifold Type II (Tecator, USA). All spectral measurements and real-time data acquisition of flow injection peaks were obtained using a double beam UV/vis spectrophotometer (Shimadzu UV-1601, Kyoto, Japan) fitted with a flow through cell. The instrument was interfaced to a computer equipped with UV Probe 2.31 software provided by Shimadzu.

Adjustments and measurements of pH were carried out using a Mettler Toledo SevenMulti potentiometer (Mettler Toledo, Schwerzenbach, Switzerland) equipped with combined glass electrode Mettler Toledo InLab®413. In the optimization part of experiment a temperature-controllable water bath accurate to ±0.5 °C was used.

2.3. Flow injection procedure

In the developed flow system, depicted in Fig. 1, the loop (500 μL) of the rotary valve was filled with the sample (or standard solution) while the ultra pure water carrier stream (CS) was mixed with the reagent stream (RS). RS consisted of 1.6×10^{-3} M iron(III) and 8.0×10^{-4} M TPTZ in acetate buffer, pH 3.6. CS and RS yielded the final stream that allowed the establishment of the baseline. By valve switching, the sample or standard solutions were injected in the carrier stream, and thus formed sample zone flowed to the confluence point (CP) where it was mixed with reagent stream. For confluence point Chemifold Type II (Tecator, USA) has been used. The final stream subsequently flowed to the reaction coil (RC) (length: 50 cm, i.d. 0.8 mm, which corresponds to a volume of 250 μL) where the coupled reaction took place. After colour development, the dispersed sample zone reached the flow cell unit positioned in the optical path of the spectrophotometer (D) and the absorbance as a transient signal was continuously monitored at 593 nm. Using a cycle time of 20 s, 180 injections per hour were made. The peak height was employed as the quantitative variable.

3. Results and discussion

The proposed indirect FIA method for determination of ascorbic acid was based upon the coupled redox-complexation reaction. In the first (redox) step of the reaction ascorbic acid reduces iron(III) to iron(II) as it is described in Eq. (1):

$$H_2A + 2Fe^{3+} \rightleftarrows DA + 2Fe^{2+} + 2H^+ \quad (1)$$

where H_2A is the reduced form of AA and DA is dehydrogenized (oxidized) AA. Formed iron(II) is conveniently measured after its complexation (second step of the reaction) with the chromogenic reagent TPTZ:

$$Fe^{2+} + 2TPTZ \rightleftarrows Fe(TPTZ)_2^{2+} \quad (2)$$

Iron(II) forms coloured (deep blue) complex at pH 3.6 whose absorption spectra measured against a reagent blank show its maximum absorption at 593 nm [20].

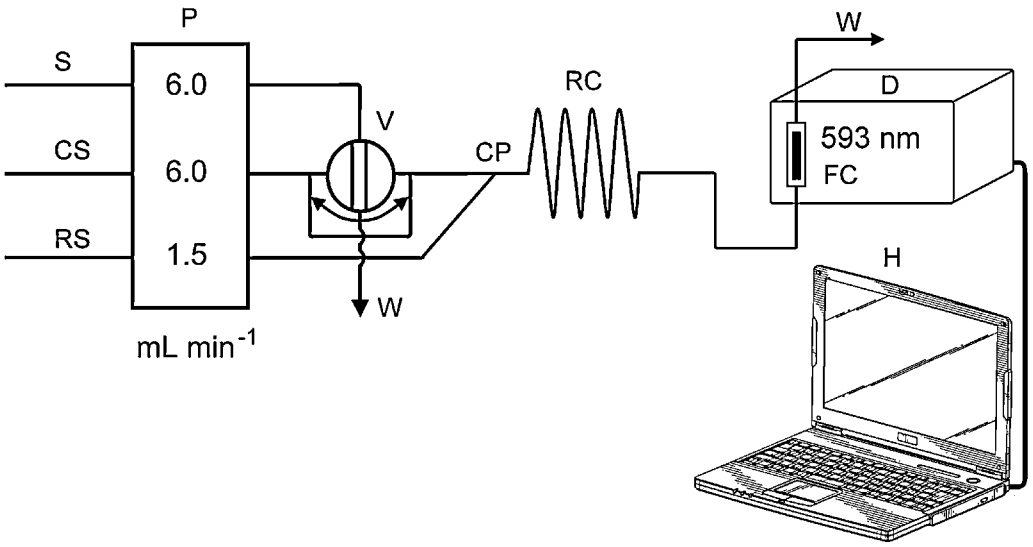

Fig. 1. Flow-injection manifold configuration. S: sample or standard solution (ascorbic acid), CS: carrier stream (ultra pure water), RS: reagent stream (1.6×10^{-3} M iron(III) and 8.0×10^{-4} M TPTZ in acetate buffer, pH 3.6), P: peristaltic pump, V: injector valve (loop = 500 μL), CP: confluence point, RC: reaction coil (length = 50 cm, i.d. = 0.8 mm), D: spectrophotometric detector equipped with flow cell (FC), (V_{FC} = 160 μL), H: computer, W: waste.

3.1. Optimization of the FIA system and reaction conditions

Optimization of manifold parameters and experimental conditions were carried out by means of univariate method [21] at the fixed concentration of ascorbic acid (1.0×10^{-5} M). Each variable was altering in turn while maintaining the other variables at their constant values (selected by random).

At lower pH value the reducing ability of AA is decreasing because its formal potential turns out to be more positive. Furthermore, iron(II) reacts with TPTZ to form deep blue $Fe(TPTZ)_2^{2+}$ complex in the pH range 3.4–5.8 [20]. The effect of pH on the peak height was examined in the pH range 3.2–3.8 using acetate buffer (0.5 M). The absorbance increased simultaneously with increasing pH up to 3.6. However, precipitation of iron hydroxide occurred at pH above 3.8. Therefore, buffered solution with a pH of 3.6 has been chosen as a compromise of preventing the formation and precipitation of iron hydroxide and achieving quantitative $Fe(TPTZ)_2^{2+}$ complex formation.

The influence of iron(III) concentration was studied in the range from 1.0×10^{-4} M to 3.2×10^{-3} M. Also, the studied concentration range of TPTZ was from 5.0×10^{-5} M to 1.6×10^{-3} M. The results showed that the peak heights increased with increasing iron(III) concentration to 1.6×10^{-3} M and were constant thereafter. Also, the largest absorbance was obtained at TPTZ concentration of 8.0×10^{-4} M. Therefore the iron(III) and TPTZ concentrations of 1.6×10^{-3} M and 8.0×10^{-4} M, respectively, were selected as the optimal values.

In order to study the effect of reaction temperature, reaction coils and reservoirs of carrier, reagent and sample were immersed in a temperature controllable water bath. The results indicated that there was no significant difference for the temperature tested, i.e. 20, 25, 30, 40, 50, 60 and 70 °C. At temperatures higher than 50 °C the baseline signal was not stable as a result of the air bubbles formed in the flow system. Thus, the room temperature was used for the convenience of operation.

The flow rate of the carrier stream was studied in the range from 0.5 mL min^{-1} to 8.0 mL min^{-1} keeping the flow rate of reagent stream at 1.5 mL min^{-1}. The peak heights increased non-linearly with increasing the carrier flow rate. The absorbance value reaches its maximum and remains constant for the flow rates higher then 2.0 mL min^{-1}.

The return time is defined as the period between the appearance of the signal maximum at concentrations $\leq 1.0 \times 10^{-5}$ M of AA and the return to the baseline. The return time was decreasing with increasing of the carrier stream flow rate. However, at flow rate higher than 7 mL min^{-1} the flow system was unstable and the return time was increasing. The carrier stream flow rate of 6.0 mL min^{-1} was chosen as a compromise between sensitivity, reproducibility and sampling frequency. Also, the flow rate of carrier stream was set relatively high to reduce the dilution of sample when the carrier stream was merged with the reagent stream.

The flow rate of the reagent stream was studied in the range from 1.5 mL min^{-1} to 6.0 mL min^{-1} keeping the flow rate of the carrier stream at 6.0 mL min^{-1}. Consequently, the total flow rate, after confluence point, was changing from 7.5 mL min^{-1} to 12.0 mL min^{-1}. The peak heights decreased at high total flow rates due to incomplete oxidation of ascorbic acid. At low reagent stream flow rates, consumption of reagent was minute (only 500 μL in each determination for the reagent flow rate of 1.5 mL min^{-1}). Therefore, a reagent stream flow rate of 1.5 mL min^{-1} was chosen as optimal. Under optimized conditions sampling rate was 180 determinations per hour. The return time, was less than 15 s. No baseline shift or absorbance drift was observed throughout the 1 h run.

The influence of the sample volume was investigated by injecting volumes in the range of 100–1000 μL. The peak heights

Table 1
Optimization of manifold parameters and experimental conditions of the proposed method for ascorbic acid determination.

Variable	Studied range	Optimum conditions
Wavelength (nm)	400–800	593
Carrier stream flow rate (mL min^{-1})	0.5–6.0	6.0
Reagent stream flow rate (mL min^{-1})	1.5–6.0	1.5
Injection volume (μL)	100–1000	500
Reaction coil length (cm)	30–525	50
Temperature (°C)	20–70	25
pH of buffer solution	3.2–3.8	3.6
Iron (III) concentration (M)	1.0×10^{-4}–3.2×10^{-3}	1.6×10^{-3}
TPTZ concentration (M)	5.0×10^{-5}–1.6×10^{-3}	8.0×10^{-4}

Table 2
Recovery measurements of samples of ascorbic acid spiked with the reducing substances (thiols).

Reducing substance added to the ascorbic acid in a molar ratio 1:1	Recovery of the spiked sample found using the proposed method[a] (%)	Recovery of the spiked sample found using the standard method[a] (%) [19]
D-Penicillamine	103.1 (0.4)	294.6 (0.7)
L-Glutathione	100.5 (0.6)	151.0 (0.5)
L-Cysteine	101.3 (0.4)	152.0 (0.3)
N-Acetyl-L-cysteine	102.1 (0.8)	152.1 (0.5)
N-(2-Mercaptopropionyl)glycine	101.8 (0.9)	147.9 (0.6)

[a] Values in parentheses are relative standard deviation in % (proposed method $n = 5$, standard method $n = 3$).

increased non-linearly with increasing sample volume as it is inversely proportional to sample dispersion. A sample volume of 500 μL was selected as compromise with respect to the method sensitivity and the high sample throughput.

The effect of the length of the reaction coil was examined in the range of 30–525 cm (i.d. = 0.8 mm). The results indicated that the proposed coupled redox-complexation reaction was really fast because the influence of reaction coil length, in the range between 30 and 60 cm, was not significant. By increasing the reaction coil length the baseline became more stable. The signals decreased above 60 cm due to the increase of sample plug dispersion. Therefore, 50 cm was chosen as the optimal reaction coil length from consideration of peak height, baseline stability and return time (Table 1).

3.2. Calibration, precision and repeatability

Under optimal conditions, the obtained calibration curve was linear in the concentration range from 8.0×10^{-8} M to 1.0×10^{-5} M of AA with a correlation coefficient of 0.9996. Calibration graph obeyed the equation: $y = 3.14 \times 10^4 x - 0.0007$, where y is peak-height absorbance and x is AA concentration expressed in mol L^{-1} (Fig. 2, inset). The limit of detection (LOD) was 2.4×10^{-8} M based on 3σ of the blank solution ($n = 12$). The limit of quantification (LOQ), calculated as 10σ of the blank solution, was 8.0×10^{-8} M,

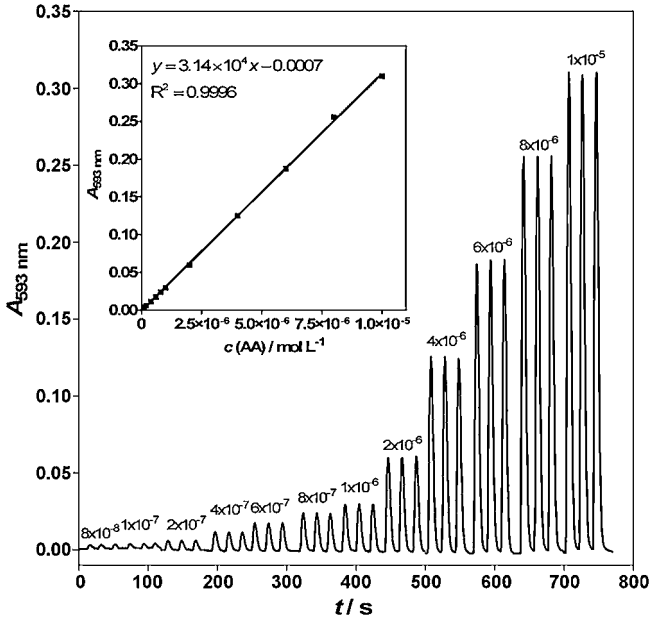

Fig. 2. Diagram chart and calibration curve (inset) for spectrophotometric determination of ascorbic acid over the concentration range from 8.0×10^{-8} M to 1.0×10^{-5} M. Experimental conditions: $c(Fe^{3+}) = 1.6 \times 10^{-3}$ M; c(TPTZ) = 8.0×10^{-4} M; pH = 3.6; $t = 20$ °C; carrier stream flow rate 6.0 mL min^{-1}; reagent stream flow rate 1.5 mL min^{-1}; sample loop volume 500 μL; reaction coil length 50 cm.

which is equal to the lowest standard on the calibration curve. At this point it should be stressed that the relative standard deviation of the slope of three calibration curves obtained on different working days with freshly prepared reagents, was smaller than 0.5%. The sample throughput was 180 per hour, i.e. about 60 samples per hour measured in triplicate.

The precision of the proposed method was checked by periodic injection of the same standard of AA solutions. The relative standard deviations of 50 injections of each solution containing 0.1, 0.4, 0.8, 1.0 and 4.0 μM of AA were 2.4, 2.0, 1.5, 0.8 and 0.8 respectively. Furthermore, the repeatability of the proposed method was assessed by carrying out more than 100 consecutive injections of 1.0 μM ascorbic acid standard solution on a single occasion. The RSD of the measured peak heights was 0.82%. This was considered to be satisfactory.

3.3. Selectivity

The advantage of TPTZ over other frequently used chromogenic reagent, 1,10-phenantroline is enhanced selectivity of redox-reaction. The calculated formal potential of the iron(III)/iron(II) couple in solution with TPTZ (0.578 V), indicates that its oxidizing power is poorer than in solution with 1,10-phenanthroline (1.197 V) [22]. This means that the proposed method with the TPTZ as a chromogenic reagent is very selective. Only the reducing substances with standard (formal) potentials lower than 0.6 V would have the thermodynamic predisposition to interfere in the proposed method. In addition, even if they fulfill this requirement, because of the specificity of flow injection analysis, the slow reactions are kinetically discriminated.

In order to evaluate the selectivity of the developed method for the analysis of AA, the influence of some reducing substances (thiol compounds) was examined. Synthetic sample solutions, containing 10.0 μM of AA and the same concentration of other reducing substance, have been analysed. For comparison the synthetic sample solutions containing 7.1 mM of AA and the same concentration

Table 3
Content of ascorbic acid in pharmaceutical preparations determined by the method reported in the literature [19] and the proposed flow-injection spectrophotometric method.

Ascorbic acid content			
Sample	Stated[a] (mg)	Proposed method[b] (mg)	Standard method[b] [19] (mg)
Plivit C 500 tablets	500	498 (0.2)	504 (0.4)
Plivit C 50 tablets	50	49 (0.2)	51 (0.3)
Plivit C 1000 tablets	1000	1020 (0.4)	1002 (1.4)
Hair factors tablets	500	501 (0.2)	701 (1.2)
Aspirin plus C	240	264 (0.6)	258 (2.1)
Efferalgan plus C	200	204 (0.5)	206 (0.4)
Lekadol plus C	300	301 (0.1)	302 (2.8)
Childlife	250	215 (0.3)	208 (0.3)

[a] Values for samples in mg per tablet, sachet or dose.
[b] Values in parentheses are relative standard deviation in % (proposed method $n = 5$, standard method $n = 3$).

Table 4
Comparison between reported indirect flow injection spectrophotometric methods for ascorbic acid determination and the proposed method.

Reference	Reagent(s) used	λ_{max} (nm)	Beer's law range (μM)	Detection limit (μM)	Sample frequency (h^{-1})	RSD (%)	Sample
[5]	Iron (III) and 1,10-phenanthroline	510	280–2800	No data	60	0.5	Fruit juice
[6]	Tris,1-10-phenanthroline–iron(III) complex	510	285–2270	No data	100	0.88	Pharmaceuticals
[13]	Iron (III) and hexacyanoferrate(III)	700	5.0–100	0.3	140	0.65	Pharmaceuticals
[7]	Iron (III) and 1,10-phenanthroline	510	5.0–60	0.5	No data	0.56	Pharmaceuticals
[8]	Iron (III) and 1,10-phenanthroline	510	114–1703	No data	No data	1.2	Pharmaceuticals
[14]	Iron (III) and ferrozine	562	2.8–57	0.16	90	0.19	Pharmaceuticals, soft drinks, urine
[9]	Iron (III) and 1,10-phenanthroline	512	28–454	15	60	1.6	Soft drinks, beer
[2]	Iron (III) and 2,2′-dipyridyl-2-pyridylhydrazone (DPPH)	535	32–3407	10	120	0.1	Pharmaceuticals
[10]	Iron (III) and 1,10-phenanthroline, Cu(II)	510	0.2–40	0.1	45	0.48	Pharmaceuticals
[15]	Iron (III) and 2,2′-dipyridyl	510	2.8–114	1.1	40	1.2	Biological matters
[11]	Iron (III) and bathophenanthroline	535	5.7–57	No data	80	4.0	Citrus fruits
[12]	Iron (III) and 1,10-phenanthroline	510	1.1–170	0.2	72	1.2	Pharmaceuticals
Present work	Iron (III) and 2,4,6-tripyridyl-s-triazine	593	0.08–10	0.03	180	0.82	Pharmaceuticals

of reducing substance (7.1 mM) were also examined using the standard method [19] (Table 2). Due to lower sensitivity of the standard method in comparison to proposed method, almost 1000-fold higher concentrations of AA and reducing substances were examined. The standard method was not selective for the AA since other reducing substances also increased the analytical signal. Aforementioned notion was also confirmed throughout the analysis of commercial pharmaceutical sample which contained ascorbic acid and glutathione (Hairfactors) (Table 3).

3.4. Interferences

The effect of some possible interfering species was studied by analysing synthetic sample solutions containing 10.0 μM of AA and various concentrations of the interfering substances. An error of 5% was considered to be tolerable. At the 1000-fold excess (10.0 mM), examined ions (Na^+, K^+, NO_3^-, SO_4^{2-}) and reducing agents of sugars (glucose, fructose, sucrose and lactose) did not interfere. The influence of excipients that can commonly accompany AA in pharmaceutical preparations was also studied. Boric acid was tolerable in 500-fold excess (5.0 mM), tartaric acid in 50-fold excess (0.5 mM). Citric acid and acetylsalicylic acid were tolerable in 10-fold excess (0.1 mM). It should be emphasized that this contaminant/analyte concentration ratio studied is much higher than those normally found in commercial pharmaceutical products. Of the species tested, the only major interference was PO_4^{3-}, most likely due the complexation of iron(III).

3.5. Applications

The applicability of the proposed method for the determination of ascorbic acid was checked by analysing several types of pharmaceutical formulations (syrup, dispersible tablets and tablets). Those formulations contained different amounts of ascorbic acid and even some other active substances beside excipients. Results were compared with those obtained by standard method recommended by the European Pharmacopoeia [19] based on titration of AA with iodine (Table 3).

The proposed method is simple to operate and more rapid (3 injections per minute, i.e. 1 sample per minute measured in triplicate) than the standard method (at least 30 min for triplicate measurements of sample). Also, the reagent consumption is minimal, only 1.5 mL per sample measured in triplicate. On the other hand, for analysis of one sample in triplicate using standard titrimetric method, at least 50 mL of highly concentrated reagents are required.

Performance characteristic of reported indirect flow injection methods, for the determination of ascorbic acid using spectrophotometric detectors, and the proposed method, are compared in Table 4.

4. Conclusions

The proposed flow injection spectrophotometric method fulfills all the main demands of routine analysis of AA. It has low instrumentation and operational costs in comparison to chromatographic methods and significant sample analysis frequency in comparison to conventional batch methods. Furthermore, no one of the previously reported flow injection spectrophotometric methods offers so sensitive determination of AA. In addition, the use of TPTZ as chromogenic reagent made the proposed method very selective with a wide linear dynamic concentration range. It is also worth mentioning that the proposed method was performed in the visible region (λ = 593 nm) away from the UV-absorbance of the UV-absorbing interfering excipient materials, which might be dissolved from pharmaceutical preparations. Application of the proposed method to the determination of ascorbic acid in pharmaceutical samples produced excellent results in terms of accuracy.

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References

[1] M.C. Yebra-Biurrun, Talanta 52 (2000) 367–383.
[2] D.G. Themelis, P.D. Tzanavaras, F.S. Kika, Talanta 55 (2001) 127–134.
[3] K.K. Verma, A. Jain, A. Verma, A. Chaurasia, Analyst 116 (1991) 641–645.
[4] A. Molina-Diaz, A. Ruiz-Medina, M.L. Fernandez-de Cordova, Fresenius J. Anal. Chem. 363 (1999) 92–97.
[5] J.M. Alamo, A. Maquieira, R. Puchades, S. Sagrado, Fresenius J. Anal. Chem. 347 (1993) 293–298.
[6] S.M. Sultan, A.M. Abdennabi, F.E.O. Suliman, Talanta 41 (1994) 125–130.
[7] A.V. Pereira, O. Fatibello-Filho, Talanta 47 (1998) 11–18.
[8] S.M. Sultan, N.I. Desai, Talanta 45 (1998) 1061–1071.
[9] E. Luque-Perez, A. Rios, M. Valcarcel, Fresenius J. Anal. Chem. 366 (2000) 857–862.
[10] N. Teshima, T. Nobuta, T. Sakai, Anal. Chim. Acta 438 (2001) 21–29.
[11] N. Memon, M.A. Memon, M.I. Bhanger, M.H. Memon, Nucleus 40 (2003) 115–118.
[12] M. Zenki, A. Tanishita, T. Yokoyama, Talanta 64 (2004) 1273–1277.
[13] J.A. Nobrega, G.S. Lopes, Talanta 43 (1996) 971–976.
[14] A. Molina-Diaz, I. Ortega-Carmona, M.I. Pascual-Reguera, Talanta 47 (1998) 531–536.
[15] T. Kleszczewski, E. Kleszczewska, J. Pharm. Biomed. Anal. 29 (2002) 755–759.
[16] B.R. Day, D.R. Williams, C.A. Marsh, Clin. Biochem. 12 (1979) 22–26.
[17] T.Z. Liu, N. Chin, M.D. Kiser, W.N. Bigler, Clin. Chem. 28 (1982) 2225–2228.

[18] J.V. Lloyd, P.S. Davis, H. Lander, J. Clin. Pathol. 22 (1969) 453–457.
[19] European Pharmacopoeia, Council of Europe, Nordlingen, 2008.
[20] P.F. Collins, H. Diehl, G. Frederick Smith, Anal. Chem. 31 (1959) 1862–1867.
[21] A. San Vicente, A. Arranz, J.M. Moreda, J.F. Arranz, Anal. Chim. Acta 298 (1994) 87–90.
[22] L. Kukoc-Modun, N. Radić, Int. J. Anal. Chem. (2011), dx. doi org/10.1155/2011/1407560.