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Original article

Simultaneous determination of iron (II) and ascorbic acid in pharmaceuticas based on flow sandwich technique

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ABSTRACT

The simple and easy performed flow system based on sandwich technique has been developed for the simultaneous separate determination of iron (II) and ascorbic acid in pharmaceuticals. The implementation of sandwich technique assumed the injection of sample solution between two selective reagents and allowed the carrying out in reaction coil two chemical reactions simultaneously: iron (II) with 1,10-phenanthroline and ascorbic acid with sodium 2,6-dichlorophenolindophenol. For achieving of excellent repeatability and considerable reagent saving the various parameters such as flow rate, sample and reagent volumes, reaction coil length were also optimized. The limits of detection (LODs) obtained by using the developed flow sandwich-type approach were 0.2 mg L^{-1} for iron (II) and 0.7 mg L^{-1} for ascorbic acid. The suggested approach was validated according to the following parameters: linearity and sensitivity, precision, recoveries and accuracy. The sampling frequency was 41 h^{-1}.

1. Introduction

Pharmaceuticals are the chemical substances containing one or several pharmacologically active ingredients and excipients — substances which are added to a prescription in order to confer a suitable consistency or form of the drug as well as to affect the bioavailability of the active ingredients (Jackson, Young, & Pant, 2000; Pifferi & Restani, 2003; World Health Organization, 2007). The quality control of pharmaceuticals is a key factor at all stages of their development and production. In recent years, the international pharmacopoeia requirements and standards related to the quality control of pharmaceuticals become stricter (Pimenta, Montenegro, Araujo, & Martınez Calatayud, 2006; World Health Organization, 2007). This demands the modern pharmaceutical analysis to develop rapid, reliable, selective and sensitive analytical methods for the determination of active ingredients and excipients in pharmaceuticals (David & Webb, 2003).

Nowadays, by the reason of the accelerated pace of life, hyponutrition, people are exposed by a lacking of essential trace elements, among which the most common is iron deficiency. Iron is of vital importance in numerous biological processes, primarily involved oxygen binding and transport, muscle oxygen use and storage, gene regulation, cell growth and differentiation, enzyme reactions, neurotransmitter synthesis, and protein synthesis (Beard, 2001; Crichton, Wilmet, Legssyer, & Ward, 2001; Halliberg, 2001; Schumann, Ettle, Szegner, Elsenhans, & Sololmons, 2007). The lack of this element in the human body causes a widespread disease — iron-deficiency anemia (IDA) (Clark, 2008). The treatment of IDA is provided by the antianemic drugs which contain iron (II) compounds and ascorbic acid as the mostly used excipient, which improves the gastrointestinal absorption of iron (Killip, Bennett, & Chambers, 2007). However, such pharmaceuticals often cause side effects: nausea, anorexia, diarrhea and metal taste in the mouth (Beutler, Hoffbrand, & Cook, 2003). Thus, it is important to carry out the quality control of antianemic drugs and determine the concentrations of iron (II) and ascorbic acid.

Traditionally, most methods of pharmaceutical analysis for separate determination of iron (II) and ascorbic acid are focused on the spectrophotometry (Davies & Masten, 1991; Guclu, Sozgen, Tutem, Ozyurek, & Apak, 2005; Karpinska & Kulikowska, 2002; Zargba & Hopkata, 1996) and electrochemistry (Mahmoud, 2001; Mary Nancy, Anithakumary, & Kumara Swamy, 2014; Merli, Profumo, & Dossi, 2012; Thangamuthu, Senthil Kumar, & Chandrasekara Pillai, 2007) (Table 1). These methods are characterized by high selectivity, sensitivity, rapidity and the possibility of automation. However, these techniques as well as atomic absorption and emission spectrometry (Zachariadis & Michos, 2007; Zachariadis, Raidou, Themelis, & Stratis, 2002) are primarily focused on the determination of iron. In turn, the chromatography (Gioia, Andreatta, Boschetti, & Gatti, 2008; Khuhawar & Lanjwani, 1998; Pengfei et al., 2012) and the capillary electrophoresis (Fotsing, Fillet, Bechet, Hubert, & Crommen, 1997; Lin Ling, Baeyens, Van Acker, & Dewaele, 1992) are focused on the determination of iron (II) or ascorbic acid.

Inevitably, automation plays an important role in pharmaceutical analysis, especially when a lot of samples have to be analyzed in the

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Table 1
Reported methods for determination of iron (II) and ascorbic acid in pharmaceuticals.

Method of analysis	Analyte	Detection	Matrix	Linear range, mg L^{-1}	LOD, mg L^{-1}	Sample frequency (h^{-1})	R.S.D., %	Reference
Spectrophotometry	Iron (II)	With 4-(2-pyridylazo) resorcinol at λ = 537 nm, pH = 9.1 (borate buffer)	Tablets	0.025–0.2	0.008	10	2.2	Karpinska & Kulikowska (2002)
		With three azoderivatives of pyrocatechol at λ = 490; 560; 600 nm, pH = 10.0;	Tablets	0.05–2.11 0.05–0.89 0.05–1.00	0.01 0.01 0.01	10	3.0 2.7 1.9	Zargba & Hopkata (1996)
	Ascorbic acid	With neocuproine at λ = 450 nm, pH = 7.0	Tablets	1–15	0.3	10	2.0	Guclu et al. (2005)
		With sodium 2,6-dichlorophenolindophenol at λ = 520 nm, pH = 4.2	Tablets	0.3–20	0.08	10	1.8	Davies & Masten (1991)
Electrochemical methods	Iron (II)	Differential pulse voltammetry, pH = 7.0	Tablets	0.05–5	0.017	5–10	3.6	Merli et al. (2012)
		Potentiometric detection with ion-selective electrode, pH = 7.0	Tablets	0.028–560	0.006	5–10	2.6	Mahmoud (2001)
	Ascorbic acid	Amperometric detection using glassy carbon electrode modified by $K_4Mo(CN)_8$, pH = 7.0	Tablets	2–100	0.5	5–10	3.5	Thangamuthu et al. (2007)
		Differential pulse voltammetry with glassy carbon electrode modified by graphene, pH = 7.0	Tablets	8–80	0.7	5–10	3.0	Mary Nancy et al. (2014)
AAS/AES	Iron (II)	AES with inductively coupled plasma	Tablets	0.01–0.5	0.002	10	2.3	Zachariadis & Michos (2007)
		AAS with flame atomization (air-acetylene flame)	Tablets	0.5–4	0.15	10–20	1.1	Zachariadis et al. (2002)
Chromatography	Iron (II)	Preliminary derivatization HPLC complexing agent (2-thiophene aldehyde-4-phenyl-3-tiosemicarbazone), stationary phase — C-18; mobile phase — a mixture of methanol, acetonitrile, water, sodium acetate and ammonium tetra butylbromide (78:10:10:1:1)	Tablets	0.5–2.5	0.025	4	4.8	Khuhawar & Lanjwani (1998)
	Ascorbic acid	HPLC stationary phase — C-18; mobile phase — a mixture of phosphate buffer and acetonitrile (95:5)	Tablets	0.4–1.2	0.13	10	2.0	Pengfei et al. (2012)
		Couple-ion reversed liquid chromatography; stationary phase — C-18, mobile phase — cetyltrimethylammonium bromide solution	Injection solutions	14–45	2	10	1.8	Gioia et al. (2008)
Capillary Electrophoresis	Ascorbic acid	Detection in the medium of a borate buffer solution (pH = 8.5)	Tablets	0.2–1	0.02	6	4.3	Fotsing et al. (1997)
		Detection in the medium of a phosphate buffer solution (pH = 5.0)	Tablets	10–1000	0.5	12	5.0	Lin Ling et al. (1992)
Flow analysis	Iron (II)	FIA, reagent — salicylic acid, λ = 520 nm, pH = 4.0	Tablets	1–20	0.3	100	3.4	Udnana et al. (2004)
		SIA, reagent — 1,10-phenanthroline,λ = 510 nm, pH = 5.5	Tablets	0.25–5	0.02	40	3.0	Tesfaldet et al. (2004)
		SIA, reagent — 2,2-bipyridyl, λ = 523 nm, pH = 4.5	Tablets	5.0–40	1.0	100	5.0	Oliveira & Masini (2001)
	Ascorbic acid	FIA, reagent — ferrozine, λ = 562 nm, pH = 5.5	Tablets	0.1–20	0.005	90	1.9	(Molina-Diaz et al., 1998)
		FIA, reagent — tripiridilttriazin iron (III), λ = 593 nm, pH = 3.6	Tablets	0.015–2	0.005	180	0.8	Kukoc-Modun et al. (2012)
		SIA, reagent — $KMnO_4$, λ = 525 nm	Tablets	0.1–1000	0.03	60	2.0	Lenghor et al. (2002)
	Iron (II) and Ascorbic acid simultaneously	Flow sandwich technique, reagent for Iron (II) — 1,10-phenanthroline, λ = 510 nm, pH = 5.5 reagent for Ascorbic acid — sodium 2,6-dichlorophenolindophenol, λ = 512 nm, pH = 4.5	Tablets	0.5–4 2–20	0.2 0.7	41	3.0 3.0	This work

minimum of time. The most widespread in pharmaceutical analysis are the flow injection (FIA) (Ruzicka & Hansen, 1975) and sequential injection (SIA) analysis (Ruzicka & Marshall, 1990). Coupling to FIA and SIA manifolds selective detectors make the developed approaches simple and easy implemented in pharmaceutical analysis (Horstkotte & Cerdà, 2009; Mervartova, Polasek, & Martınez Calatayud, 2007). Thus, flow analysis with spectrophotometric detection offers practically endless possibilities to the automation of pharmaceutical analysis procedures. Moreover it also provides miniaturization of analysis (Tzanavaras & Themelis, 2007).

For the determination of iron (II) and ascorbic acid in pharmaceuticals the flow injection (Alonso, Bartrolí, del Valle, & Barber, 1989; Alonso, Bartroli, Delvalle, Escalada, & Barber, 1987; Alonso-Chamarro, Bartroli, & Barber, 1992; Araujo, Lima, Alonso-Chamarro, Bartroli, & Poch, 1991; Cerda & Cerda, 2009; Falkova, Pushina, Bulatov, Alekseeva, & Moskvin, 2014; Horstkotte & Cerdà, 2009; ISO 5725-1, 1994; IUPAC, 1997; Kukoc-Modun, Biocic, & Radic, 2012; Lenghor et al., 2002; Mervartova et al., 2007; Molina-Diaz, Ortega-Carmona, & Pascual-Reguera, 1998; Mortatti, Krug, Pessenda, & Zagatto, 1982; Ruzicka & Hansen, 1975; Ruzicka & Marshall, 1990; Tzanavaras & Themelis, 2007; Udnana et al., 2004) and sequential injection (Lenghor et al., 2002; Oliveira & Masini, 2001; Tesfaldet, van Staden, & Stefan, 2004) techniques were developed, which provide high sensitivity (Table 1) in combination with high sampling frequency (up to 180 h^{-1}). However, they are focused only on the determination of iron (II) or ascorbic acid. To the best of our knowledge, simultaneous separate determination of iron (II) and ascorbic acid based on flow methods has not been reported.

This present work describes a new automated procedure for simultaneous separate determination of iron (II) and ascorbic acid in pharmaceuticals. For this purpose the flow system based on sandwich technique is implemented. At first the application of this technique in flow analysis was presented by Alonso and co-authors and was applied

for the determination of iron (II) and total iron in a ground water samples (Alonso et al., 1989). The sandwich technique provides the strict order of reagents and sample injection into the flow system and means the sample "sandwiching" between two selective reagents (Alonso et al., 1987; Alonso-Chamarro et al., 1992; Araujo et al., 1991). During the moving thought the reaction coil two analytical products are formed. Despite the necessity to use the relatively large sample volume for preventing the overlapping of the colored zones, the sandwich technique is characterized by satisfactory sampling frequency because of the high flow rate using. Moreover, because of the optimizing of physical parameters of the flow systems it become possible to achieve the excellent repeatability of analysis and considerable reagent saving (Cerda & Cerda, 2009).

2. Materials and methods

2.1. Reagents and solutions

All chemicals were of analytical reagent grade. Ultra pure water from Millipore Milli-Q RG (Millipore, USA) was used throughout the experiment. The stock solution of 0.045 M of iron (II) was prepared by dissolving of the corresponding weight of $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ in 0.1 M H_2SO_4. The stock solution of 0.1 M of ascorbic acid was prepared by dissolving of the corresponding weight in the water. The working solutions of analytes were prepared by appropriate dilution of the stock solutions with the water. The solution of 1 μM 1,10-phenanthroline and sodium 2,6-dichlorophenolindophenol were prepared by dissolving of the corresponding weights of reagents in the acetate buffer solution (pH = 4.5) and the water, respectively. The 1,10-phenanthroline solution was colorless, stable within a week when stored in the dark place. The solution of sodium 2,6-dichlorophenolindophenol was brightly colored, stable within a week when stored at temperature from 2 to 5 °C. The working solutions of reagents were prepared by appropriate dilution of the stock solutions with acetate buffer solution.

2.2. Manifold and apparatus

The flow system based on sandwich technique includes (Fig. 1): an eight-port valve (Cole-Parmer, USA), a peristaltic pump MasterFlex L/S (Cole-Parmer, USA) (flow rate 3.0 mL min^{-1}), a reaction coil (80 cm length and 1 mm in i.d.), home-made PTFE filter prepared according to Falkova et al. (2014) for filtering a sample solution and communication tubes (PTFE 1 mm in i.d.). It is equipped with a source of visible light LS-1 and a USB 4000 spectrometer (Ocean Optics, USA) with a 50 mm path length flow cell FIA-Z-SMA-50-TEF (Fialab, USA). The analyzer was operated automatically by means a computer.

To carry out the reference analyses of pharmaceuticals a capillary electrophoresis system Capel-105M (Lumex, Russia) and an atomic absorption spectrometer AA-7000 (Shimadzu, Japan) were used. To carry out the sampling preparation of pharmaceuticals an ultrasonic bath Sapphire (Sapphire, Russia) was used.

2.3. Samples

Three different samples of antianemic drugs at solid dosage form "Sorbifer Durules", "Fenules" and "Tardyferon" were analyzed. All these antianemic drugs contain ferrous sulfate as active ingredient and ascorbic acid as excipient, with dosage form 500 mg.

2.4. Sample preparation

The sample preparation was performed by ultrasound-assisted dissolution of solid dosage form of antianemic drugs in the medium of 0.1 M HCl. Three tablets of antianemic drugs were grounded to a homogeneous powder in a ceramic mortar. Then, 0.01 g of the powder was mixed with 1 g of potassium chloride. This step is carrying out because of the high content of iron (II) and ascorbic acid in antianemic drugs. Further, 0.01 g of the obtained mixture was placed in a glass vessel and the 5 mL of 0.1 M HCl was added. Afterwards the dissolution of antianemic drugs took place under ultrasonication (325 W, 35 kHz) at 20 °C for 10 min. The prepared solution was then analyzed by flow system based on sandwich technique. While the calculation of the content of the iron (II) and ascorbic acid in the tablet, the mass of the tablet and the analyte content using the calibration curve were taken into consideration.

2.5. The procedure of flow determination of iron (II) and ascorbic acid

To implement the flow system based on sandwich technique (Fig. 1) the portions of sample and reagent solutions were sequentially delivered into the reaction coil by movement of the peristaltic pump through a eight-port valve in strict order: 75 μL of acetate buffer solution as the carrier solution (*g*), 150 μL of 1,10-phenanthroline solution (*b*), 1 mL of sample solution (*a*), 150 μL of sodium 2,6-dichlorophenolindophenol solution (*d*) and again 500 μL of carrier solution (*g*). The high flow rate (3.0 mL min^{-1}) and a large volume of the sample eliminate overlapping

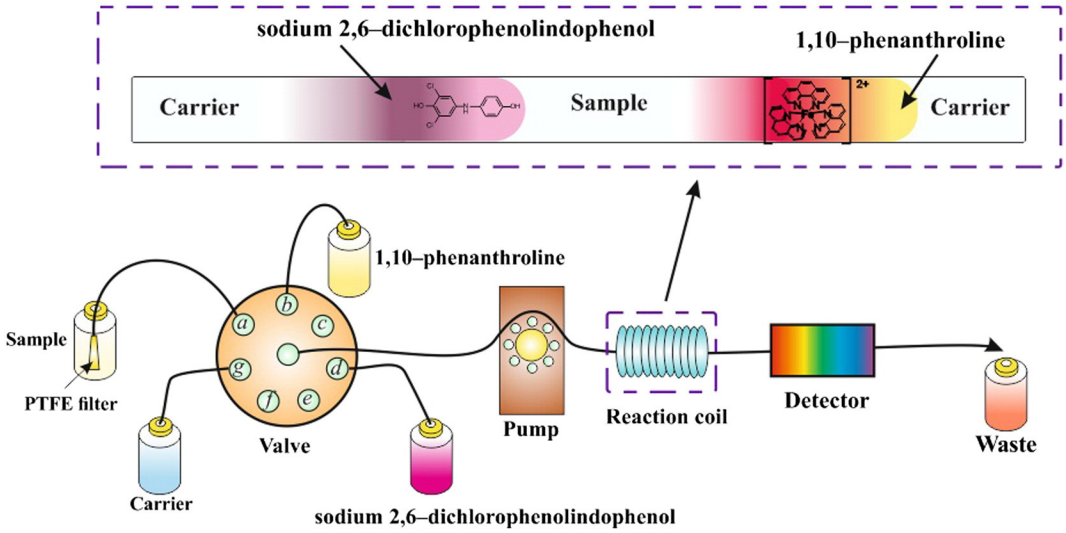

Fig. 1. The manifold of the flow system based on sandwich technique.

of the colored zones of reaction products. The analytical signals were measured in the flow-stop condition for 3 s by means of the spectrophotometer at the wavelength 510 nm for iron (II) and 512 nm for ascorbic acid. The first analytical signal corresponds the complex of iron (II) with 1,10-phenanthroline, the second one — the product of ascorbic acid with sodium 2,6-dichlorophenolindophenol. To avoid the memory effect the manifold was washed out with carrier solution. To ensure the strict order of zones formation, sequence and time of performing of all analysis steps the special program was set up.

2.6. Procedure for the determination of iron (II) and ascorbic acid by reference methods

For the AAS determination of iron (II) in antianemic drugs the pre-treatment stage was performed by ultrasound-assisted dissolution of powdered tablets in 50 mL of 1 M HNO_3 for 10 min. Then, the mixture was filtered through the paper filter and farther analyzed by AAS with atomization in a continuous flame.

For the CE determination of ascorbic acid in antianemic drugs the pre-treatment stage was performed by ultrasound-assisted dissolution of powdered tablets in 5 mL of 0.1 M HCl for 10 min. For the stabilization of ascorbic acid in the prepared solution 10 mL of 0.05 M EDTA was added. Then, the mixture was filtered through the paper filter and farther analyzed by capillary electrophoresis. The length of used quartz capillary was 50 cm with 75 μm in i.d. The measurements were performed at 254 nm using $Na_2B_4O_7$ — sodium dodecyl sulfate solution as a supporting electrolyte.

3. Results and discussion

3.1. Theoretical aspects

For the spectrophotometric determination of iron (II) a well-known color-forming reagent 1,10-phenanthroline was used. This reaction proceeds in wide range of pH from 2.0 to 9.0 in a molar ratio 3:1 ($R:Fe^{2+}$) (Mortatti et al., 1982). The complex absorbance maxima is observed at the 510 nm ($\varepsilon_{510} = 1.1 \times 10^4$ L mol^{-1} cm^{-1}).

For the spectrophotometric determination of ascorbic acid the reagent sodium 2,6-dichlorophenolindophenol was chosen, because of the reducing properties of the analyte. This reaction proceeds in a molar ratio 1:1 according to the following schematic representation:

In the acidic medium the reagent solution has a purple color, which is discoloring during the interaction with ascorbic acid. Absorbance maximum of sodium 2,6-dichlorophenolindophenol is observed at the 512 nm ($\varepsilon_{512} = 3.2 \times 10^4$ L mol^{-1} cm^{-1}).

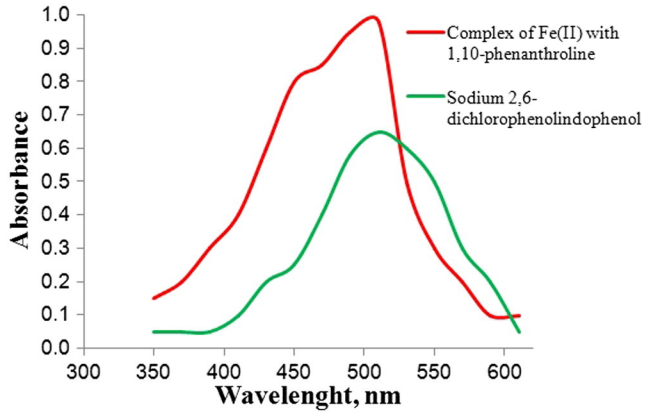

Fig. 2. Absorbance spectra of complex of Fe (II) with 1,10-phenanthroline ($C_R = 10^{-4}$ M, $CFe^{2+} = 10^{-4}$ M, pH = 4.5) and sodium 2,6-dichlorophenolindophenol ($C_R = 10^{-4}$ M, ascorbic acid = 10^{-4} M, pH = 4.5).

The absorbance spectra of complex of Fe (II) with 1,10-phenanthroline and sodium 2,6-dichlorophenolindophenol are presented in the Fig. 2.

The reaction between ascorbic acid and sodium 2,6-dichlorophenolindophenol is strongly dependent on the acidity of the medium. However, solving the problem of choosing the optimal pH of the reaction, it was necessary to take into account the presence of iron (II) in the antianemic drugs and its possible influence on the reaction. According to Davies & Masten (1991), it was decided to carry out the reaction in the presence of acetate buffer solution. In the pH range from 4.0 to 4.5 the interference effect of Fe^{2+} ions is not found. Thus, the acetate buffer solution (pH = 4.5) was used as an appropriate carrier-solution.

3.2. Optimization of parameters

The conditions for the determination of iron (II) and ascorbic acid were optimized by studying the influence of the various parameters such as flow rate, sample and reagent volumes, reaction coil length and reagent concentration. 4×10^{-5} M of iron (II) and ascorbic acid, 5×10^{-3} M of 1,10-phenanthroline and 5×10^{-4} M of sodium 2,6-dichlorophenolindophenol were used to optimize these parameters. In all cases both the relative peak height and percentage of RSD were used as criteria for establishing the most appropriate parameters.

The flow rate is a very important parameter to be optimized because it influences to the products formation. The reactions between iron (II) and 1,10-phenanthroline as well as ascorbic acid and sodium 2,6-dichlorophenolindophenol are rapid, resulting in an almost instant products formation. The flow rate was varied between 0.5 and 3.5 mL min^{-1} by changing the speed of the pump. The better repeatability and lower consumption of carrier-solution was achieved at 3.0 mL min^{-1} and this flow rate was chosen as optimum working condition (Fig. 3A).

The aim of optimization of sample and reagent volumes is to minimize the consumption of sample and reagents, maintaining the best sensitivity and repeatability and to prevent the mixing of products while moving them in the reaction coil into the detector. The injected sample volume was varied from 0.5 to 1.5 mL and the volumes of 1,10-phenanthroline and sodium 2,6-dichlorophenolindophenol were varied from 50 to 250 μL. The results presented in the Fig. 3B shown that the injected sample volume less than 1 mL leads to decreasing of repeatability because product zone mixing is observed. It was also found that optimal reagent volumes are 150 μL for both reagents (Fig. 3C).

The reaction coil is usually kept as short as possible to avoid the product dispersion but sufficient to provide the product formation. The length of this coil was varied from 50 to 100 cm. The optimum length of the reaction coil was 80 cm, which ensures the effective overlapping zones of reagents with the sample and prevent the mixing of products zones (Fig. 3D).

The concentrations of the reagents were varied from 1×10^{-3} to 1×10^{-2} M of 1,10-phenanthroline and from 1×10^{-4} to 1×10^{-3} M of sodium 2,6-dichlorophenolindophenol. It was found that the optimal concentrations of 1,10-phenanthroline and sodium 2,6-dichlorophenolindophenol are 5×10^{-3} and 5×10^{-4} M, respectively.

3.3. Optimization of dissolution

Most of antianemic drugs are in a solid dosage form with a relatively high content of iron (II) and ascorbic acid (13–20%). Therefore, it is important to provide the effective dissolution as well as dilution of the sample. It is known that H_2SO_4 and HNO_3 have strong oxidizing properties, which contribute to the destruction of ascorbic acid and oxidation of Fe^{2+} to Fe^{3+}. Thus for investigating of the sample dissolution the deionized water and HCl were used. The effects of temperature, thermostating and ultrasonication time were investigated to increase

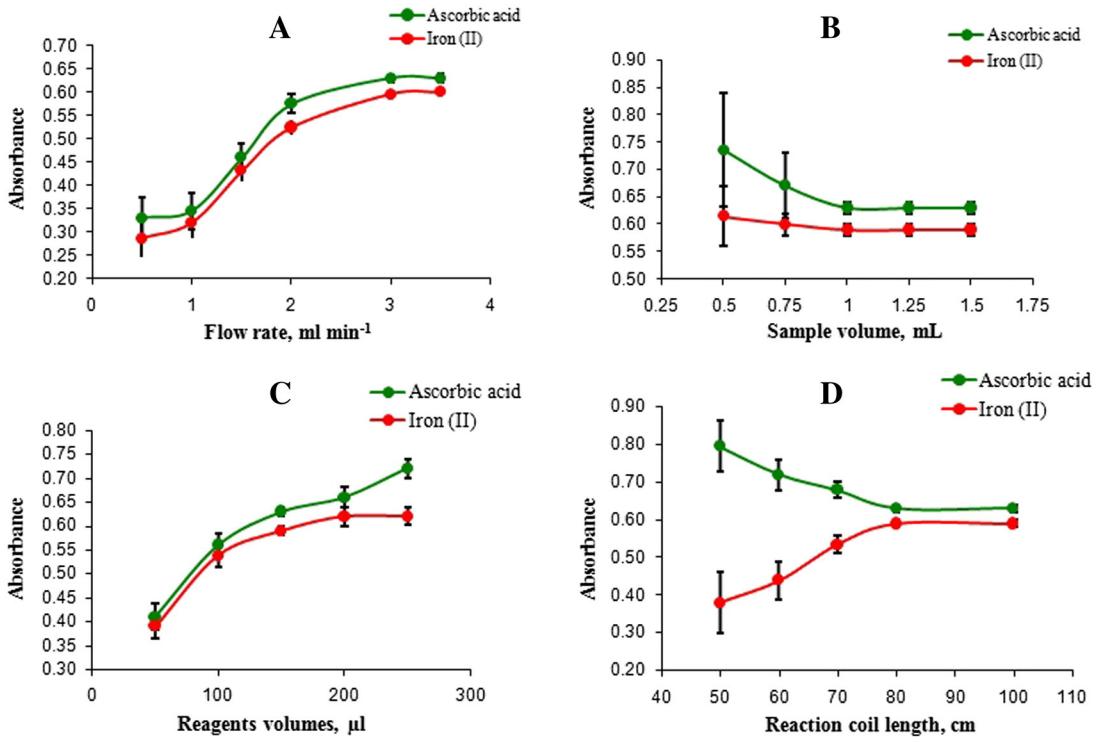

Fig. 3. Investigation of flow system based on sandwich technique experimental conditions: (A) effect of flow rate, (B) effect of sample volume, (C) effect of reagents volumes, (D) effect of reaction coli length.

the efficiency of dissolution. Thus the temperature was varied from 20 to 50 °C and the thermostating and ultrasonication time — from 2 to 20 min. Two parallel experiments were performed — with and without ultrasound assistants. The results showed that effective dissolution of antianemic drugs is observed in the media of 0.1 M HCl at 50 °C for 10 min without ultrasonication and at 20 °C for 10 min with ultrasonication.

3.4. Interference effect

The effect of potentially interfering components presented in antianemic drugs (Mg^{2+}, Co^{2+}, Cu^{2+}, SO_4^{2-} ions and riboflavin, pyridoxine, thiamine and pantothenic acid) on the determination of analytes was investigated. It was performed by addition of known concentration of each component into a model solution, containing iron (II) ions and ascorbic acid at concentration of 4×10^{-5} M in 0.1 M hydrochloric acid. The tolerable excess of each taken foreign species is considered to be less than 5% of relative error in the signal. The greatest influence (less than 10-fold excess) on the product formation has riboflavin, thiamine, pyridoxine and pantothenic acid, but in the antianemic drugs a content of these components is much lower than the content of iron (II) and ascorbic acid. Thus it should be concluded with the absence of interference effect.

3.5. Validation

For validation, the following parameters were evaluated for linearity and sensitivity, precision, recoveries and accuracy.

3.5.1. Linearity and sensitivity

Under the optimized reaction condition according to the previously described procedure, the calibration curves for iron (II) and ascorbic acid determination were constructed from ten data points using the standard solution of analytes (Fig. 4). The relationship obtained between the relative peak height and concentrations of iron (II) as well as ascorbic acid were given by the follow equations:

$$\text{Relative peak height} = 0.231 \times \left[\text{Concentration of Fe (II), mg L}^{-1}\right] + 0.062, r^2 = 0.996;$$

$$\text{Relative peak height} = -0.046 \times \left[\text{Concentration of ascorbic acid (II), mg L}^{-1}\right] + 0.0968, r^2 = 0.995.$$

The absorbance of colored products at the wavelength 510 nm and 512 nm for iron (II) and ascorbic acid, respectively, obey the Beer's law in the range of 0.5–4.0 mg L^{-1} for iron (II) and of 2.0–20 mg L^{-1} for ascorbic acid.

The sensitivity was characterized by the limit of detection (LOD). It was measured by standard IUPAC method as 3 standard deviation of the blank (3 s) (IUPAC, 1997). The LODs calculated from the calibration plots based on 3 s were 0.2 mg L^{-1} for iron (II) and 0.7 mg L^{-1} for ascorbic acid. The results indicated that the LODs both for iron (II) and ascorbic acid are satisfied for their determination in antianemic drugs.

3.5.2. Precision

The precision of the method was evaluated with regard to its repeatability and reproducibility (ISO 5725-1, 1994).

The repeatability of the developed approach was determined by analyzing 10 replicates of model solutions which contain iron (II) and ascorbic acid in the range from 0.5 mg L^{-1} to 4.0 mg L^{-1} and from 2 mg L^{-1} to 20 mg L^{-1}, respectively. It was studied that the developed flow sandwich-type approach provides satisfactory results. The RSD values were <3.0%. This level of precision of the proposed flow sandwich-type approach was adequate for quality-control of antianemic drugs.

The reproducibility of the proposed methods was assessed by applying the developed flow sandwich-type approach with the use of 2 different instruments in 2 different laboratories at 2 different times. Results

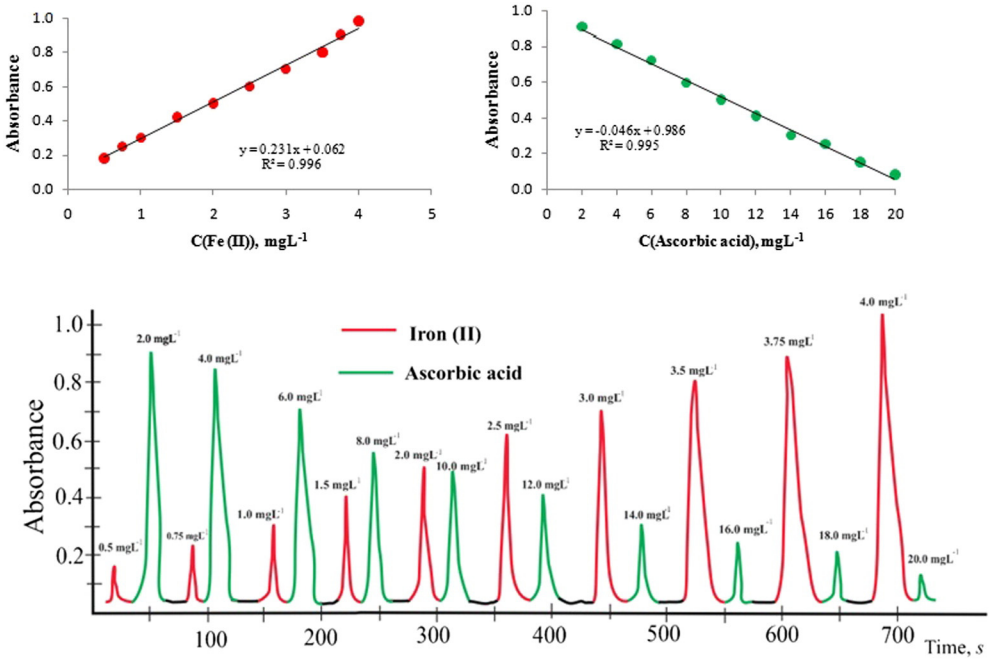

Fig. 4. Calibration curves and depiction of absorbance peaks obtained by suggested flow system based on sandwich technique.

obtained with the laboratory-to-laboratory and day-to-day variations were found to be reproducible because the RSD values under conditions were 5.0%.

3.5.3. Recovery

Recovery values were determined for each analyte at 2 mg and 5 mg concentrations in dosage form of antianemic drugs. The investigation was performed for 3 replicate of three samples ("Sorbifer Durules", "Fenules", "Tardyferon"). The standard additions of analytes were placed into the samples of antianemic drugs before dissolving. It is shown in Table 2 that recovery values for both analytes are between 98 and 102%.

3.5.4. Accuracy

The available solid dosage form of antianemic drugs was analyzed by proposed approach and by reference methods: AAS for iron determination and CE for ascorbic acid determination. It should be pointed out that in the presence of ascorbic acid iron exists in the form of Fe (II). The accuracy of the approach was evaluated by the comparison of the results receiving by both approaches. The obtained results represent no significant differences in iron (II) and ascorbic acid concentrations obtained by suggested and reference methods (Table 2). Student's *t*-test of statistical hypotheses about the equality of the means was used to test the equality of the means of the experimental data obtained by developed method and by the reference method. Hypothesis about the equality of the means of the experimental data obtained by the developed method and by the reference method was taken as the null hypothesis (H_0). Hypothesis about the difference of mentioned means was taken as an alternative hypothesis (H_1). The significance was taken equal to the 0.05 level. Based on the obtained results, the observed differences were not contrary to the hypothesis H_0 and the obtained discrepancies with the 0.05 significance level could be considered insignificant.

4. Conclusion

In comparison with the previously reported flow approaches (Kukoc-Modun et al., 2012; Lenghor et al., 2002; Molina-Diaz et al., 1998; Oliveira & Masini, 2001; Tesfaldet et al., 2004; Udnana et al., 2004) the flow system based on sandwich technique has been proposed for the simultaneous separate determination of iron (II) and ascorbic acid in antianemic pharmaceuticals for the first time. Taking into account high content of analytes (13–20%) in the pharmaceuticals the LOD values of the developed approach are quite satisfactory. The sample frequency of the developed approach is comparable with the previously reported flow approaches, because during the one flow cycle it is

Table 2
The results of the iron (II) and ascorbic acid determination in antianemic pharmaceuticals (F-critical = 19.0, t-critical = 4.3, P = 0.95, n = 3).

Drug	Labeled value, mg/tablets		Added amount, mg/tablets	The developed method, mg/tablets	AAS, mg/tablets	F-value	t-value	Recovery, %	The developed method, mg/tablets	CE, mg/tablets	F-value	t-value	Recovery, %
	Fe (II)	AA		Iron (II)					Ascorbic acid				
Sorbifer Durules	100	60	0	95 ± 5	96 ± 8	2.5	0.5	–	58 ± 4	57 ± 2	7.0	1.1	–
			2	96 ± 5	97 ± 7	1.1	0.3	99	61 ± 5	60 ± 6	1.6	0.7	101
			5	101 ± 5	100 ± 8	2.3	0.5	101	63 ± 5	62 ± 5	1.7	0.5	101
Fenules	55	50	0	58 ± 3	60 ± 5	4.3	1.8	–	66 ± 5	67 ± 3	5.2	0.5	–
			2	61 ± 4	62 ± 6	2.7	0.6	98	69 ± 4	70 ± 4	1.0	0.8	99
			5	64 ± 5	65 ± 6	1.3	0.8	99	72 ± 5	73 ± 4	4.0	0.8	99
Tardyferon	80	–	0	69 ± 4	70 ± 5	1.9	0.7	–	86 ± 4	87 ± 3	1.8	0.3	–
			2	72 ± 4	71 ± 4	0.7	0.5	101	88 ± 4	90 ± 5	1.3	1.3	102
			5	74 ± 5	75 ± 6	1.6	0.4	99	91 ± 4	93 ± 5	1.7	1.1	98

possible to analyzed two analytes. The proposed procedure is of great value in quality-control analysis of the developed pharmaceuticals, because it improves automation, miniaturization, simplicity, sensitivity, flexibility and low cost.

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