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Stepwise injection spectrophotometric determination of epinephrine

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ABSTRACT

Simple, rapid and fully automated methods for the manual and automated spectrophotometric determination of epinephrine have been developed by using schemes of stepwise injection (SWIA) and sequential injection analysis (SIA) implemented in the same manifold. The determination is based on the formation of reduced form of 18-molybdodiphosphate heteropoly anion by its reaction with epinephrine. Using of the reaction vessel in the general SWIA configuration instead of a holding and reaction coil in the SIA manifold provides several essential advantages, including higher sensitivity and lower reagent consumption. The linear dependence of the analytical signal on the epinephrine concentration was preserved over the range of 1.5–30, 3.0–30, and 1.5–25 μmol L^{-1} by using of SWIA, SIA and spectrophotometric analysis, respectively. The relative standard deviation for the SWIA determination of 10 μmol L^{-1} epinephrine was 1.8% (n=10).

1. Introduction

Flow-injection analysis (FIA) [1] and sequential injection analysis (SIA) [2–5] are the well-established automated methods used in the analysis of pharmaceuticals. However, both methods have certain drawbacks. At least a part of them are caused by used non-equilibrium approach involving registration of the analytical signal by flow detector in the non-equilibrium conditions. Dispersion of reactants and reaction products zones along the hydraulic routes results in decreased sensitivity of FIA and SIA methods in comparison with similar non-automated methods. Besides, FIA and SIA are less effective in the case of the automation of multistage or slow reactions or when the redesign of the manifold is necessary by changing from one application to another.

To eliminate the above mentioned disadvantages, methods limiting the dispersion of the analytical form were proposed, including FIA/SIA with a mixing chamber [6,7], using of an automated micro batch analyzer [8], segmented flow analysis [9], or stepwise injection analysis (SWIA) [10,11]. SWIA provides the widest possibilities of solving the problems of the analysis automation without loss in the sensitivity due to dispersion as well as unification of the design of hydraulic schemes.

The SWIA and SIA manifolds are close to each other in many aspects; both of them allow easy manipulation with the direction of the flow. The SWIA and SIA manifolds include reversible pump and multiposition switching valve in which several inlets are commutated to a single outlet. As opposite to the SIA manifold, holding and reaction coil are replaced in the SWIA configuration by the cylindrical reaction vessel (RV) with a funnel-shaped inlet at the bottom. Finally, one of the inlets of switching valve is connected to the atmosphere or to a gas container with an inert gas ensuring a possibility for the intense and effective mixing of a reagent and sample solutions in the reaction vessel. One of the main distinctions between SWIA and previously known FIA/SIA techniques consists in the change of diffusion mass transfer between sample and reagent zones to more effective convective stirring. Thus, an analytical signal is measured under conditions, when it reaches a maximum value in the given analytical procedure.

By using of a SWIA method, the optimization of the flow variables of the manifold can be greatly simplified because the parameters of the analytical method found in batch conditions can be used almost without any changes. The combination of the SWIA manifold with such modules as an auxiliary vessel or a sorption column allows effectively to preconcentrate the analyte. By the carrying out of the extraction process or the absorption of the gaseous species in the reaction vessel, the configuration of the system is significantly simplified [11–14]. The configuration of the flow system is in many situations more flexible than that for the FIA/SIA methods and shows greater analytical efficiency. New possibilities are given by the integration of the reaction vessel with a measurement cell. At the same time, SWIA essentially concedes to the known flow methods in throughput but this feature is not always asked for in real analysis.

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Epinephrine is employed in cardiac resuscitation and in veterinary medicine as a treatment for anaphylaxis. Because of its vasoconstrictive properties, epinephrine is also added to local anesthetics to retard systemic absorption and prolong effect. It works by narrowing the blood vessels, increasing blood pressure and blood glucose levels.

Several flow injection procedures have been reported for epinephrine determination using different detectors such as spectrophotometric [15–20], chemiluminescent [7,21–23], spectrofluorimetric [24] and potentiometric [25]. The numerous color reactions for adrenaline determination described in the literature can be divided in three groups: (a) those depending mainly on the formation of a red oxidation product; (b) those depending on the presence of the catechol grouping, (c) miscellaneous. The reaction of oxidation is slow and 15–30 min is necessary for the complete development of the coloration. Several oxidants such as permanganate [26]; iodine [20], metavanadate [27], periodate [28], bromine [29] and phenanthroline-iron(III) complex [30] have been proposed.

The heteropoly anions were among first colorimetric reagents proposed for the determination of epinephrine. Folin uric acid reagent 18-tungstophosphate heteropoly acid rapidly reacts with adrenaline in strongly basic solution [31]. In these conditions color produced fades quite rapidly because heteropoly complexes are unstable in basic medium due to progressive destruction of the reagent by the alkaline hydrolysis [32]. In such conditions reaction is not specific. Besides uric acid, most easily oxidizable substances, particularly aromatic polyhydroxy compounds, give some blue color [33]. The reaction is much more sensitive than that based on the formation of orange adrenochrome.

The purpose of this work was to develop a simple, rapid and fully automated methods for the routine manual and automated spectrophotometric determination of epinephrine by using schemes of stepwise injection and sequential injection analysis. The determination is based on the formation of reduced form of recently proposed reagent – 18-molybdodiphosphate heteropoly anion (18-MPA) – by its reaction with epinephrine [34,35].

2. Materials and methods

2.1. Reagents and solutions

The procedure for the synthesis of ammonium salt of 18-MPA described in [36] was modified as follows. Dissolve 100 g of $Na_2MoO_4 \times 2H_2O$ in 400 mL of H_2O, add 15 mL of 85% H_3PO_4 and 80 mL of concentrated HCl. Boil for 8 h with return condenser. When the solution turns from yellow to green due to reduction, add several mL of 3% H_2O_2. After cooling add sufficient solid NH_4Cl assuring the precipitation of as much as possible of light-yellow ammonium salt of $PMo_{12}O_{40}^{3-}$. Usually, depending on the quantity of the 12-molybdophoshate HPA formed, about 20–30 g of NH_4Cl is enough to ensure complete separation of 18-MPA from the main impurity. The yellow crystalline precipitate, after settling, is filtered on a Buchner funnel and discarded. To the clear solution add enough solid NH_4Cl (~100 g) to saturate the solution. Every portion of the NH_4Cl added must have been dissolved before adding a new one. To do this, the solution can be slightly heated (40–50 °C). The precipitated orange ammonium salt of 18-MPA is filtered on a Buchner funnel and sucked as dry as possible. The obtained preparation can be usually used without further purification. Yield: ~40 g.

0.01 mol L^{-1} solution of 18-MPC is prepared by dissolving 0.7855 g of the synthesized salt and diluting to 25 mL with distilled water. If there is a small insoluble residue, filter the solution. The stock solutions of 2.5×10^{-3} mol L^{-1} epinephrine nitrate (Sigma-Aldrich) was daily prepared by dissolving accurately weighed amounts in 0.01 mol L^{-1} hydrochloric acid, and stored in a refrigerator. 0.1 mol L^{-1} sodium phosphate buffer (pH=7.0) was used for adjusting the pH of the samples to an optimum value.

2.2. Apparatus

Absorption spectra and absorbances were measured using a SF-26 (LOMO, Russia) and SHIMADZU UVmini-1240 (Shimadzu Scientific Instruments, Japan) spectrophotometers equipped with 10 or 50 mm light-path cell. The pH of solutions was measured using an EV-74 ion meter (ZIP, Homel, Belarus) equipped with glass and Ag/AgCl reference electrodes.

The flow systems for the SIA (Fig. 1(a)) and SWIA (Fig. 1(b)) determination of epinephrine were based on flow injection analyzer PIAKON-30-1 (Rosanalit, Saint-Petersburg, Russia). They included a bidirectional peristaltic pump ensuring a reverse flow, a six-port titanium valve, a 50 mm optical Z-flow through cell, and communication tubes (PTFE, 0.5 mm in inner diameter). A tungsten light source and a USB650 UV–VIS fibre optic CCD detector (OceanOptics, USA) were connected to the flow system via 600 μm i.d. optical fibres having SMA connectors.

In the case of exploitation of the analyzer in SIA mode (Fig. 1(a)), it included a holding coil (HC) (diameter 10 mm, length 40 mm) and reaction coil (diameter 10 mm, length 45 mm). In the other case, by exploitation of the analyzer in SWIA mode (Fig. 1(b)), the system included a reaction vessel (RV) which had cylindrical shape and was funnel-shaped at the bottom (glass tube 350 mm in height and 10 mm in inner diameter). The analyzer was controlled by the homemade programme written in QuickBasic language.

2.3. Spectrophotometric procedure for the determination of epinephrine

In a 25 mL volumetric flask, 1 mL of 2.5 mmol L^{-1} 18-MPA, 1 mL of 0.1 mol L^{-1} phosphate buffer, and the required amount of epinephrine were mixed. The flask was then filled with distilled water to the mark. Absorbance was measured after 5 min at 820 nm against water in a 50 mm glass cell.

2.4. Procedure for the SWIA determination of epinephrine

At the first stage of the measurements, the components of the reaction mixture are sequentially delivered through the ports of multiselection valve into reaction vessel in the following order: 170 μL of 0.2 mmol L^{-1} 18-MPC (port **a**), 130 μL of sample solution (port **b**), 210 μL of phosphate buffer with pH 7.0 (port **c**). To stir the reaction mixture, a flow of argon gas was passed through the port **d** at a rate of 6 mL min^{-1} into the reaction vessel for a 60 s. Then the reaction mixture was moved at 30 μL s^{-1} from the reaction vessel into the photometric detector flow cell through port **e** by reverse movement of the peristaltic pump. The absorbance is measured under stopped-flow conditions for a 15 s and solution is discharged to waste. The system lines, reaction vessel and flow cell are washed with distilled water and argon gas using port **f, d** and **e**. At the second stage, the described sequence of the operations is repeated with the exception that distilled water is pumped instead of sample solution. The absorbance of the blank is measured. The difference between the absorbances measured at the second and first stages is used as analytical signal. The sequence and time of performing all steps of analysis were set by the program as a matrix and presented in Table 1.

2.5. Procedure for the SIA determination of epinephrine

Before the carrying out the measurement, the system is washed and filled with phosphate buffer used as carrier solution. The

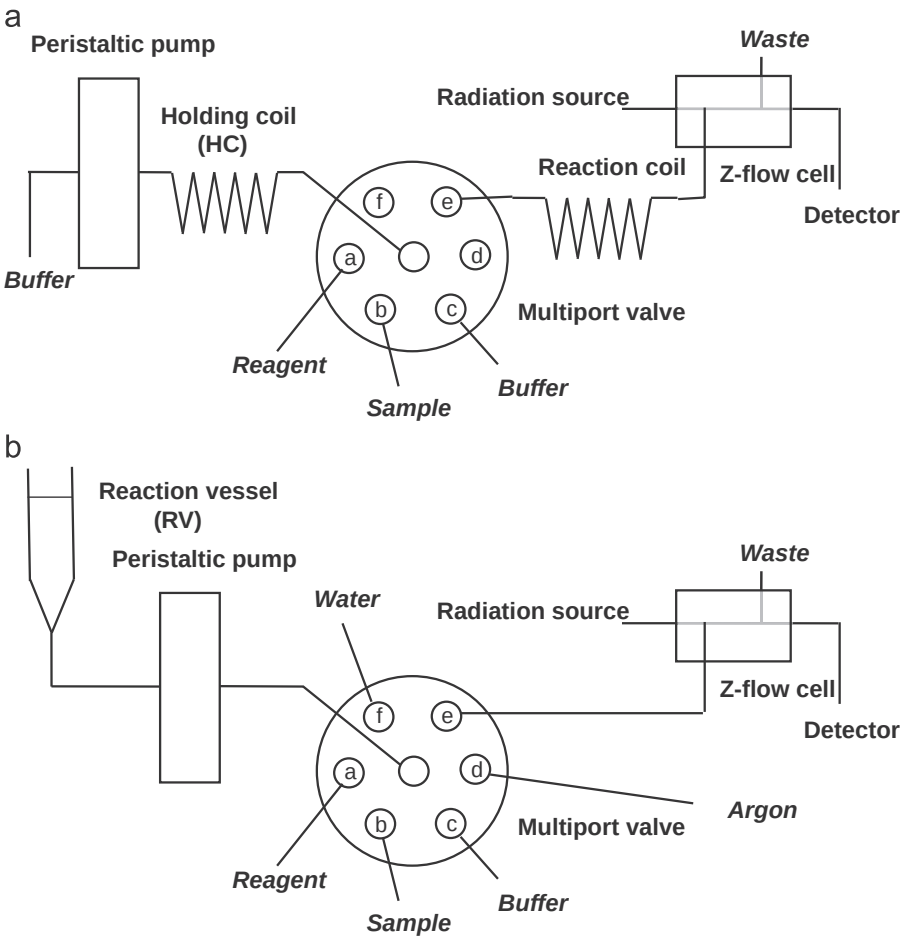

Fig. 1. The schemes of the manifold for SIA (a) and SWIA (b) determination of epinephrine.

analytical cycle begins by aspirating 170 μL of 1 mmol L^{-1} 18-MPA solution into the holding coil through port **a** of the multiselection valve. Then 130 μL of sample solution and 220 μL of carrier solution are injected into the holding coil through the ports **b** and **c**, respectively. The entire volume is moved by peristaltic pump in counterclockwise direction at 22 μL s^{-1} through port **e**, reaction coil and then through flow cell. At this stage, the response signal is measured at 820 nm by detector and system is washed with phosphate buffer solution. Signal of the detector corresponding to a baseline is measured using the same sequence of the operations. For this purpose, distilled water used in place of a sample solution. Analytical signal is measured as a concentration peak. The sequence and time of performing each step of analysis were set by the program as a matrix and presented in Table 1.

3. Results and discussion

3.1. Color reaction and optimization of chemical variables

Recently, the applicability of the Wells-Dawson polyoxometallate $P_2Mo_{18}O_{62}^{6-}$ for the determination of ascorbic acid in batch and sequential injection systems was shown [34,35]. Further study

Table 1
Sequence of the steps in the optimized control programs for SWIA and SIA determination of epinephrine.

Time (s)	Volume (μL)	Valve position	Direction of the flow[a]	Measurement of the absorbance	Description of the operations
SWIA					
8	240	a	+1	Off	Filling of RV with 18-MPC
6	180	b	+1	Off	Filling of RV with sample solution
10	300	c	+1	Off	Supply of buffer solution into RV
60	–	d	+1	Off	Stirring of the reaction mixture with argon
30	900	e	−1	On	Moving of the reaction mixture into the flow cell
15	450	f	+1	On	Absorbance measurement. Washing of the RV with distilled water
30	–	d	+1	Off	Stirring with argon
40	1200	e	−1	Off	Washing of the flow cell
SIA					
8	170	a	+1	Off	Filling of HC with 18-MPC
6	130	b	+1	Off	Filling of HC with sample solution
10	220	c	+1	Off	Supply of buffer solution into HC
25	550	e	−1	On	Absorbance measurement. Washing of the system with buffer solution

[a] −1, +1 refer to the clockwise and counterclockwise rotation of the pump, respectively.

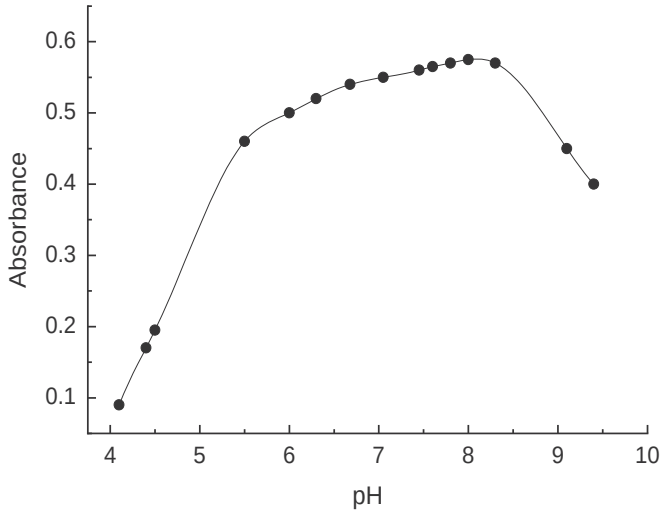

Fig. 2. Dependence of the absorbance of heteropoly blue formed in the reaction between epinephrine and 18-MPC on the pH of the solution. C(Ep)=2×10^{-5} mol L^{-1}, C(18-MPC)=8×10^{-5} mol L^{-1}, pH=7.0; λ=820 nm, *l*=1 cm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

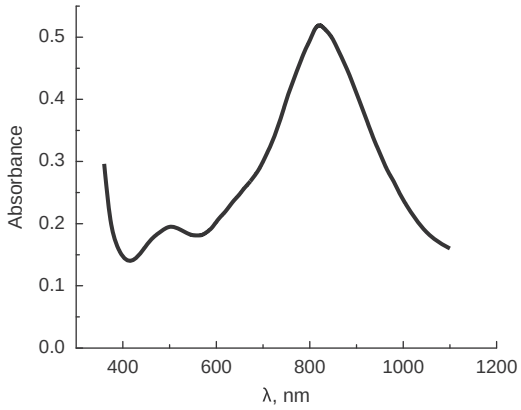

Fig. 3. Absoprtion spectrum of heteropoly blue obtained by reduction of 18-MPC with excess of epinephrine. C(18-MPC)=4×10^{-5} mol L^{-1}, C(Ep)=2×10^{-4} mol L^{-1}, pH=6.86, *l*=1 cm.

has demonstrated that catecholamines can be successfully determined using 18-MPA.

The completeness and the rate of the reaction between 18-MPA and epinephrine strongly depend on the solution pH (Fig. 2). At pH ≥4.0, the redox potential of the 18-MPA/$P_2Mo_2{}^{V}Mo_{16}{}^{VI}O_{62}{}^{8-}$ couple remains constant and is equal to 0.57 V. At the same time, redox potential of epinephrine/adrenochrome system becomes progressively more negative with increase in the pH. Beginning from pH 6.0, the power of the epinephrine as reducer becomes so high that its reaction with 18-MPA goes to the completion. The region from the pH 6 to pH 8 is favorable for the analytical reaction.

The rate of the reaction between epinephrine and 18-MPA should be taken into account. At pH <6.5 the reaction is rather slow. Though at the pH >7 the reaction is fairly rapid, the absorbance of the solution is slowly decreases in the time. The rate of the hydrolytic destruction of 18-MPA becomes inadmissible high at pH >8. In such conditions, the concentration of the 18-MPA left in the solution is already insufficient for the complete oxidation of the epinephrine. Considering both rates of the reactions of formation of heteropoly blue and destruction of the reagent, pH 7.0 was chosen as optimal. At this pH, the formed colored product is stable at least during 30 min.

Stoichiometry of the reaction was studied using the molar ratio method. The change in the absorbance of heteropoly blue was measured using constant concentration of 18-MPA and varying concentration of epinephrine, and vice versa. In both cases, only one intersection point was found on the experimental saturation curve at the ratio of 2 mol 18-MPA to 1 mol epinephrine. Then the absorbance remained constant even in the big excess of epinephrine.

The spectrum of heteropoly blue obtained in the reaction between epinephrine and 18-MPC has the absorbance maximum at λ=820 nm (Fig. 3). The shape of the basic band and its position indicate that two-electron heteropoly blue is formed at any studied ratio of the reactants. It should be mentioned that more deeply reduced heteropoly blues can be obtained by reduction with other reducing agents. Four- and six-electron heteropoly blues were obtained by the reduction of 18-MPC with excess of ascorbic acid [34] or chromium(II) [37], respectively. The molar absorptivity of two-electron reduced form of 18-MPC is equal to 1.3×10^4 mol^{-1} L cm^{-1} at 820 nm. In correspondence with this, molar absorptivity for the epinephrine calculated from the slope of the calibration curve was equal to 2.18×10^4 mol^{-1} L cm^{-1}. This value is significantly higher than molar absoprtivities calculated at the maximum absorbance for adrenochrome (ε_{490}=4.05×10^3 mol^{-1} L cm^{-1} [38] or complex of adrenaline with Fe(II) (910 mol^{-1} L cm^{-1} [15]).

Such stoichoimetry of the reaction is consistent with generally accepted scheme for the reaction of epinephrine with most of oxidizing agents [39]. It includes the formation of adrenochrome at the final stage of the reaction and can be formulated by the following equation (Fig. 4).

The formation of the adrenochrome is confirmed not only by the fact that the epinephrine acts as four-electron reducer in the reaction but also by the presence of the band with maximum at approximately 500 nm in the spectrum of the mixture obtained by the oxidation of epinephrine with 18-MPC (Fig. 3). The presence of this band can be used to check if the other reducers or oxidants besides epinephrine are present in the solution. The ratio of absorbances at 820 and 500 nm should be equal to 2.60 in the absence of interferents.

3.2. SWIA and SIA procedures: Optimization of manifold parameters

Typical feature of the optimization process used in the flow methods is the fact that the optimization of the chemical variables should be repeated again even if such parameters were found previously when the corresponding manual spectrophotometric procedure was developed. In addition, the search of optimal values is as a rule more complex than for the batch methods, often needing the multivariate optimization to be carried out. The found optimal ranges of the variables are usually narrower. These features make the developed method less reliable. In the present work, the chemical variables were primarily found during the development of the batch spectrophotometric procedure and then used almost without any changes in SWIA procedure.

The concentration of 18-MPA used in spectrophotometric and SWIA procedures can be varied in a wide range. Two conditions have to be taken into account. On the one hand, it is necessary to limit the amount of the reagent used. On the other hand, the range of analyte concentrations determined by the given procedure should be extended as much as possible. As a compromise value, concentration of 0.2 mmol L^{-1} of 18-MPA was chosen.

The time of mixing of reagents with a flow of argon gas should be enough to completely mix the reactants in the reaction vessel

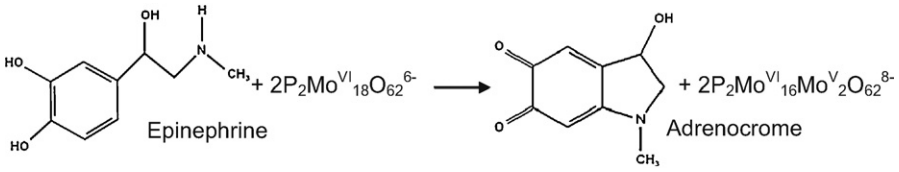

Fig. 4. Schematic representation of the reaction between epinephrine and 18-MPC. The intermediates adrenalinequinone and leucoadrenochrome are not shown.

Table 2
Figures of merit for epinephrine determination by SWIA, SIA and spectrophotometric methods.

Parameter	SWIA method	SIA method	SP method
pH	7.0	7.0	7.0
18-MPA concentration (mmol L^{-1})	0.20	1.0	0.10
Volume of sample (μL)	180	130	150–2500[a]
Volume of 18-MPA reagent (μL)	240	170	2500
Time of stirring with argon in RV (s)	60	–	–
Flow rate through the detector (μL s^{-1})	30	22	–
Linear range (μmol L^{-1})	1.5–30	2.8–30	1.5–25
Calibration graph			
Intercept	0.037	0.021	−0.015
Slope (L μmol^{-1})	0.0289	0.0125	0.109
Correlation coefficient	0.984	0.992	0.996
Detection limit (μmol L^{-1})	0.5	0.9	0.5
Quantification limit (μmol L^{-1})	1.5	2.8	1.5
RSD (%) (n=10)[b]	1.8	1.2	2.7
Sampling frequency (per hour)	20	73	10

[a] 0.25 mmol L^{-1} solution of epinephrine.
[b] 10 μmol L^{-1} of epinephrine.

and to make the reaction go to completion. The constant value of the absorbance is reached after 60 s of stirring with argon in the studied system.

Optimization of instrumental variables (volume of injected sample, buffer and reagent, flow rate) for the SWIA manifold operated in the SIA mode was performed using the univariate optimization procedure. The optimum values finally chosen for the manual and both flow methods developed are compared in Table 2. The order of mixing of reactants is important in SIA method. The obtained signal is much higher when solution of 18-MPA was injected first in holding coil and only after that the sample solution. For the general SWIA method, the order in which the species are mixed in the RV has no influence on the final absorbance.

Typical recorder outputs obtained by SWIA and SIA methods are shown in Fig. 5. A stopped flow is the preferred method for analytical signal measurements in SWIA. Thus, the analytical signal measured in the SWIA procedure corresponds to the difference between average outputs of sample and blank solutions. In the

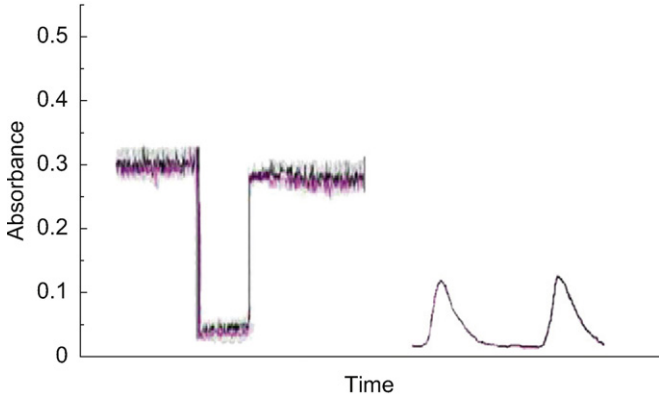

Fig. 5. The typical recorder outputs obtained by the proposed SWIA (a) and SIA (b) methods.

Table 3
Influence of some interfering species on the determination of 10 μmol L^{-1} of epinephrine.

Species	Tolerable concentration (mol L^{-1})
Na^+, K^+, Mg^{2+}, Ca^{2+}	0.1[a]
NO_3^-, SO_4^{2-}, PO_4^{3-}, Cl^-	0.1[a]
Cu^{2+}, Fe^{2+}	8×10^{-5}
$Na_2SO_3{\cdot}H_2O$	7×10^{-3}[a]
$Na_2S_2O_3{\cdot}5H_2O$	3×10^{-3}[a]
$KNaC_4H_4O_6{\cdot}4H_2O$	7×10^{-3}[a]
Saccharose, paracetamol	0.01[a]
Nicotinic acid, caffeine	0.01[a]
Uric acid	5×10^{-5}
Citric acid	7×10^{-3}[a]
Salicylic acid, acetylsalicylic acid	7×10^{-3}[a]
Norepinephrine, methyldopa	2×10^{-6}
Ascorbic acid, cysteine	2×10^{-6}

[a] Highest studied concentration of the interferent.

SIA mode ordinary concentration peak is registered. Average value of the absorbance for the blank solution is subtracted from the absorbance measured in the peak maximum and taken as analytical signal.

3.3. Calibration graphs, interference study and application

The optimized chemical and physical parameters, analytical performance of the proposed methods including linearity ranges, the equations for the dependence of the recorded analytical signal on the concentration of epinephrine (in mol L^{-1}), detection limits, sampling frequency, and precision are summarized in Table 2. The detection limit was defined as the concentration of epinephrine yielding an analytical signal equal to three-times the standard deviation of the blank absorbance.

The influence on the epinephrine determination of some compounds possessing reducing properties, including those having phenolic, carboxylic, and hydroxylic groups, as well as common excipients was studied in synthetic mixtures (Table 3). No interferences were found for saccharose, acetylsalicylic acid, paracetamol, nicotinic acid, caffeine, and common excipients (sodium chloride, EDTA, magnesium stearate, lactose, talc and starch) at ratios [interferent]/[epinephrine] much higher than those found commonly in pharmaceuticals. At least 10-fold excess of inorganic reducers such as Fe(II) or Cu(II) had no influence on the determination of adrenaline. The monopenols with the exception of most active species such as *p*-aminophenol show no interference. Most serious interference is caused by the presence of polyphenolic compounds, ascorbic acid, uric acid. Cysteine rapidly reacts with 18-MPA but it can be effectively masked with formaldehyde. All standard local anaesthetics contain the preservative sodium bisulfite or metabisulfite which strongly interfere to the adrenaline determination. It was found that by passing the sample solution through the cation ion-exchange column in the Na^+ form this interferent is completely removed. Another possibility for the removing of the bisulfite can be realised by using an SWIA analyzer. Sulfur dioxide is completely removed from the solution by passing of the flow of the inert gas through acidified solution of the sample.

Table 4
Assay of some drug forms (mg of epinephrine/ampule±confidence intervals of the method for n=7 and 95% confidence level).

Drug	Nominal value	SWIA method	SIA method	SP method	Reference method [39]
Adrenaline hydrochloride	1.00	0.95±0.08	0.96±0.04	1.04±0.07	0.987±0.014
Adrenaline hydrotartrate	1.82	1.75±0.04	1.80±0.05	1.79±0.03	1.794±0.011

Two pharmaceutical formulations were commercially obtained at a local drug store, including adrenaline hydrochloride (〈Moscow Endocrine Plant〉, Russia) and adrenaline hydrotartrate ("Health", Kharkov, Ukraine) in 1 mL ampules. The epinephrine concentrations were determined using all three proposed methods and reference method based on the oxidation of epinephrine to adrenochrome with iodine [40] (Table 4). In all instances studied, there was no significant difference between the results of the analysis of epinephrine by three suggested methods and content provided by the referenece method, thus confirming the accuracy of the developed methods (Table 3). In addition, the results of the analysis correspond well with the claimed values of producers. The method is characterized by acceptable precision. The relative standard deviation for ten successive measurements of 10 μmol L^{-1} adrenaline was 1.8%. A sample throughput of 73 h^{-1} achieved for the SIA method is much higher than that for the SWIA method (20 h^{-1}) but in SWIA method the significant saving of the reagent is attained in view of the fact that lower reagent concentration is used in this case.

4. Conclusions

Simple, rapid and highly sensitive spectrophotometric and SWIA methods for the determination of epinephrine have been developed by using the newly proposed reagent 18-molybdodiphosphate heteropoly anion.

Utilization of reaction vessel in the general SWIA configuration instead of a holding coil has as a result several essential advantages. In the optimal conditions, the absorbance obtained by SWIA method running in general mode was at least twice higher as compared to that achieved in the SIA mode. Though the reagent volumes used in both procedures are comparable, the optimal concentration of 18-MPA exploited in general SWIA method is five-times lower. Thus, the amount of the reagent used for a determination is strongly reduced. Due to high mass concentration of the reagent used, Schlieren effect is quite big in the studied system by using the manifold in SIA mode limiting the sensitivity and reproducibility. On the contrary, this effect was completely excluded by working in the usual SWIA configuration. At the same time, by using SWIA method, most of the advantages of the SIA method are retained, including easy manipulation by the direction of the flow, simple reconfiguration of the system for implementation of the new task, full automation of the analytical procedure. The sampling frequency of the method by using SWIA manifold in SIA mode is much higher −73 to 20 determinations per hour.

Acknowledgement

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.03.059.

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