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Determination of bopindolol by sequential injection technique with spectrophotometric detection

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Abstract

In the proposed procedure, the determination of bopindolol using a sequential injection technique (SIA) with spectrophotometric detection at 560 nm is described. The new method of determination is based on the color reaction of the indole group in the molecule of bopindolol with 4-dimethylaminobenzaldehyde (Ehrlich's reagent) in acidic medium with production of a violet water-soluble complex. Due to the kinetic standpoint of reaction, the ''stopped flow'' technique with mixing coil between the valve and detector was tested and optimized. The proposed SIA system was used for the direct determination of bopindolol in tablets, negative effects of interfering substances (excipients of tablets) were not observed. The selectivity of the proposed method of determination was tested in the presence of seven interfering substances from the group of β-blockers with good results. The interference effect was observed only in the presence of pindolol. The sample throughput with stopped flow technique was 40 samples per hour. Bopindolol was determined in the linear range from 1 to 10 μg ml^{-1}, RSD was less than 1% ($n = 10$), with limit of detection (3σ) 0.1 μg ml^{-1} and limit of quantification 0.5 μg ml^{-1}. Obtained results were compared with conventional HPLC method, both analytical techniques were in good agreement.

Keywords: Sequential injection analysis; Stopped flow technique; Bopindolol; 4-Dimethylaminobenzaldehyde

1. Introduction

Sequential injection analysis (SIA), developed by Ruzicka and Marshall in 1990 [1], represents advanced form of solution manipulation available to analytical chemists for mixing and transport of samples, reagents and products of chemical reactions to the measurement point. The volume of reagents used, and thus produced waste, is decreased compared to conventional analytical techniques (FIA, LC, HPLC). The SIA response is a result of two processes, both kinetic in nature, physical process of dispersion of the sample zone within the carrier stream and chemical process of formation of chemical species. These two processes occur simultaneously, and yield, together with dynamic characteristics of detectors, the typical SIA response.

Bopindolol (4-[2′-benzoyloxy-3′-(*tert*-butylamino) propoxy]-2-methylindole), depicted in Fig. 1, belongs to the group of beta-blockers and acts as a slowly dissociating β_1-adrenergic receptor antagonist. Beta-blockers are drugs reducing heart workload and lower blood pressure. They are commonly used to relieve angina, to treat congestive heart failure or high blood pressure (hypertension). Bopindolol is clinically useful in the treatment of hypertension and this drug is a prodrug, which is metabolized to benzoic acid and the non-selective β-adrenoreceptor antagonist ''hydrolyzed bopindolol''. This metabolite is further converted into active metabolite 4-(3-*t*-butylaminopropoxy)-2-carboxyl indole, by oxidation. The anti-hypertensive effects of bopindolol are in part indebted to these metabolites through a strong β-blocking action [2,3]. Pharmaceutical formulations contain low amount of bopindolol (usually about 1 mg per tablet). The determination of bopindolol has been rarely studied. There is only limited number of related works, e.g. determination of bopindolol meta-

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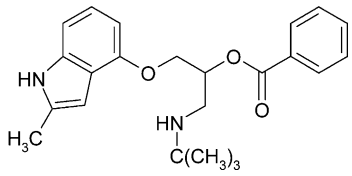

Fig. 1. Chemical structure of bopindolol.

bolite by HPLC [4–6]; enantiomeric separation of bopindolol [7]; separation of bopindolol, its precursor and degradation product by supercritical-fluid chromatography [8] and its isotachophoretic determination [9]. Detection techniques applied for bopindolol assays are fluorescence [4], electrochemical detection [5], coulometry [6], UV spectrophotometry [7] or conductimetry [9].

Some drugs from the group of the beta-blockers are native fluorescent compounds. The fluorescence detection according Oddie et al. [4] for sequential injection application was tested, but only metabolite of bopindolol was native fluorescent. Bopindolol showed only very poor fluorescence in acidic medium (HCl, H_2SO_4 or H_3PO_4) and the fluorescent product was very unstable (change of the fluorescence response of the standard solution within the day).

Some beta-blockers produce a green, water-soluble complex with Fe (III) chloride in acidic medium with a maximum absorbance (λ_{max}) at 635 nm. This reaction was previously applied for the determination of pindolol [10,11]. There was observed that also bopindolol produces a similar green complex with Fe (III). This reaction was test to use it in the flow system and there was found out detection limit in the range of 70–100 μg ml^{-1}.

The possibility for the determination of low amount of bopindolol in pharmaceuticals with sufficient selectivity and sensitivity was to find appropriate derivatization reaction. The different reagents for amino-group derivatization were tested and the reaction with 4-dimethylaminobenzaldehyde (Ehrlich's reagent–reagent usually used for derivatization of secondary aromatic amines) was found to be the most suitable. The same reaction was described Banerjee [12] for batch spectrophotometric determination of pindolol. Highly cited determination was based on drastic reaction conditions (sample mixed with 0.007% 4-dimethylaminobenzaldehyde solution in anhydrous acetic acid–HCl, 17:3, (v/v) and heated on a boiling-water bath for 1.5 min), which were difficult to carry out in the SIA system.

Our working strategy was the modification of the mentioned reaction and then the automatization of the method for using in the sequential injection technique. The optimized method was applied for quick and precise determination of bopindolol in a pharmaceutical formulation containing 1 mg of the drug in one tablet.

2. Experimental procedures

2.1. SIA apparatus

An overall schematic view of the sequential injection system is shown in Fig. 2. A FIAlab® 3000 system (FIAlab® Instruments, USA) is a commercially produced instrument and consists of a syringe pump (syringe reservoir 2.5 ml) and an 8-port selection Cheminert valve (Valco Instrument Co., USA). FIAlab® 3000 was equipped with fiber-optic UV–vis diode array detector USB 2000 (Ocean Optics, Inc., USA) connected with SMA flow Z-cell (10 mm optical path length designed to minimize bubble entrapment during flow measurements) and visible light source LS-1 tungsten halogen lamp (Ocean Optics) was employed for detection step. The whole SIA system was controlled by the latest version of program FIAlab for Windows 5.0. All connections and reaction coils (140 and 120 cm) were made of a 0.75 mm i.d. PTFE tubing.

2.2. HPLC apparatus

HPLC determination was chosen as a comparative technique. The HPLC system consisted of LCP 4100 pump (Ecom Prague, Czech Republic), Waters 717 plus autosampler, Waters 486 UV detector and controlled by CSW software v. 1.7 for Windows. Stationary phase was end-capped Purospher RP-18 column (LichroCART, 125 mm, 4 mm, 5 μm) with pre-column of the same material (LichroCART, 4 mm, 4 mm, 5 μm).

2.3. Chemicals

All solutions were prepared from analytical grade chemicals and a Millipore Milli-Q RG ultra pure water. Stock solution 1.00 mg ml^{-1} of bopindolol (Leciva a.s., Czech Republic) was prepared dissolving the appropriate amount in methanol. Working standard solutions were prepared by dilution of the stock standard solution with water. Stock solution of 10% of 4-dimethylaminobenzaldehyde (Chemapol, Czech Republic) was prepared dissolving of 1.00 g of reagent in 5 ml of 96% (v/v) ethanol and adding 10% (v/v) H_2SO_4 to 10 ml. Working solutions of Ehrlich's reagent were prepared by dilution of the stock solution with the carrier solution. The carrier stream was a mixture of 5% (v/v) of ethanol and 1% (v/v) H_2SO_4 in distilled water. All the solutions were filtered and degassed under reduced pressure.

2.3.1. SIA procedure

The SIA system described in Section 2.1 was used for automated aspiration of the optimized volume of standards and samples; the reagent and sample zones were dispensed by flow reversal and stopped in the holding coil; after defined time the zone of reaction

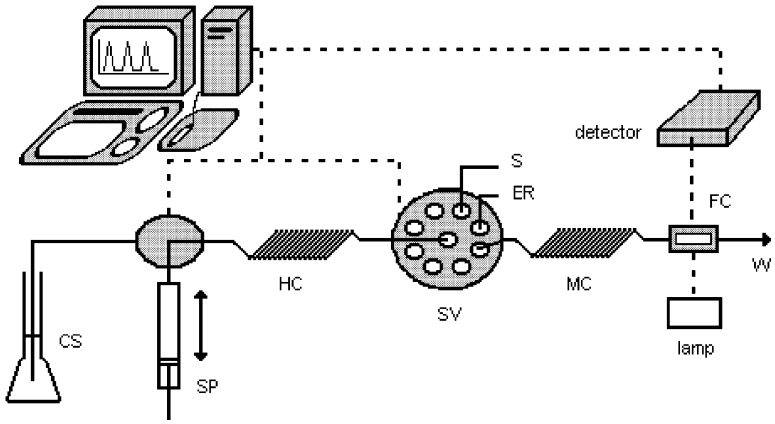

Fig. 2. Scheme of SIA set-up for spectrophotometric determination of bopindolol. CS, carrier stream; SP, syringe pump; HC, holding coil (140 cm); SV, selection valve; MC, mixing coil (120 cm); W, waste; FC, flow cell; ER, Ehrlich's reagent; S, sample or standard solution.

product was dispensed through the flow cell of the spectrophotometric detector. The resulting signal was recorded in the form of peaks, the peak heights were calculated automatically and data were stored by the computer for subsequent processing.

Standards and samples were measured in triplicates and the mean peak height values were used for data acquisition. The typical sequence of particular steps of the programme is mentioned in Table 1.

Table 1
The sequence of particular steps of the SIA control programme for bopindolol determination (a single cycle)

Unit	Command	Parameter	Action
Syringe pump	Valve position IN		Set valve position
Syringe pump	Set flow rate (μl s^{-1})	100	
Syringe pump	Aspirate (μl)	1500	
Syringe pump	Valve position OUT		Set valve position
Multi-port valve	Set port position	3	
Syringe pump	Set flow-rate (μl s^{-1})	50	
Syringe pump	Aspirate (μl)	80	Reagent aspirated
Multi-port valve	Set port position	2	
Syringe pump	Aspirate (μl)	100	Sample aspirated
Multi-port valve	Set port position	3	
Syringe pump	Aspirate (μl)	80	Reagent aspirated
Multi-port valve	Set port position	8	
Syringe pump	Set flow-rate (μl s^{-1})	50	
Syringe pump	Dispense (μl)	100	Mixing
Syringe pump	Aspirate (μl)	100	Mixing
Syringe pump	Delay (s)	10	Syringe pump stopped
Syringe pump	Empty syringe		Mixing+detection

2.3.2. HPLC procedure

Composition of mobile phase was optimized to the mixture of methanol and water 80:50 (v/v) with pH adjustment to 4.1. Sample volume was 20 μl, flow rate 0.5 ml min^{-1} and detection wavelength 264 nm. Standard and samples of tablet mass were prepared under the same conditions as for the SIA determinations. All samples were measured in triplicate.

2.4. Determination in pharmaceutical formulation

The pharmaceutical formulation analyzed was Sandonorm® 1 mg tablets (Leciva a.s., Czech Republic). Determination of the active substance in tablets was done by weighting 10 tablets (calculation of average weight of one tablet), crashing the tablet mass and, using an average weight of one tablet, dissolving it in water to get the concentration of 5 μg ml^{-1}. For the sample dissolution, 10 min of ultrasonic bath and 5 min of centrifugation (3000 rpm) were used. The supernatant was then injected directly to the flow system and analyzed.

3. Results and discussion

3.1. Optimization

The detection wavelength was optimized with respect to the absorption maximum of the reaction product of bopindolol with Ehrlich's reagent. Detection wavelength was chosen using diode array spectrum mode of the flow spectrophotometer. This mode enabled to see the optimum wavelength for determination in real conditions of flow measurement (scanning the whole spectrum during the sample position in the flow cell of the UV–vis detector). The other parameters optimized were: flow rate, time of reaction, number of mixing in flow reversal mode, volume of sample, concentration

and volumes of the zones of Ehrlich's reagent and composition of the carrier stream. The univariant method of procedure optimization was used in our study.

The main parameters to choose the optimal values were maximum response of the detector, minimum noise of the baseline and relative standard deviation of the respective measurement. Ranges and optimal values of volume of the reagent (relating to the sample volume in the mode of *reagent–sample-reagent*), flow rate through the detector and concentration of the reagent are mentioned in Table 2 and Fig. 3. The concentrations of Ehrlich's reagent tested were in the range of 0.5–4.0%. The experiments tested out of this range resulted in low analytical response due to the low concentration of the reagent or low solubility of the reagent in the carrier stream (concentration of the reagent $>5\%$). The best analytical signal was observed for 2% Ehrlich's reagent concentration (Fig. 3).

Due to the kinetic standpoint of reaction, the "stopped flow" technique with mixing of sandwiched zones in holding coil to prolong the reaction time and to improve reaction conditions was tested and optimized. Table 3 shows several experiments for optimization of reaction time. The mixing was performed dispense of 200 μl volume of the syringe pump by reverse direction. The reaction time of 10 s with one mixing in holding coil was chosen as optimal for SIA bopindolol determination, because the best signal of bopindolol was obtained. On the other side, the experiments showed that more important parameter to get improved sensitivity is mainly the total time of the reaction and the mixing was used to accelerate the reaction. The number of mixing in holding coil more than 3 was meaningless, because the zone of product was diluted and it was resulted in the decrease of the signal.

The carrier stream was a mixture of 5% (v/v) of ethanol and 1% (v/v) H_2SO_4 in distilled water. This composition of the carrier stream was applied to achieve better stability of the baseline. In the case of the water carrier stream, the false positive peak was observed after injection of blank sample zone. This phenomenon was caused by disturbing effect of the refractive index between zone of reagent and carrier. Good stability of the baseline was achieved using the almost identical background in the carrier stream and reagent. The second possibility to decrease the noise of the baseline was possible by incorporating of mixing coil in front of the detection point.

Table 2
Parameters and optimal values for SIA spectrophotometric determination of bopindolol optimized by the univariant method

Variable	Range	Optimum values
Wavelength (nm)	400–800	560
Flow rate through the detector (μl s^{-1})	30–70	50
Volume of the sample	10–100	100
Volume of the reagent	10–100	80
Concentration of Ehrlich's reagent (%)	0.5–4.0	2

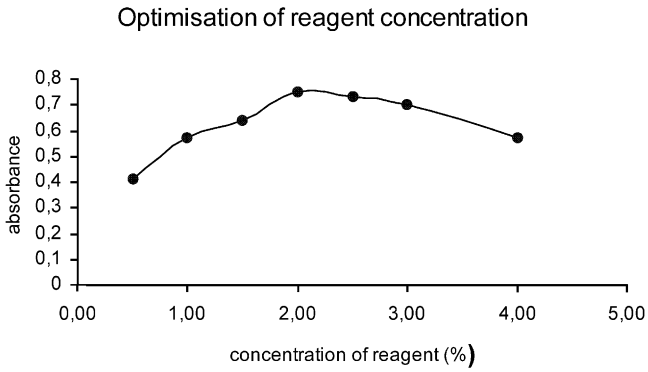

Fig. 3. Optimization of Ehrlich's reagent concentration. The optimization measurement was carried out under the following conditions: concentration of bopindolol 20 μg ml^{-1}, in the mode of 40 μl of reagent–50 μl of sample–40 μl of reagent, time of reaction was 10 s, and flow rate through the detector 50 μl s^{-1}.

Table 3
Optimisation of the reaction time

Reaction time (s) without the mixing	Number of mixing	Response of the detector (absorbance)
0	0	0.15
5	0	0.28
10	0	0.61
10	1	0.62
10	2	0.61
20	2	0.60
30	2	0.58

The optimization measurement was carried out under the following conditions: concentration of bopindolol 20 μg ml^{-1}, in the mode of 40 μl of reagent–50 μl of sample–40 μl of reagent, mixing was carried out inside the holding coil in reverse flow mode.

3.2. Calibration

Calibration experiments were carried out with six standard solutions of following concentrations of bopindolol: 10, 5, 3, 2, 1 and 0.5 μg ml^{-1}. Each standard solution was measured in triplicate. The calibration graph was described by the following equation $A = 0.0878c + 0.1104$ (where A is the absorbance and c is bopindolol concentration) the correlation coefficient was 0.9998. The linear range of the calibration was 0.5–10 μg ml^{-1} with limit of detection (3σ) 0.1 μg ml^{-1} and limit of quantification 0.5 μg ml^{-1} for 100 μl sample injection.

The repeatability of bopindolol determination was measured at three concentration levels—0.1, 1 and

Table 4
Bopindolol assays carried out in the SIA and HPLC systems

Assay technique		Bopindolol concentration (% of the prescribed amount ± RSD)[a]	
		SIA	HPLC
Samples	1	97.7 ± 0.7	98.9 ± 0.6
	2	98.7 ± 0.7	99.2 ± 0.7
	3	97.9 ± 0.6	98.6 ± 0.5
Average		98.1 ± 0.7	98.9 ± 0.6

[a] All samples were measured in triplicate.

10 μg ml^{-1}. The relative standard deviations (RSD) ($n = 10$) were 1.30%, 0.89% and 0.26%, respectively.

3.3. Assay of bopindolol in Sandonorm tablets and interference studies

The proposed system was applied for the determination of bopindolol in the commercially available tablets Sandonorm® (declared content 1mg of bopindolol in one tablet). In order to determine the content of bopindolol in Sandonorm® 1 mg tablets, signals of the analyzed solution and standard with the expected concentration of bopindolol were compared. The analyzed solution was prepared following the procedure described in Section 2. Both solutions should contain bopindolol in a concentration of about 5 μg ml^{-1}.

The results are in good agreement with the pharmacopoeial requirements on the active compound content in tablets with low amount of pharmaceuticals (range 95.0–105.0%). Results of bopindolol assays in the SIA system were compared with the conventional HPLC determination and are summarized in Table 4. The statistical t-test (95% level) revealed no significant difference between the found average values of both methods.

The interference effect of excipients (glucose, lactose, starch and magnesium stearate) was not observed. The selectivity of the proposed method of determination was tested in the presence of seven interfering substances from the group of β-blockers (Table 5). The interference effect was observed in the presence of pindolol only.

4. Conclusion

The described SIA system with stopped flow technique was tested for determination of bopindolol in pharmaceutical formulation Sandonorm®. The color reaction, based on the formation of violet, water-soluble complex with 4-dimethylaminobenzaldehyde in acidic medium, was applied for the assay of bopindolol in tablets. Although the reaction speed is relatively low, the mixing and standing of reaction zone in holding coil makes possible to improve the sensitivity of determination.

Table 5
Influence of interfering substances from the group of β-blockers on the determination of bopindolol (10 μg ml^{-1})

Interfering substance (IF)	Concentration ratio bopindolol/IF (w/w)	Found amount of bopindolol (%)
Bopindolol	0	100
Desacetylmetiprolol	1:10	99.71
Methypranolol	1:10	96.07
Desacetylmethypranolol	1:10	100.38
Atenolol	1:10	95.16
Metoprolol	1:10	102.39
Acebutolol	1:10	102.45
Pindolol	1:3	> 200

Results of the bopindolol assay were in good agreement with both comparative HPLC method and pharmacopoeial requirements. In comparison with published analytical methods applied for bopindolol determination, the SIA system shows extremely fast analysis with good sensitivity. The sample throughputs of previously published methods were in the range 8–14 min. The principles of SIA fulfill economic aspects of modern analyses, e.g. simple and quick (40 samples per hour) determination and low consumption of sample and reagents. Due to the selectivity of the mentioned reaction this SIA technique could be successfully applied to the direct determination of bopindolol in the presence of other β-blockers except of pindolol. The proposed method is suitable for determination of bopindolol in pharmaceutical formulations with very low cost per analysis and without expensive instrumentation.

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