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Short communication

Determination of bopindolol using the flow injection technique coupled with solid phase extraction

Joana Meireles [a,b], Hana Sklenářová [b], Dalibor Šatínský [b], Petr Solich [b,*], Alberto N. Araújo [a], Maria Conceição B.S.M. Montenegro [a]

[a] *CEQUP/Department of Physical Chemistry, Faculty of Pharmacy, University of Porto, Rua Aníbal Cunha 164, 4050 Porto, Portugal*
[b] *Laboratory of Flow analysis, Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Heyrovského 1203, 500 05 Hradec Králové, Czech Republic*

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Abstract

In the proposed procedure, the determination of bopindolol using a flow injection analysis (FIA) technique, with spectrophotometric detection at 635 nm, is described. The method is based on the production of a green, water-soluble complex with ferric ions in acid medium. The automated lab-made FIA system was used for the direct determination of bopindolol in tablets. Bopindolol was adsorbed onto the solid phase in a mini-column, which was integrated directly into the flow system. The positive feature of the use of solid phase extraction (SPE) was the pre-concentration of bopindolol (seven times). The sample throughput was 50 samples per hour. Using the SPE method, bopindolol was determined with a linear range from 125 to 1000 μg ml^{-1} (Relative standard deviation (R.S.D.) = 1.87%), with a detection limit (3σ) of 70 μg ml^{-1}. The method was applied to the determination of bopindolol in Sandonorm® tablets. The results obtained were compared with a conventional HPLC method, both analytical techniques were in good agreement.

Keywords: Flow injection analysis; Solid phase extraction; Pre-concentration; Bopindolol

1. Introduction

Flow injection analysis (FIA) [1] represents an advanced form of solution manipulation available to analytical chemists for the mixing and transport of samples, reagents and products of chemical reactions to the measurement point. All the features of FIA contribute to a simple way of sample handling and reduce environmentally harmful effects.

Solid phase extraction (SPE) has become a very popular technique for "separation" and pre-concentration of the analytes. This technique widely used for the preparation of samples before analysis increases specificity and sensitivity of the analytical method. On the other hand, off-line SPE is

* Corresponding author. Tel.: +420-49-506-7294; fax: +420-49-551-8718.
E-mail address: solich@faf.cuni.cz (P. Solich).

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both time consuming and complicated and, thus, it is usually the slowest step of the analysis.

One of the possibilities to facilitate and accelerate the analysis is to integrate the sample preparation step directly into the flow injection system. SPE could be applied to the elimination of interferences in complex sample matrices and for the pre-concentration of analytes as well. For the determination of drugs in tablets with a low content of one active substance, the effect is mainly pre-concentration.

Bopindolol belongs to the group of β-blockers and acts as a slowly dissociating β_1-adrenergic receptor antagonist. Pharmaceutical formulations contain low amount of bopindolol (usually about 1 mg per tablet). The structure of bopindolol (4-[2′-benzoyloxy-3′-(tert-butylamino) propoxy]-2-methylindole) is shown in Fig. 1.

The determination of bopindolol has been rarely studied. There are only a limited number of related works, e.g. determination of bopindolol metabolites by HPLC [2–4]; enantiomeric separation of bopindolol [5]; separation of bopindolol, its precursor and degradation product by supercritical-fluid chromatography [6] and its isotachophoretic determination [7]. Detection techniques applied for bopindolol assay are fluorescence [2], electrochemical detection [3], coulometry [4], UV spectrophotometry [5] or conductimetry [7]. In many methods, the total time of analysis is usually prolonged by the addition of time-consuming sample pre-treatment.

Some β-blockers produce a green, water-soluble complex with Fe(III) chloride in acid medium with a maximum absorbance (λ_{max}) at 635 nm. This reaction was previously applied to the determination of pindolol [8,9]. We have found that bopindolol also produces a similar green complex with Fe(III). The resulting method was then automated using flow injection technique as described below.

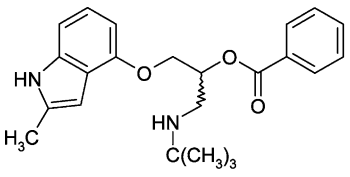

Fig. 1. Chemical structure of bopindolol.

Although bopindolol could be determined by UV spectrophotometry, we chose the visible area of spectra to obtain more specific detection (colour reaction) using sophisticated analytical equipment (miniaturised fibre optic spectrophotometer).

2. Experimental procedures

2.1. Apparatus

The FIA system consisted of a peristaltic pump (4-channel, Gilson, France) equipped with Tygon tubes and an injection valve (double, 6-port, Vici-Valco, USA). All connections and reaction coil (120 cm) were made of a 0.75 mm i.d. PTFE tubing. S2000/SAD500 spectrophotometer with optical fibres (Ocean Optics Inc., USA) connected with SMA flow Z-cell (10 mm optical path length designed to minimise bubble entrapment during flow measurements) and visible light source LS-1 tungsten halogen lamp (Ocean Optics) were employed for detection step. The control unit consisted of a Pentium PC 75 MHz with lab-made program (LPM-FIA 1.0) written in LABVIEW®.

The SPE procedure was carried out in a mini-column packed with material that retains the analyte. The material used was Bakerbond C-18 (USA), the grain size 40–60 μm, packed into a PTFE tubing with 2 mm i.d. and 10 mm length and retained using plugs of cotton wool.

2.2. Chemicals

All solutions were prepared from a Millipore Milli-Q RG ultra pure water. 1.50 mg ml^{-1} of bopindolol (Leciva a.s., Czech Republic) standard solution and 0.5 M Fe(III) chloride (Lachema, Czech Republic) reagent solution were prepared dissolving the appropriate amount of chemicals in 0.3 M hydrochloric acid (Lachema). Working standard solutions were prepared by dilution of the stock standard solution with 0.3 M HCl. The carrier stream, which also served as the elution solvent for retained bopindolol from the column, was 17% (v/v) of methanol (Lachema). All chemicals were of analytical-reagent grade and solutions were filtered and degassed under reduced pressure.

2.3. Sample preparation

The pharmaceutical formulation analysed was Sandonorm® 1 mg tablets (Leciva a.s.). Determination of the active substance in tablets was done by weighting ten tablets, crushing the tablet mass and, using an average weight of one tablet, dissolving it in 5.0 ml of 0.3 M hydrochloric acid. For 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 analysed.

2.4. FIA system and procedure

The FIA system described was designed for a simple and fast measurement procedure. The peristaltic pump generated continuous flow of the carrier, sample and reagent streams. In the sampling step (injection valve in load position) the sample was propelled through the sampling coil where the SPE column was inserted. In this period, the carrier stream was merged with the reagent flow and pushed through the detector (setting the baseline signal). The sample volume was controlled by measuring software and set in between beginning the measurement cycle and changing the injection valve position into the injection step.

Injection was carried out by washing the sampling coil with the carrier stream. In this step, bopindolol, retained in the SPE column, was eluted. Then the carrier stream was mixed with the reagent and pushed through the detector (sample signal). While the injection valve was in the injection position sample was discarded to waste. The injection step and signal integration were controlled by the software as well. Configuration of the FIA system is shown in Fig. 2.

2.5. HPLC measurements

As a comparative technique an HPLC determination was developed. 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, 125×4 mm, 5 μm) with pre-column of the same material (LichroCART, 4×4 mm, 5 μm).

The composition of the mobile phase was optimised to a mixture of methanol and water 8:5 (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 FIA-SPE determinations but manual SPE was used in the sample preparation step. All samples were measured in triplicate.

3. Results and discussion

3.1. Optimisation

The detection wavelength was optimised with respect to the maximum sample signal. The detection wavelength was chosen using the spectrum mode of the flow spectrophotometer. This mode enabled the determination of the optimum wavelength in real conditions during flow measurement. The other parameters optimised were: pump

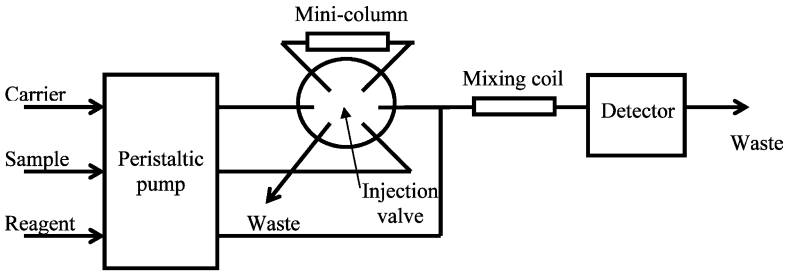

Fig. 2. FIA system with SPE mini-column.

Table 1
Optimised parameters for bopindolol FIA

Variable	Range	Optimum values
Wavelength (nm)	400–800	635
Pump speed-reaction (ml min^{-1})	0.31–1.07	0.88
Sampling time (s)	5.0–15.0	10.0
Methanol concentration (%)	10–20	17

speed, pre-concentration time and concentration of methanol used as elution solvent. Ranges and optimal values found are detailed in Table 1. Tables 2 and 3 show several experiments for optimisation of pre-concentration time (relating to the sample volume) and pump speed. The main parameters to choose the optimal values were detector response and R.S.D. of the respective measurement.

The optimisation of methanol concentration in the eluent was carried out in the range 10–20%. The final measurement conditions were 635 nm as detection wavelength, 0.88 ml min^{-1} as the pump speed, 10.0 s as sampling time and 17% (v/v) of methanol for the elution step.

3.2. Calibration

Calibration experiments were carried out with five standard solutions of following concentrations of bopindolol: 1000, 500, 250, 200 and 125 μg ml^{-1}. Each standard solution was measured in triplicate. The regression equation ($y = ac + b$) was $y = 0.00094c - 0.036$ with correlation coefficient 0.9995. The linear range of the calibration was 125–1000 μg ml^{-1} and detection limit for bopindolol determination was 70 μg ml^{-1} (3σ).

The pre-concentration capacity of the mini-column was tested by comparison of the calibration curve achieved with and without the column. The linear range of the calibration without the SPE column was 0.5–4.0 mg ml^{-1}. With on-line SPE the signal was increased by seven times in comparison.

3.3. Assay of bopindolol in Sandonorm tablets

To determine the content of bopindolol in Sandonorm® 1 mg tablets, signals of the analysed solution and standard with the expected concentration of bopindolol were compared. The analysed solution was prepared following the

Table 2
Optimisation of the pre-concentration time for bopindolol FIA

Pre-concentration time (s)	Samples ($A_{635\ nm}$)			Mean	R.S.D. (%)
	1	2	3		
5	0.4409	0.4276	0.4615	0.4433	4.51
10	0.6323	0.6446	0.6579	0.6449	2.34
15	0.6430	0.6409	0.6390	0.6409	0.62

Table 3
Optimisation of the pump speed for bopindolol FIA

Pump speed (ml min^{-1})	Samples ($A_{635\ nm}$)			Mean	R.S.D. (%)
	1	2	3		
0.31	0.6323	0.6446	0.6579	0.6449	2.34
0.88	0.9127	0.9044	0.8970	0.9047	1.03
1.07	0.9207	0.9998	0.8798	0.9334	7.59

Table 4
Bopindolol assays carried out using the FIA-SPE and HPLC systems

Assay technique		Bopindolol concentration (% of the prescribed amount ± R.S.D.)[a]	
		FIA-SPE	HPLC
Samples	1	101.35 ± 1.32	102.88 ± 0.59
	2	101.82 ± 1.40	102.55 ± 0.72
	3	100.80 ± 1.38	102.59 ± 0.45
Average		101.32 ± 1.37	102.67 ± 0.59

[a] All samples were measured in triplicate.

procedure described in Section 2. Both solutions should contain bopindolol at concentrations of about 200 μg ml^{-1}.

The results were in a good agreement with the required range of active compound content in the tablets. Results of the bopindolol assays in the FIA-SPE system were compared with the conventional HPLC determination and the results summarised in Table 4, show good correlation of the two techniques. The main difference between FIA-SPE and HPLC method was the sample throughput because samples for HPLC analysis were prepared by manual SPE that was time consuming.

4. Conclusion

The described FIA system with on-line SPE mini-column was tested for determination of bopindolol in pharmaceutical formulation Sandonorm®. The colour reaction, based on the formation of a green, water-soluble, complex with Fe(III) in an acid medium, was applied to the assay of bopindolol in tablets. Although the detection limit of the mentioned colour reaction is relatively poor, the SPE technique improves this critical parameter and thus the sensitivity of the determination was increased by about seven times. The SPE column was renewable and there was no requirement to change the column during our study.

Results for the bopindolol assay compared well with both comparative HPLC method and pharmacopoeial requirements. In comparison with published analytical methods applied to bopindolol determination, the FIA-SPE system showed extremely fast analysis (compared with the manual SPE step that is principally required in the HPLC analysis). The principles of FIA fulfil the economic requirements of modern analyses by providing simple and quick (50 samples per hour) determinations and low consumption of sample and reagents.

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