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Determination of fenoterol hydrobromide by sequential injection analysis with spectrophotometric detection

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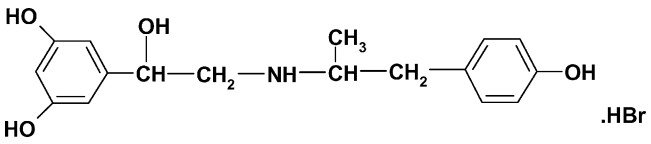
Abstract

A rapid, economical and automated sequential injection spectrophotometric determination of the phenolic sympathomimetic drug, fenoterol hydrobromide, is reported. The method is based on the reaction of fenoterol hydrobromide with 4-aminoantipyrine and potassium hexacyanoferrate and the absorbance of the colored product monitored at 505 nm. Chemical as well as physical SIA parameters that affect the signal response have been optimized in order to get better sensitivity, higher sampling rate and better reagent economy. Using the optimized parameters, a linear relationship between the relative peak height and concentration was obtained in the range 0.5–40 mg L^{-1}. The detection limit (as 3σ value) was 0.1 mg L^{-1} and precision was 1.8 and 1.6% at 2 and 5 mg L^{-1}, respectively. As compared to previous works, wide linear range, low detection limit, and highly economical reagent consumption are the advantages of our method.

Keywords: Flow techniques; Sympathomimetic drugs; Anti-asthmatics; Bronchodilator

1. Introduction

Fenoterol hydrobromide (**I**) [1-(3,5-dihydroxyphenyl)-2-(4-hydroxy-α-methyl-phenethylamino) ethanol] is a β_2-selective adrenergic receptor agonist. It is a direct-acting sympathomimetic stimulant mostly used as bronchodilator. Fenoterol (brand name Berotec®) is used to relieve and prevent bronchial asthma, chronic and acute bronchitis, emphysema, exercise-induced bronchospasm, and other pulmonary disorders that cause bronchospasm [1,2].



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The analytical methods so far reported for the determination of fenoterol in dosage forms and biological samples include: spectrophotometry [3–6], colorimetric flow injection [7], coulometry [8], voltammetry [9], differential pulse stripping with Nafion-modified carbon paste electrode [10], HPLC [11,12], capillary electrophoresis [12,13] and isotachophoresis [12]. Most of these methods suffer from drawbacks of undesirable sensitivity, high technical demand, high cost or inconvenience to perform in a high throughput format. In relative terms, the spectrophotometric method reported by El-Gendy is better with respect to simplicity, sample throughput, sensitivity, and cost [4]. This method is based on the flow injection spectrophotometric detection of the condensation reaction product of fenoterol with 4-aminoantipyrine in the presence of an oxidant, potassium hexacyanoferrate (**III**) [4]. El-Gendy used the ferricyanide and 4-aminoantipyrine reagents in a continuous flow mode, which is uneconomical with respect to reagent consumption and waste generation. SIA is known for its economical use of reagents and minimal waste generation since it is based on a discontinuous flow that takes up the reagents sequentially and only when required. Moreover, it avoids multiple flow channels by employing a single channel with the help of a centrally located multi-position selection valve and a single pump that can be manipulated as stopped–reversed–forward mode.

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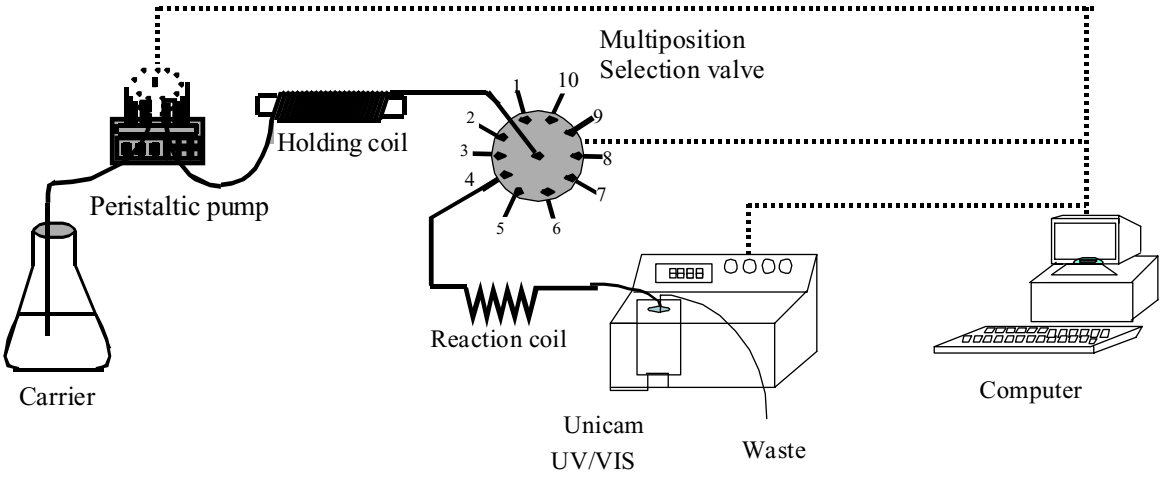

Fig. 1. A schematic diagram of the SIA system used for the determination of fenoterol hydrobromide.

This paper presents an automation of the ferricyanide-aminoantipyrine reaction described above for the sequential injection spectrophotometric determination of fenoterol hydrobromide.

2. Experimental

2.1. Apparatus

Fig. 1 illustrates the SIA manifold used. Solutions were driven by a Gilson minipuls 3 peristaltic pump (Villiers-le-Bel, France) using a 1.29 mm i.d. pump tubing (Jobling, Staffordshire, UK). A 10-position micro-actuated selection valve from Valco Instruments (Houston, TX, USA) was used to select the solutions. The holding coil was made by winding a 5 m, 0.89 mm i.d. Tygon tube on a glass rod. A single wavelength Unicam 8625 UV–vis spectrophotometer (Unicam Ltd., Cambridge, UK) fitted with a 10 mm flow through cell that has a volume of 80 μL (Hellma, Mullheim, Baden, Germany) was employed. Data acquisition and device control was accomplished by using a PC30-B interface board (Eagle Electric, Cape Town, South Africa) in combination with an assembled distribution board (Mintek, Randburg, South Africa). The FlowTEK software package (version 1.3a) from Mintek was used throughout. The analog spectrophotometric response was automatically converted to digital, amplified and the response given in mV versus time profile. Data were evaluated using the relative peak heights in mV.

2.2. Reagents and samples

Deionized water was produced using a Modulab apparatus (Continental Water System, San Antonio, TX, USA). 4-Aminoantipyrine, fenoterol hydrobromide and potassium ferricyanide were obtained from Sigma-Aldrich. The carrier was 1.0% (w/v) Na_2CO_3 (Sigma-Aldrich). Stock solutions of 4-aminoantipyrine (1%, w/v) and potassium ferricyanide (5%, w/v) were prepared in 1.0% (w/v) Na_2CO_3. Stock solution (100 mg L^{-1}) of fenoterol hydrobromide was prepared in deionized water.

Three samples of Berotec® syrup with different batch number (Batch Nos. 229A, 231A, and 233A) and one formulated by a professional pharmacist were obtained from a local pharmacy. Before analysis, the syrups were diluted 50-fold in deionized water.

2.3. Procedure

Wavelength scan between 360 and 750 nm was run using an Agilent 8345 diode array spectrophotometer (Waldbronn, Germany) and maximum absorbance was obtained at 505 nm. The device sequence and corresponding timing are shown in Table 1. To compare results obtained by SIA, fenoterol hydrobromide in the samples was determined by a reported spectrophotometric method [5]. Precision was

Table 1
Device sequence and timing to change pump direction and valve position

Time (s)	Pump	Valve
0.00	Off	Starting position (position 1, reagent 1)
1.00	Reverse-draws reagent 1	
2.72	Off	
4.00		Advance to position 2 (sample)
5.00	Reverse-draws sample	
6.72	Off	
8.00		Advance to position 3 (reagent 2)
9.00	Reverse-draws reagent 2	
9.86	Off	
11.00		Advance to position 4 (to detector)
12.00	Forward	
58.00	Off	
59.00		Return to position 1

evaluated by performing five SIA measurements in comparison to three spectrophotometric measurements. Recovery test was made by spiking 10 and 20 mg L^{-1} standard fenoterol hydrobromide in the diluted Berotec® syrup (Batch No. 229A).

3. Results and discussion

3.1. Selection of carrier solution and aspiration order of reagents and sample

In general phenols react with aminoantipyrine in the presence of alkaline oxidizing agents [14]. To make the medium slightly alkaline, El-Gendy employed borate buffer solution (pH 9.5) [4]. Others used 2% sodium carbonate [15] and 0.01 mol L^{-1} sodium bicarbonate [16] for the same reaction but different analyte with a phenolic moiety. Preliminary investigation comparing the use of borate buffer, 0.01 mol L^{-1} bicarbonate and 1% sodium carbonate solutions as a carrier showed that using the latter gives better signal. Alternatively, distilled water could be used as a carrier and the alkalinizing reagent could be aspirated through one of the channels of the selection valve. However, this option was not tested for fear that it significantly extends the analysis time and in turn reduce the sample throughput. Thus, 1% sodium carbonate solution was used as a carrier. However, no attempt was made to optimize the concentration of the carrier solution.

In reactions involving multiple zone penetrations, it is important to investigate the aspiration order of reagents and sample. In this work maximum peak height and excellent repeatability were observed when the sample zone is sandwiched between the two reagent zones (Table 2). This is in agreement with previous observations on three zone penetrations [17,18]. In a recent report dealing with phenylephrine, it has been shown that the order aminoantipyrine–sample–potassium ferricyanide and potassium ferricyanide–sample–aminoantipyrine resulted in almost similar responses [18], whereas in this work interchanging the order of the two reagents (sandwiching the sample zone) exhibited significant difference (a difference of 60%). Apparently, the order aminoantipyrine–sample–potassium ferricyanide was chosen.

Table 2
Aspiration order of reagents and sample

Aspiration order	Relative peak height (mV) [mean ± S.D. (R.S.D%)]
AP–sample–ferricyanide	4.63 ± 0.03 (0.5)
AP–ferricyanide–sample	1.31 ± 0.02 (1.6)
Ferricyanide–AP–sample	1.05 ± 0.04 (3.8)
Ferricyanide–sample–AP	1.87 ± 0.02 (1.2)
Sample–AP–ferricyanide	1.52 ± 0.01 (0.7)
Sample–ferricyanide–AP	1.27 ± 0.02 (1.4)

Experimental conditions were: reaction coil: 0.89 mm i.d. and 90 cm in length, aspiration volume 100 μL in each case, concentration of standard 25 mg L^{-1}, flow rate 3.5 mL min^{-1}, concentration of AP 0.4% (w/v), concentration of ferricyanide 1% (w/v).

3.2. Optimization of parameters

3.2.1. Flow rates

In any flow-based analysis the response is dependent on the flow rate and thus it is necessary to optimize it. The effect of the flow rate on the peak height was investigated from 1 to 5 mL min^{-1} at every 0.5 mL min^{-1} range (Fig. 2). The sample and reagent volumes aspirated were kept constant by changing the aspiration time in accordance with the flow

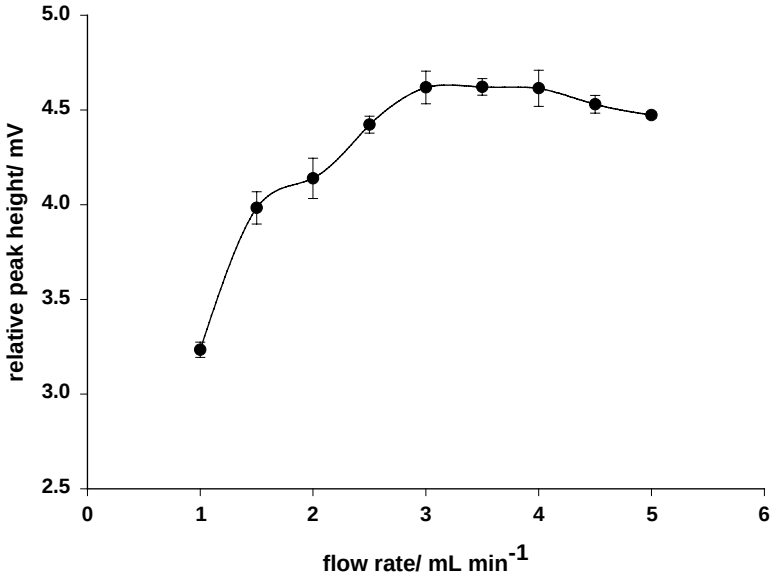

Fig. 2. Effect of flow rate on the relative peak height. Experimental conditions were: reaction coil: 0.51 mm i.d. and 90 cm in length, aspiration volume 100 μL in each case, concentration of standard 20 mg L^{-1}, concentration of AP 0.4% (w/v), concentration of ferricyanide 1% (w/v).

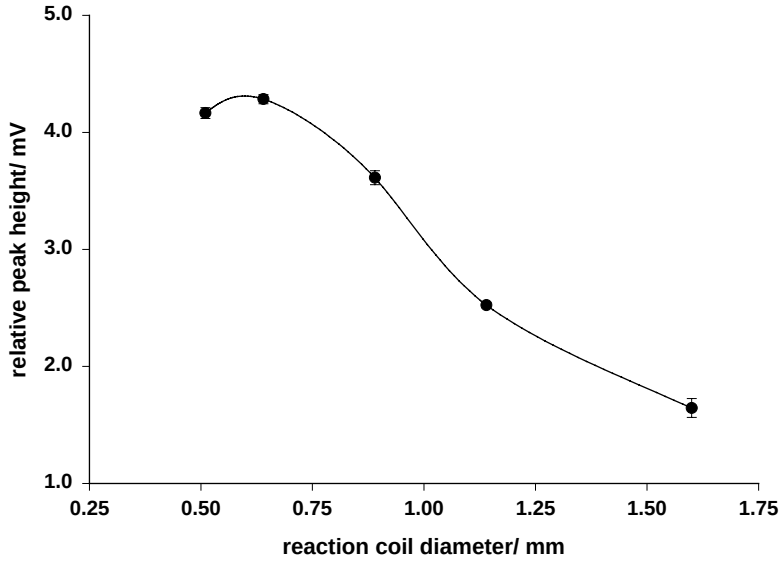

Fig. 3. Effect of reaction coil diameter on the peak height. Experimental conditions were: flow rate 3.5 mL min^{-1}, aspiration volume 100 μL in each case, concentration of standard 20 mg L^{-1}, concentration of AP 0.4% (w/v), concentration of ferricyanide 1% (w/v).

rate. As the flow rate increases the peak height increases up to 3.5 mL min^{-1}, kept almost constant till 4.0 mL min^{-1}, and then started to level off. Better repeatability (lower RSD) was observed at a flow rate of 3.5 mL min^{-1} and thus was chosen for subsequent measurements. At this flow rate the sample throughput was found to be around 60 per hour.

3.2.2. Reaction coil internal diameter

One of the factors that affect dispersion (SIA's main mode of mixing) is reaction coil diameter. Theoretically, dispersion is minimal at lower coil diameter resulting in higher response and shortest peak time [19]. However, depending on the kinetics of the chemical reaction the highest response may be obtained at a larger coil diameter. The effect of reaction coil diameter on peak height was studied from 0.51 to 1.60 mm (all lengths were 90 cm) at five different diameters based on availability (Fig. 3). A coil diameter of 0.64 mm gave the highest signal. As the diameter increases the relative peak height decreases and the peak time increases.

3.2.3. Reaction coil length

The other parameter that affects dispersion and hence SIA measurement is reaction coil length. In order to have a

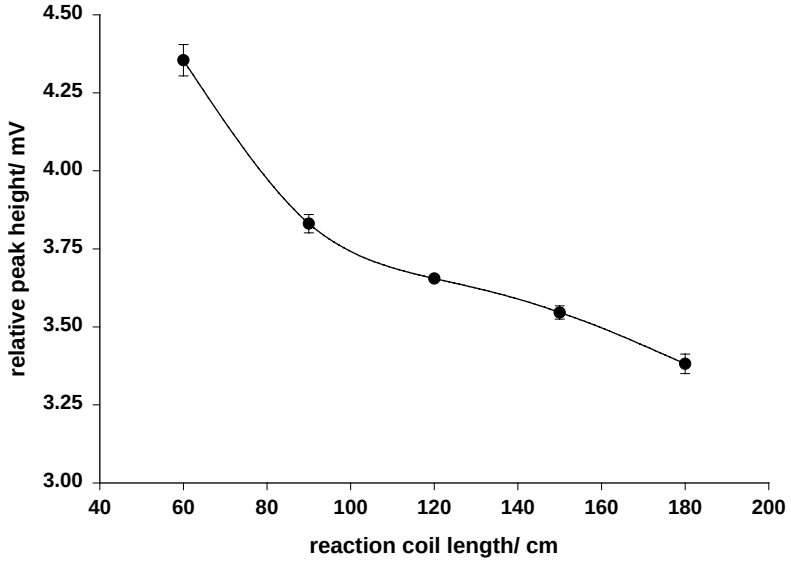

Fig. 4. Effect of reaction coil length on the peak height. Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil diameter 0.64 mm, aspiration volume 100 μL in each case, concentration of standard 20 mg L^{-1}, concentration of AP 0.4% (w/v), concentration of ferricyanide 1% (w/v).

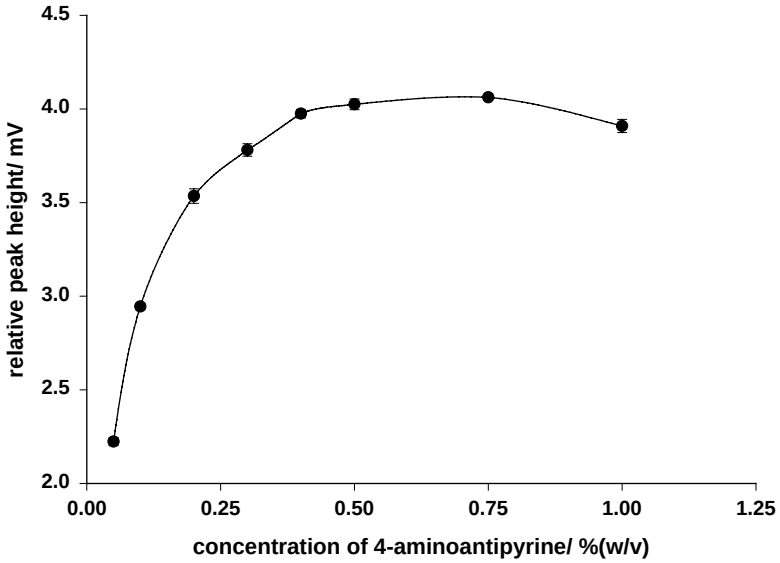

Fig. 5. Effect of concentration of aminoantipyrine. Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil 60 cm and 0.64 mm i.d., aspiration volume 100 μL in each case, concentration of standard 20 mg L^{-1}, concentration of ferricyanide 1% (w/v).

better sensitivity and higher peak height, a reaction coil dimension that gives minimum dispersion (shortest in length) is required. The effect of reaction coil length on peak height was assessed from 60 to 180 cm at every 30 cm range (Fig. 4). In agreement with theory, the highest signal and the shortest peak time were observed at a coil length of 60 cm.

3.2.4. Concentration of 4-aminoantipyrine

The effect of concentration of 4-aminoantipyrine was studied from 0.05 to 1% (w/v) (Fig. 5). The peak height increases with increasing concentration up till 0.4%, kept almost constant till 0.75% and started levelling off. Thus, concentration of 0.4% (w/v) was taken for subsequent measurements.

*3.2.5. Concentration of potassium hexacyanoferrate (**III**)*

The effect of concentration of potassium hexacyanoferrate (**III**) was investigated from 0.05 to 4% (w/v) (Fig. 6). The peak height increases with increasing concentration. However, the increase above 2% was not significant when the increase in concentration is taken into consideration, and thus a concentration of 2% (w/v) was chosen for subsequent experiments.

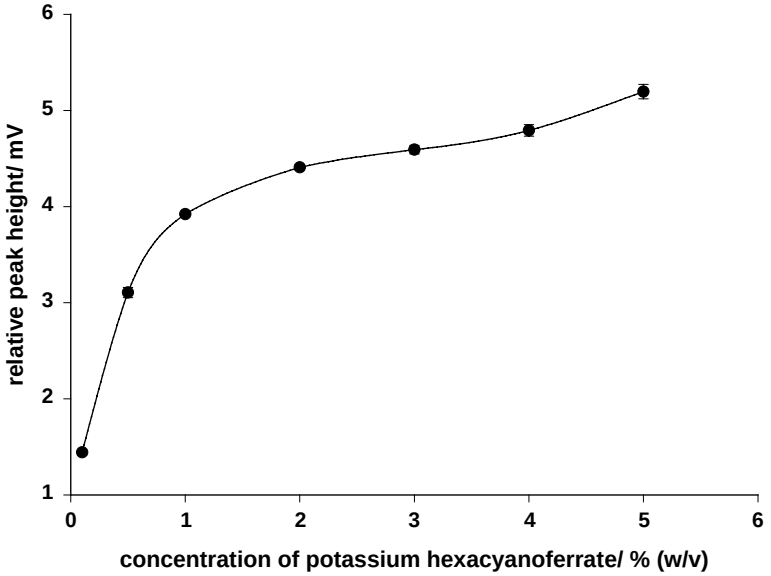

Fig. 6. Effect of concentration of potassium hexacyanoferrate. Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil 60 cm and 0.64 mm i.d., aspiration volume 100 μL in each case, concentration of standard 20 mg L^{-1}, concentration of aminoantipyrine 0.4% (w/v).

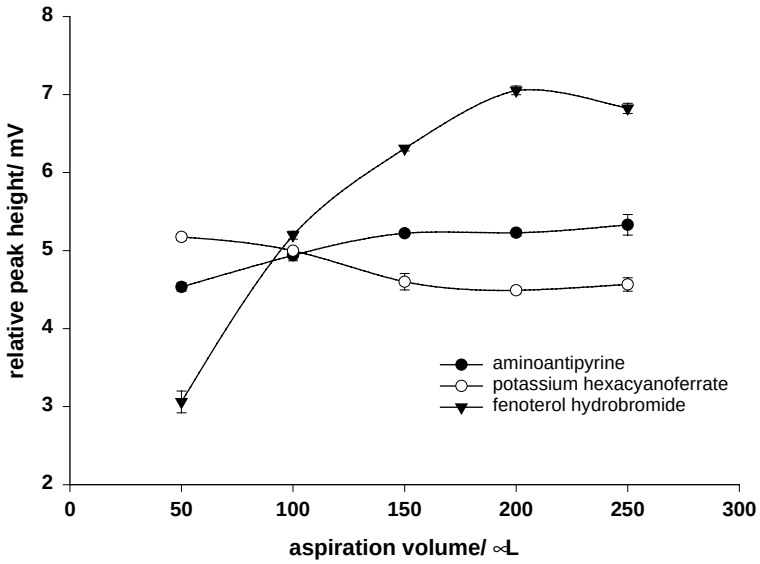

Fig. 7. Optimization of aspiration volumes of sample and reagents. Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil 60 cm and 0.64 mm i.d., concentration of standard 20 mg L^{-1}, concentration of ferricyanide 2% (w/v), concentration of 4-aminoantipyrine 0.4% (w/v).

3.2.6. Aspiration volumes

One of the parameters that affect SIA measurements is the volume of reagents and sample drawn up. In this work the effect of aspiration volumes of the two reagents and the sample were studied in the range 50–250 μL at every 50 μL interval. When varying the volume of solution in question the other two were kept at 100 μL. First, volume of 4-aminoantipyrine was considered. As the aspiration volume increases the peak height increases up to 150 μL and kept almost constant afterwards (Fig. 7). Since the increase in response while increasing the volume from 100 to 150 μL is insignificant (5.4%) 100 μL was taken for subsequent measurements. Again keeping the others at 100 μL, that of ferricyanide varied as aforesaid. The response decreases as the volume increases (Fig. 7) and thus 50 μL was taken as optimum. When the volume of the sample varied from 50 to 250 μL, maximum response was obtained at a volume of 200 μL (Fig. 7). Using this volume gives better sensitivity but narrow linear range due to the limitation of the FlowTEK program that has a fixed scale of 10 mV for the response axis. Any reading above this goes as an offset. To compromise the effect on sensitivity and linear range 100 μL was chosen and acceptable sensitivity and better linear range could be obtained at this value. Table 3 gives the summary of the optimized parameters discussed above.

3.3. Figures of merit

Using the aforementioned parameters the SIA system was evaluated for its response for different concentrations of fenoterol hydrobromide. Linear calibration was found from 0.5 to 40 mg L^{-1} with an excellent correlation (relative peak height $= 0.202C$ (mg L^{-1}) $+ 0.338$, $r^2 = 0.999$). The linear range obtained in this work is far better than the one reported earlier [3–5,9,10]. At concentrations of 2 and 5 mg L^{-1}, RSDs of 1.8 and 1.6%, respectively, were registered ($n = 10$ measurements in each case). The detection limit (as 3σ value at a concentration of 0.5 mg L^{-1} for 10 determinations) [20] was found to be 0.1 mg L^{-1}.

3.4. Analysis of pharmaceutical sample

Three samples of Berotec® syrup with different batch number (Batch Nos. 229A, 231A, and 233A) and one formulated by a professional pharmacist were analysed by the SIA method as well as with a reported spectrophotometric method [5]. The samples were diluted 50-fold in deionized water. Table 4 presents the results obtained by the two methods as well as the manufacturers' claimed value. A *t*-test was used to determine whether the results obtained by the two methods differ significantly. The calculated *t*-values in all cases were less than the tabulated value (2.45 for six degrees of freedom at 95% confidence level) showing that there is no significant difference between the values obtained by the two methods. Moreover, a *t*-test was employed to compare the results from SIA measurements with the

Table 3
Parameters optimized for the SIA determination of fenoterol hydrobromide

Parameters	Optimized value
Flow rate (mL min^{-1})	3.50
Reaction coil i.d. (mm)	0.64
Reaction coil length (cm)	60.00
Concentration of 4-aminoantipyrine (%, w/v)	0.40
Concentration of potassium ferricyanide (%, w/v)	2.00
Aspiration volume of 4-aminoantipyrine (μL)	100.00
Aspiration volume of potassium ferricyanide (μL)	50.00
Aspiration volume of sample (μL)	100.00

Table 4
Fenoterol hydrobromide in Berotec® syrups as determined by the SIA and a reported spectrophotometric methods

Sample	SIA (mg L^{-1}) ($n = 5$)	Reported method (mg L^{-1}) ($n = 3$)	Difference (%)	Calculated t-value	Claimed value (mg L^{-1})
Batch No. 229A	505.39 ± 18.36	530.33 ± 16.80	4.9	1.91	500
Batch No. 231A	502.91 ± 14.13	521.33 ± 11.59	3.7	1.89	500
Batch No. 233A	497.80 ± 8.23	505.83 ± 14.18	1.6	1.04	500
Formulated	499.70 ± 10.22	515.67 ± 17.34	3.2	1.68	500

claimed values and in all cases the calculated value were less than the tabulated (2.78 for four degrees of freedom at 95% confidence level) confirming once again the validity of the method.

4. Conclusions

The advantages of the developed SIA method over the method reported by Al-Malaq et al. [5] are wide linear range, simplicity, better repeatability, employing non-toxic reagents, and very fast since the latter requires 20 min incubation. Moreover, the reagent consumption is significantly reduced as compared to the FIA method reported by El-Gendy and waste generation is extremely minimized. Additional advantage of the SIA method is requiring less operator input since the method is automated. Therefore, it can potentially replace the poorly sensitive, labour and time-consuming titration method considered as standard method by the British Pharmacopoeia [21].

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