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Sequential injection spectrophotometric determination of ritodrine hydrochloride using 4-aminoantipyrine☆

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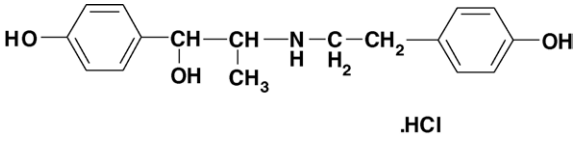
Abstract

A sequential injection spectrophotometric determination of ritodrine hydrochloride is described. The method is based on the condensation of aminoantipyrine with phenols in the presence of an alkaline oxidizing agent to yield a pink coloured product the absorbance of which is monitored at 503 nm. Different sequential injection analysis (SIA) parameters including reagent concentrations have been optimised and used to obtain the analytical figures of merit. A linear concentration range of 3.1–123.5 μmol L^{-1} and a detection limit (as 3σ-value) of 1.0 μmol L^{-1} were obtained. The precision was 2.4 and 2.3% relative standard deviation (R.S.D.) at 6.2 and 15.4 μmol L^{-1}, respectively. This method is superior over previously reported ones in terms of linear range, short analysis time, high sample throughput, excellent reagent economy and minimum waste generation.

Keywords: Flow techniques; Sympathomimetic agents; Uterine relaxant

1. Introduction

Ritodrine hydrochloride [1-(4-hydroxy phenyl)-2-[(2-hydroxy phenyl) ethyl amino] propanol-hydrochloride] is a selective β_2-adrenergic agonist solely used as uterine relaxant. It stimulates the β_2-adrenergic receptors inhibiting the contractility of uterine smooth muscle that results in the arrest of premature labour [1,2].



Several methods have been reported for the determination of ritodrine hydrochloride in pharmaceutical products as well as in biological samples. These include HPLC [3,4], fluorimetric [5], and spectrophotometric methods with different colour reagents [6–13]. Apparently, the former is expensive, time consuming and needs high technical demand. The other methods either have disadvantages of low sensitivity, very narrow linear range, very low sample throughput, or require a long incubation time. The spectrophotometric method reported by Revanasiddappa and Manju is relatively simple, fast, and does not need extraction or a heating step [9]. This method is based on the condensation reaction of 4-aminoantipyrine with phenolic moieties in the presence of an alkaline oxidizing agent yielding a pink coloured product. According to their report the probable mechanism is oxidation of aminoantipyrine with potassium hexacyannoferrate in alkaline medium causing lose of two protons from the former leading to the formation of a nucleophilic intermediate that further undergoes nucleophilic substitution with the phenolic moieties of ritodrine that in turn results in the coloured product [9]. However, this method is completely manual and thus it is labour intensive, consumes large amount of reagents and generate the corresponding amount of waste.

In general, flow analysis is known to be fast, precise, inexpensive (due to small sample and reagents volume needed), less prone to operator error, computer compatible, to enhance selectivity and sensitivity, to allow multiple analysis and easy to automate as compared to manual or batch methods [14]. Prominent among flow systems are flow injection analysis (FIA) and

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sequential injection analysis (SIA). Apparently, the main advantages of SIA over FIA are: low reagent consumption and minimum waste generation (since all solutions are pumped continuously in FIA), computer control of the pump, selection valve and the detector (easy and efficient control of hydrodynamic variables) where by minimizing operator input and ability to perform several different measurements coupled with various modes of detection without the need to reconfigure the flow manifold.

This paper utilises the aforesaid advantages of SIA in conjunction with the manual method reported earlier [9] to develop a sequential injection spectrophotometric method for the determination of ritodrine hydrochloride based on its condensation reaction with 4-aminoantipyrine.

2. Experimental

2.1. Apparatus

Fig. 1 illustrates the SIA manifold used. A Gilson minipuls 3 peristaltic pump (Villiers-le-Bel, France) with a 1.29 mm i.d. pump tubing (Jobling, Staffordshire, UK) was used to propel solutions. Reagents and sample channels selection has been done by a 10-position micro-actuated selection valve from Valco Instruments (Houston, TX, USA). The holding coil was constructed by winding a Tygon tube (5 m, 0.89 mm i.d.) on a glass rod. Spectrophotometric detection was made by using a single wavelength Unicam 8625 UV–vis Spectrophotometer (Unicam Ltd., Cambridge, UK) that was fitted with a 10 mm flow through cell that has a volume of 80 μL (Hellma, Mullheim, Baden, Germany). 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 experiment. The analog spectrophotometric response from the spectrophotometer was automatically converted to digital, amplified and the final response was given in millivolt versus time profile. Data were evaluated using the relative peak heights in millivolt.

2.2. Reagents and samples

Deionised water was produced using a Modulab apparatus (Continental Water System, San Antonio, TX, USA). 4-Aminoantipyrine, ritodrine hydrochloride and potassium ferricyanide were obtained from Sigma–Aldrich. The carrier was 94 mmol L^{-1} Na_2CO_3 (Sigma–Aldrich). Stock solutions of 4-aminoantipyrine (49.2 mmol L^{-1}) and potassium ferricyanide (151.9 mmol L^{-1}) were prepared in 94 mmol L^{-1} Na_2CO_3. A stock solution (0.62 mmol L^{-1}) of ritodrine hydrochloride was prepared in deionised water.

Samples of Yutopar® tablets were obtained from a local pharmacy in Egypt. The content of each tablet was powdered using a pestle and mortar, dissolved in deionised water, and filtered. Before analysis, the sample solution was diluted 10-fold in 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 503 nm. The device sequence and corresponding timing are shown in Table 1. To compare results obtained by SIA, ritodrine hydrochloride in the samples was determined by a previously reported manual spectrophotometric method [12]. Precision was evaluated by performing 10 SIA measurements in comparison to three measurements with the published procedure [12].

3. Results and discussion

3.1. Selection of carrier solution

The condensation reaction of phenols with aminoantipyrine in the presence of alkaline oxidizing agents was reported decades

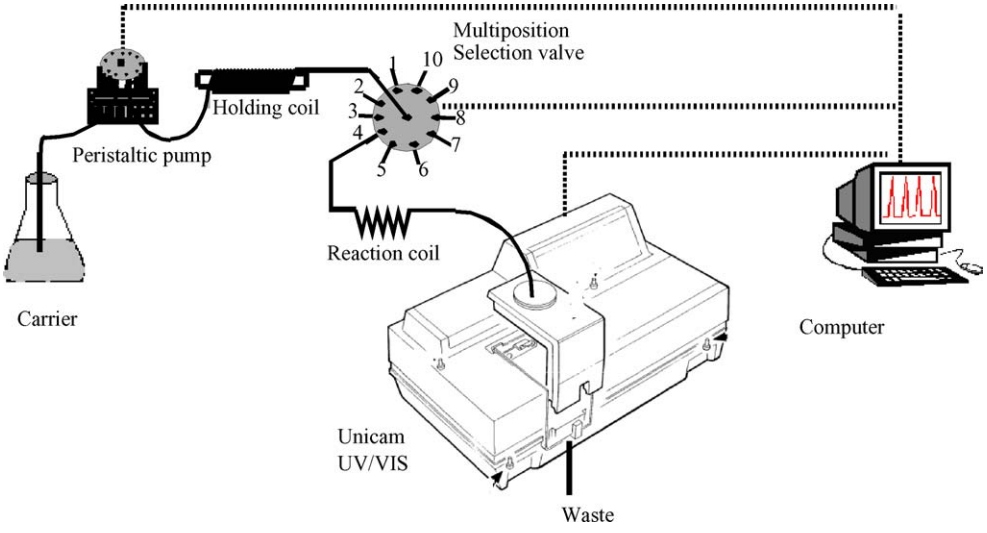

Fig. 1. A schematic diagram of the SIA system used.

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	
10.72	Off	
12.00		Advance to position 4 (to detector)
13.00	Forward	
58.00	Off	
59.00		Return to position 1

ago [15]. Since then a number of reports on the analytical application of this reaction have been published. To fulfil the alkaline requirement of the medium different workers used different alkalinising reagents. The original work emphasised that use of sodium hydrogen carbonate or sodium carbonate gives better colour development [15]. El-Gendy employed a borate buffer solution (pH 9.5) [16], Revanasiddappa and Manju used 2% sodium carbonate [9] and Rocha et al. used 0.01 mol L^{-1} sodium bicarbonate [17] for the same reaction but different analyte with a phenolic moiety. We have recently employed sodium carbonate solution (1%) for the reaction of etilefrine hydrochloride [18] and fenoterol hydrobromide [19] with aminoantipyrine. This was after a preliminary observation comparing the use of borate buffer, 0.01 mol L^{-1} bicarbonate and 1% sodium carbonate solutions. Thus, in this work 1% sodium carbonate solution was used as a carrier as well as alkalinising reagent.

3.2. Optimisation of parameters

In this report, both physical and chemical variables were optimised in a univariate (one-at-a-time) approach.

3.2.1. Aspiration order of reagents and sample

Previous reports on reactions that involve three zone penetrations showed that maximum response and excellent repeatability are obtained when the sample zone is sandwiched between the two reagent zones [18–20]. There are two possibilities to sandwich the sample: potassium ferricyanide-sample-aminoantipyrine and aminoantipyrine-sample-potassium ferricyanide. The former order gave the highest signal and better repeatability (Table 2). Apparently, the order potassium ferricyanide-sample-aminoantipyrine was chosen.

3.2.2. Flow rates

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. The sample and reagent volumes aspirated were kept constant by changing the aspiration time in accordance with the flow rate. Maximum peak height was obtained at a flow rate of 1.5 mL min^{-1}, however, the sample throughput at this flow rate is too small as compared to the value at higher flow rates. As a compromise between sample throughput, sensitivity and better repeatability (lower relative standard deviation, R.S.D.) a flow rate of 3.5 mL min^{-1} was chosen for subsequent measurements. At this flow rate the sample throughput was found to be 60 h^{-1}.

Table 2
Aspiration order of reagents and sample

Aspiration order	Relative peak height (mV) [mean ± S.D. (R.S.D.%)]
AP-sample-ferricyanide	$3.5 \pm 7.1 \times 10^{-2}$ (2.0)
AP-ferricyanide-sample	$0.3 \pm 2.1 \times 10^{-2}$ (7.7)
Ferricyanide-AP-sample	$0.5 \pm 3.6 \times 10^{-2}$ (8.1)
Ferricyanide-sample-AP	$0.8 \pm 2.3 \times 10^{-2}$ (3.1)
Sample-AP-ferricyanide	$1.0 \pm 1.8 \times 10^{-2}$ (1.8)
Sample-ferricyanide-AP	$0.3 \pm 1.8 \times 10^{-2}$ (7.0)

Experimental conditions were: reaction coil, 0.64 mm i.d. and 90 cm in length, aspiration volume 100 μL in each case, concentration of standard 61.8 μmol L^{-1}, flow rate 3.5 mL min^{-1}, concentration of AP 19.7 mmol L^{-1}, concentration of ferricyanide 30.4 mmol L^{-1}.

3.2.3. Reaction coil internal diameter and reaction coil length

The effect of the reaction coil diameter on the peak height was studied from 0.51 to 1.60 mm (all lengths were 90 cm) at five different diameters based on availability. Maximum response was obtained at a diameter of 0.51 mm in agreement with theory. The effect of reaction coil length on peak height was assessed from 60 to 180 cm at every 30 cm range and maximum response was exhibited at a coil length of 90 cm.

3.2.4. Concentration of potassium hexacyanoferrate(III)

The effect of concentration of potassium hexacyanoferrate(III) was investigated from 1.5 to 151.9 mmol L^{-1} (Fig. 2). The peak height increases with increasing concentration of potassium hexacyanoferrate(III). However, the increase in response above 60.7 mmol L^{-1} was not significant when the increase in concentration is taken into consideration, and thus

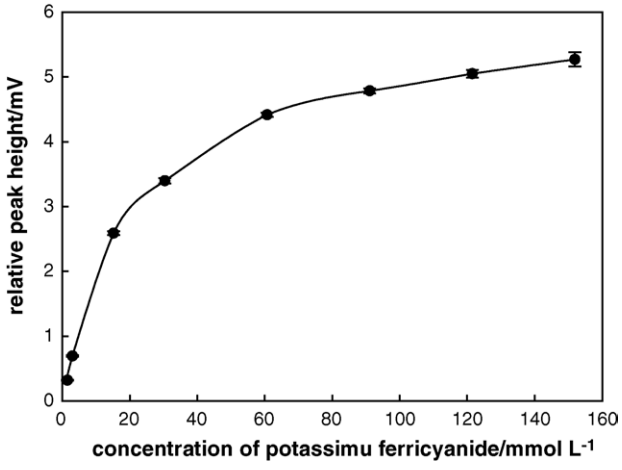

Fig. 2. Effect of concentration of potassium hexacyanoferrate(III). Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil 90 cm and 0.51 mm i.d., aspiration volume 100 μL in each case, concentration of standard 61.8 μmol L^{-1}, concentration of 4-amnioantipyrine 19.7 mmol L^{-1}.

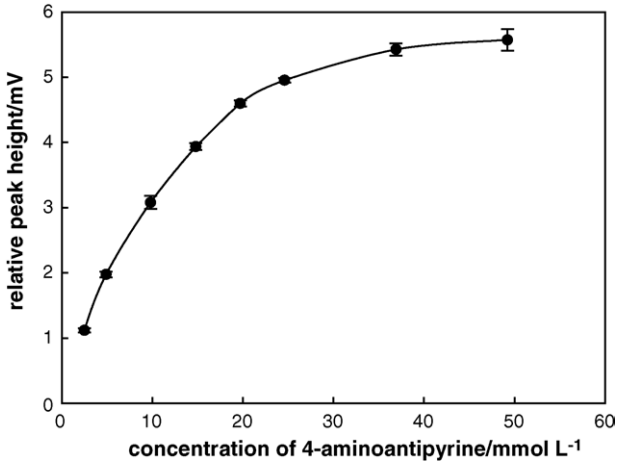

Fig. 3. Effect of concentration of 4-aminoantipyrine. Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil 90 cm and 0.51 mm i.d., aspiration volume 100 μL in each case, concentration of standard 61.8 μmol L^{-1}, concentration of potassium hexacyanoferrate(III) 30.4 mmol L^{-1}.

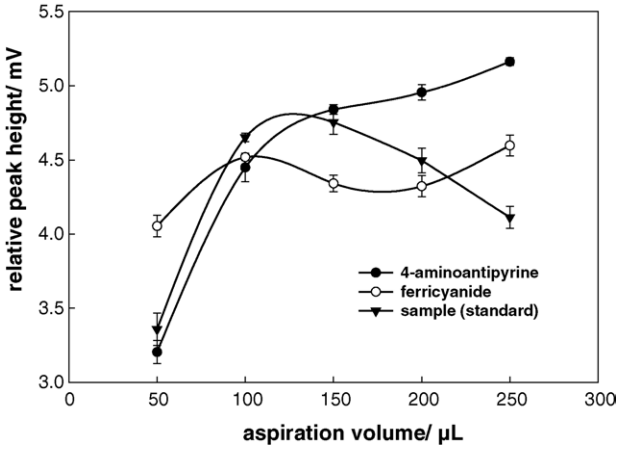

Fig. 4. Optimisation of aspiration volumes of sample and reagents. Experimental conditions were: flow rate 3.5 mL min^{-1}, reaction coil 90 cm and 0.51 mm i.d., concentration of standard 61.8 μmol L^{-1}, concentration of 4-amnioantipyrine 19.7 mmol L^{-1}, concentration of potassium hexacyanoferrate(III) 30.4 mmol L^{-1}.

a concentration of 60.7 mmol L^{-1} was chosen for the following measurements.

3.2.5. Concentration of 4-aminantipyrine

The effect of concentration of 4-aminoantipyrine was studied from 2.5 to 49.2 mmol L^{-1} (Fig. 3). The peak height increases with increasing concentration of aminoantipyrine. However, the increase in response above 19.7 mmol L^{-1} was not significant when the increase in concentration is taken into consideration, and thus a concentration of 19.7 mmol L^{-1} was chosen for subsequent experiments.

3.2.6. Aspiration volumes

The volume of reagents and sample drawn up is also affecting SIA responses. 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, the volume of 4-aminoantipyrine was considered. The response kept increasing with increasing aspiration volume that in turn increased the analysis time (Fig. 4). As a compromise between the reaction time and the response, an aspiration volume of 100 μL was chosen. Similarly, keeping the others at 100 μL, that of the ferricyanide varied as explained above. Maximum responses were obtained at aspiration volumes of 100 and 250 μL (Fig. 4) but due to the short analysis time the former is selected. In the case of the volume of sample/standard aspirated, maximum peak height was observed at a volume of 150 μL (Fig. 4) but there was no significant difference as compared to the response at 100 μL (2.2%). Moreover, the repeatability is much better at 100 μL and thus chosen for following measurements. Table 3 gives the summary of the optimised SIA parameters discussed so far.

3.3. Figures of merit

Using the abovementioned parameters the SIA system was evaluated for its response for different concentrations of standard ritodrine hydrochloride solutions. Linear calibration was found from 3.1 to 123.5 μmol L^{-1} with an excellent correlation (relative peak height = 0.069 C [μmol L^{-1}] + 0.149, r^2 = 0.999). The linear range obtained in this work is far better than those reported earlier [5–11,13]. The fixed scale of the FlowTEK program limited the extension of the range beyond 123.5 μmol L^{-1}. Our group is in the final stage to launch a better SIA program that may automatically change the scale using LabVIEW graphical programming language that may improve the linear range further. At concentrations of 6.2 and 15.4 μmol L^{-1}, R.S.D.'s of 2.4 and 2.3%, respectively, were registered (n = 10 measurements in each case). The detection limit (as 3σ-value at a concentration of 3.1 μmol L^{-1} for 10 determinations) [21] was found to be 1.0 μmol L^{-1}.

3.4. Analysis of pharmaceutical sample

Yutopar® tablets were analysed by the SIA method as well as with a reported manual spectrophotometric method [12]. The samples were diluted 10-fold in deionised water. A paired t-test [22] 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 (2.20 for 11 degrees of freedom at 95% confidence level) showing that there is no significant difference between the values obtained by the two

Table 3
Parameters optimised for the SIA determination of ritodrine hydrochloride

Parameters	Optimised value
Flow rate (mL min^{-1})	3.50
Reaction coil i.d. (mm)	0.51
Reaction coil length (cm)	90.00
Concentration of 4-aminoantipyrine (mmol L^{-1})	19.70
Concentration of potassium ferricyanide (mmol L^{-1})	60.70
Aspiration volume of 4-aminoantipyrine (μL)	100.00
Aspiration volume of potassium ferricyanide (μL)	100.00
Aspiration volume of sample (μL)	100.00

Table 4
Determination of Ritodrine hydrochloride in Yutopar® tablets (10 mg ritodrine hydrochloride/tablet) using the proposed SIA method and a reported spectrophotometric method

Sample	SIA, mg/tablet ($n = 10$)	Reported method, mg/tablet ($n = 3$)	Calculated t-value
Tablet 1	9.90 ± 0.15	10.02 ± 0.12	1.37
Tablet 2	10.03 ± 0.16	10.04 ± 0.14	0.17
Tablet 3	10.04 ± 0.10	10.14 ± 0.16	1.42

methods. Moreover, a t-test was employed to compare the results from SIA measurements with the claimed values and in all cases the calculated value were less than the tabulated (2.26 for 9 degrees of freedom at 95% confidence level) confirming once again the validity of the method. Table 4 presents the results obtained and the manufacturers' claimed value.

4. Conclusions

The advantages of the developed SIA method over the method reported by Bakry et al. [12] are better sensitivity and detection limit, simplicity, better repeatability, and being very fast since the latter requires 25 min incubation in a boiling water bath. It has additional advantage of wider linear range over the other methods [5–11,13]. Moreover, the reagent consumption is significantly reduced as compared to the same reaction but a manual method reported by Revanasiddappa and Manju [9] and thus waste generation is extremely minimised. Additional advantage of the SIA method is requiring less operator input since the method is automated.

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