Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 64 (2004) 1233–1236
Talanta
www.elsevier.com/locate/talanta

Flow-injection spectrophotometric determination of salbutamol with 4-aminoantipyrine☆

Isabel Dol, Moisés Knochen*

Departamento "Estrella Campos", Facultad de Química, Universidad de la República, Av. Gral. Flores 2124, Casilla de Correo 1157, 11800 Montevideo, Uruguay

Received 12 April 2004; received in revised form 28 April 2004; accepted 28 April 2004
Available online 11 September 2004

Abstract

A flow-injection method is proposed for the determination of salbutamol. The method involves the condensation of salbutamol with 4-aminoantipyrine in the presence of hexacyanoferrate (III) in alkaline medium, producing a coloured quinoneimide that was detected absorptiometrically at 500 nm.

The values of four variables (two reactor lengths and two reagent concentrations) were optimised by means of the sequential simplex method and their influence studied in univariant way.

The method was validated and compared with the HPLC method established in the United States Pharmacopeia (USP). Linearity was demonstrated in the range 0–74.1 mg/L of salbutamol sulfate ($r^2 = 0.9999$). Commercial samples of pharmaceuticals containing salbutamol sulfate (tablets and oral solutions) were analysed and the results obtained with the proposed method agreed with the USP method in less than 1.6%, with precision similar to the HPLC method (1%–2% R.S.D.). The sampling frequency was 75 samples/hour.

Keywords: Salbutamol; Flow-injection analysis; Spectrophotometric

1. Introduction

Salbutamol (α^1-[(tert-butylamino)methyl]-4-hydroxy-m-xylene-α,α'-diol, albuterol) is a direct-acting sympathomimethic with beta-adrenergic activity, employed as bronchodilator for the treatment of asthma and chronic obstructive pulmonary disease. It is also used to arrest premature labor in pregnancy [1]. Salbutamol sulfate is dispensed under several forms including tablets, syrups, aerosol inhalers and injections. This drug is usually administered alone, and only a few forms contain salbutamol associated with a second active ingredient.

Several different methods have been proposed for the determination of this substance in pharmaceutical dosage forms [2]. Direct spectrophotometric measurement at the maximum wavelengths of either 276 or 225 nm can be carried out only in the absence of other UV-absorbing substances. However, the fairly low absorptivity of this analyte favours the appearance of additive interferences when spectral overlap with some co-extracted excipients exists, which is the usual situation. Further, direct UV measurement does not apply to oral solutions where the combined spectra of preservatives, sweeteners and colorants produce considerable additive interference.

Selectivity may be increased by means of a chemical reaction producing a chromophore. Some of these reactions take advantage of the phenolic characteristics of salbutamol. For instance, the British Pharmacopoeia, 1988 [3], in its Salbutamol Injection monograph, included a colorimetric method based on the reaction of salbutamol with *N*,*N*-dimethyl-*p*-phenylenediamine sulfate and hexacyanoferrate(III) in the presence of sodium bicarbonate buffer. The chromogen formed was then extracted into chloroform and the absorbance measured at 605 nm. This method was replaced in later editions by a high-performance liquid chromatography (HPLC) method [4]. HPLC is also the method of choice in the albuterol monograph in the United States Pharmaceopeia [5].

☆ Presented in the 12th International Conference on Flow Injection Analysis and Related Techniques (12th ICFIA), Mérida, Venezuela, December 7–13, 2003.

* Corresponding author. Tel.: +598 2 9241808; fax: +598 2 9241906.
E-mail address: mknochen@fq.edu.uy (M. Knochen).

0039-9140/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.04.040

Other methods found in the literature include visible spectrophotometry after colour development by means of novel reagents [6] and flow injection with on-line separation and spectrophotometric detection after reaction with the Folin–Ciocalteau reagent [7].

HPLC has important advantages in terms of freedom from interferences and selectivity from degradation products and impurities. However, for some purposes, such as quality control of the production, other less expensive methods may be equally acceptable provided their figures of merit are satisfactory. In this context, a spectrophotometric method that can be automated by means of flow-injection analysis (FIA) [8–10] may be useful.

As stated before, the salbutamol molecule has a phenolic group that can be used to generate a chromophore, thus enabling a quantitative method to be developed. The reaction with 4-aminoantipyrine in the presence of an oxidant, such as hexacyanoferrate (III) in alkaline solution, is one of the most widely used reactions for the determination of phenols [11]. This reaction, first studied by Emerson [12], produces a red-orange colour attributed to the formation of a quinoneimide through a condensation process. This reaction has been exploited for the determination of phenolic substances in different matrixes, and a number of papers have been published concerning applications [13–15], as well as the mechanism and products [16]. Pharmaceutical applications of this reaction have also been dealt with [17–19], however, in a revision of the literature no application to the determination of salbutamol could be found. In spite of this, this reaction has been employed as a qualitative test for the identification of salbutamol (for instance, in the British Pharmacopoeia [3,4]).

When carried out in batch, this reaction usually requires the extraction of the coloured substance formed into chloroform prior to the spectrophotometric measurement. In the literature [14], this step is justified on the basis of enhanced colour stability and separation from the coloured reaction mixture. The use of FIA should overcome the need for such separation by virtue of the inherently accurate timing. Besides, the use of a transient signal measured from a baseline, characteristic of FIA may contribute to minimise the influence of a coloured reagent such as hexacyanoferrate (III).

In this work, a flow-injection method was developed for the determination of salbutamol. The method does not require solvent extraction and can be applied to pharmaceutical dosage forms (tablets and oral solutions).

2. Materials and methods

2.1. Apparatus

The flow system is depicted in Fig. 1. The fluids were pumped by a Rainin Dynamax (Woburn, MA, USA) RP-1 peristaltic pump employing Tygon® pump tubing. Injection was performed by means of a 6-port 2-position injection valve with microelectric actuator (Valco Cheminert; Houston, TX, USA) fitted with a 50 μL loop.

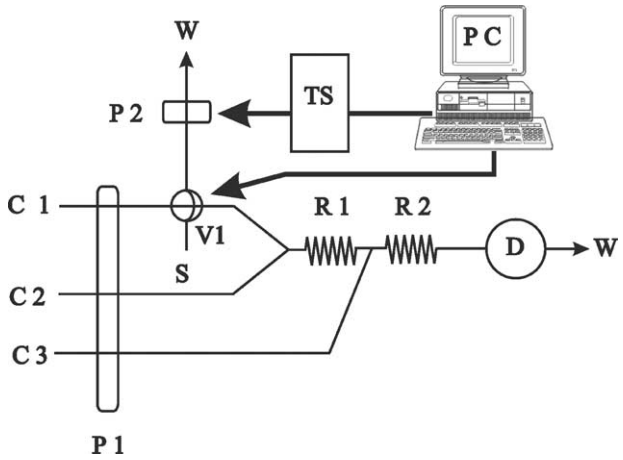

Fig. 1. Schematic diagram of the flow-injection system. P1, main peristaltic pump; P2, auxiliary peristaltic pump (2.0 mL/min); C1: water (0.77 mL/min); C2: reagent no. 1 (0.60 mL/min); C3: reagent no. 2 (0.60 mL/min); PC: personal computer; TS: Triac switch; V1: six-port injection valve with 50-μL loop; R1 and R2: reactors, internal diameter 0.8 mm (length: R1, 14 cm and R2, 50 cm); S: sample; D: detector, spectrophotometer set at 500 nm; W: waste.

An auxiliary pump (Ismatec (Zürich, Switzerland) MS-CA2/820) was used for loading the sample into the sampling loop. This pump was switched on and off as required under software control.

Reactors (coiled in "8" shape) were made of 0.8 mm ID FEP tubing (Cole Parmer, USA), and connections employed were Valco Cheminert flangeless fittings.

Detection was carried out with a Shimadzu (Kyoto, Japan) UV-240 UV-visible recording spectrophotometer with a Hellma (Müllheim, Germany) 178.010 quartz flow cell (internal volume 80 μL). Preliminary experiments showed that the absorbance maximum was located at 500 nm, and this wavelength was chosen for detection. The absorbance signal was recorded as a function of time, and peak-height measurements were obtained directly from the instrument by the peak-pick function.

The operation was controlled by software compiled in QuickBASIC 4.0 and running under MS-DOS 6.0 in a 486-based notebook computer (Canon Innova Book 10). The program provided the appropriate timing and controlled the Valco injector via the RS-232 serial port and the auxiliary pump via the LPT parallel port and a lab-made optoisolated triac switch. The injector valve was then commuted to the "load" and "inject" positions and the auxiliary pump (used only for loading the sampling loop) turned on and off as required.

2.2. Reagents

Salbutamol sulfate (Spectrum, USP grade) and 4-aminoantipyrine (Eastman) were used without further purification. Other reagents were of analytical reagent grade.

The following solutions were freshly prepared:

Sodium carbonate 1% (m/v) in water.
Reagent no. 1: 4-aminoantipyrine 0.4% (m/v) in water.
Reagent no. 2: Potassium hexacyanoferrate (III) 2% (m/v) in 1% sodium carbonate.
Salbutamol sulfate was dissolved in water and diluted with water as needed.

2.3. Analysis of dosage forms (oral solutions and tablets)

Tablets were pooled (20 units) and ground in a mortar. An amount of powder equivalent to one tablet (4.8 mg of salbutamol sulfate) was accurately weighed in a 100-mL volumetric flask and extracted for 1 h with 50 mL of water in a wrist-action shaker, followed by 10 min in an ultrasonic bath. The volume was then adjusted with water to 100 mL and the solution filtered through 0.45-μm polyamide membrane filter, discarding the first 15 mL of the filtrate. This solution was injected in the FIA system.

For oral solutions, the density was determined, and about 10 mL (equivalent to 4.8 mg of salbutamol sulfate) was accurately weighed and diluted with water to 100 mL in a volumetric flask. This solution was used as the sample.

2.4. Optimisation

The operation of the system was optimised using the sequential simplex method [20]. The following operating variables were considered: reactor length (R1 and R2), and reagent concentration (4-aminoantipyrine and potassium hexacyanoferrate (III)). For R1, the values tested ranged from 0 to 200 cm; for R2, from 20 to 500 cm. Concentration of 4-aminoantipyrine ranged from 0.20% to 0.80% (m/v); concentration of potassium hexacyanoferrate (III) ranged from 0.50 to 3.0% (m/v).

3. Results and discussion

3.1. Optimisation

Only four of the variables were chosen for formal optimisation. The values of some variables were chosen based on practical considerations and once fixed, those four variables were optimised. For instance, the sampling loop size determines the peak size, and thus a value of 50 μL was chosen in preliminary experiments to obtain appropriate peak size. Similarly, flow rates were chosen attempting to ensure excess reagent and to obtain a satisfactory sampling frequency.

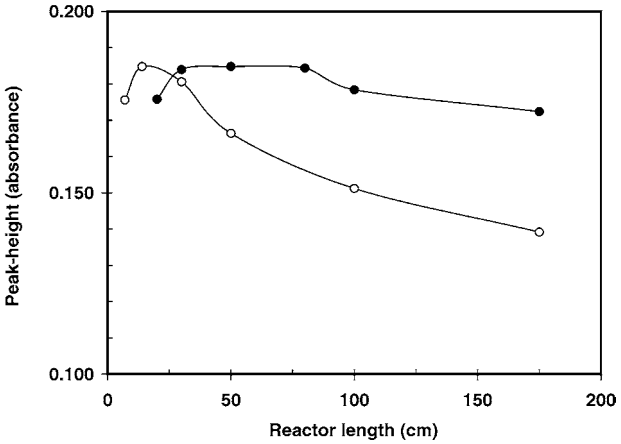

Fig. 2. Plot of variation of system response with reactor length. ○ = R1; ● = R2. Concentration of potassium hexacyanoferrate (III) = 2% m/v; concentration of 4-aminoantipyrine = 0.4% m/v. For variation of R1, R2 was kept at 50 cm; for variation of R2, R1 was kept at 14 cm.

Maximum response was obtained in the seventh simplex experiment with a system employing the following values: reactor length R1 = 14 cm and R2 = 50 cm; reagent concentrations: 4-aminoantipyrine 0.4% (m/v) and potassium hexacyanoferrate (III) 2% (m/v).

Figs. 2 and 3 show univariant response curves for the four variables in reduced ranges encompassing the optimum values found. The optimum conditions arising from these univariant experiments agreed with those found by means of the multivariant sequential simplex approach.

3.2. Validation

Linearity was investigated by means of a six point calibration curve in the range 0–74.1 mg/L of salbutamol sulfate. The best-fit regression line was $A = 0.0031C - 0.00006$ ($r^2 = 0.9999$).

In order to investigate accuracy and precision in commercial samples, tablets (Tarison®, ICU-Vita, and Salbutamol Lazar, both with a label claim of 4.8 mg per tablet of salbuta-

Table 1
Contents found (average of six injections) and relative standard deviation (s_r (%)) obtained for commercial samples analyzed by the FIA and (HPLC) methods

	Label claim (salbutamol sulfate)	Contents found and relative standard deviation (FIA) (%)	Contents found and relative standard deviation (HPLC) (%)	Relative difference of contents (%)
Solutions (mg/dL)				
Ventolin	48	48.2 (1.74)	49.0 (0.56)	−1.6
Tablets (mg/tablet)				
Tarison	4.8	4.40 (0.98)	4.42 (1.59)	−0.4
Salbutamol Lazar	4.8	4.60 (2.23)	4.59 (1.86)	0.2

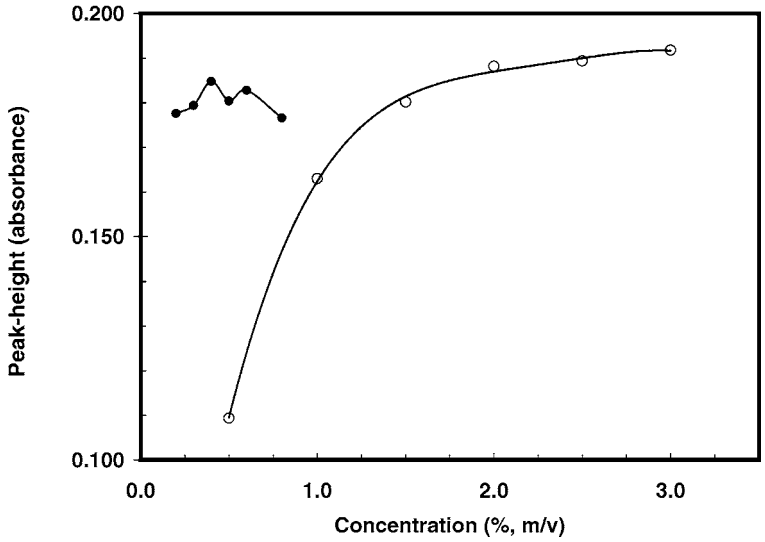

Fig. 3. Plot of variation of system response with reagent concentration. ○ = potassium hexacyanoferrate (III) (PHCF); ● = 4-aminoantipyrine (4-AAP). R1 = 14 cm and R2 = 50 cm. For variation of PHCF, 4-AAP was kept at 0.4%; for variation of 4-AAP, PHCF was kept at 2%.

mol sulfate) and oral solutions (Ventolin®, GlaxoWellcome, label claim 48 mg/dL of salbutamol sulfate) were analysed by the proposed method and by a standard HPLC reference method [5]. The results obtained by both methods (Table 1) were compared.

The close agreement observed between both methods suggests that no significant interferences existed in these determinations. Table 1 also shows the precision ($n = 6$) obtained, which was similar to that of the HPLC method and appropriate for the purpose. Under these conditions, the sampling frequency obtained was 75 samples per hour.

4. Conclusions

The use of the sequential simplex method of optimisation allowed to find the optimum conditions for the system in a fast and efficient way.

The figures of merit exhibited by the proposed method suggest that it could be used in routine pharmaceutical quality control without the need of resorting to more expensive and complex procedures such as HPLC.

Acknowledgements

The authors thank Comisión Sectorial de Investigación Científica de la Universidad de la República (CSIC-UDELAR) and Programa de Desarrollo de Ciencias Básicas (PEDECIBA, UNDP URU/97/016) for financial support, Leticia Vázquez for laboratory work and Javier Giglio for the HPLC determinations.

References

[1] K. Parfitt (Ed.), Martindale. The Complete Drug Reference, 32nd ed., Pharmaceutical Press, London, 1999.
[2] H.Y. Aboul-Enein, A.A. Al-Badr, S.E. Ibrahim, in: K. Florey (Ed.), “Analytical Profiles of Drug Substances”, vol. 10, Academic Press, 1981, pp. 665–689.
[3] British Pharmacopoeia, vol. II, Her Majesty’s Stationery Office, London, 1988, pp. 845–846.
[4] British Pharmacopoeia, vol. II. The Stationery Office, London, 1999, p. 2083.
[5] United States Pharmacopeia, 26th revision, United States Pharmacopeial Convention, Rockville, 2003, p. 56.
[6] G.G. Mohamed, S.M. Khalil, M.A. Zayed, M.A. El-Hamid El-Shall, J. Pharm. Biomed. Anal. 28 (2002) 1127.
[7] D. Satinsky, R. Karlicek, S. Svoboda, Anal. Chim. Acta 455 (2002) 103.
[8] J. Ruzicka, E. Hansen, Flow Injection Analysis, 2nd ed., Wiley, New York, 1988.
[9] M. Trojanowicz, Flow Injection Analysis: Instrumentation and Applications, World Scientific, Singapore, 2000.
[10] J. Martínez-Calatayud, Flow Injection Analysis of Pharmaceuticals-Automation in the Laboratory Taylor and Francis, London, 1996.
[11] M. Pesez, J. Bartos, Colorimetric and Fluorimetric Analysis of Organic Compounds and Drugs, Dekker, New York, 1974, pp. 94–95.
[12] E.I. Emerson, J. Org. Chem. 8 (1943) 417.
[13] R.W. Martin, Anal. Chem. 21 (1949) 1419.
[14] M.B. Ettinger, C.C. Ruchhoft, R.J. Lishka, Anal. Chem. 23 (1951) 1783.
[15] T.A. LaRue, E.R. Blakley, Anal. Chim. Acta 31 (1964) 400.
[16] Y. Fiamegos, C. Stalikas, G. Pilidis, Anal. Chim. Acta 467 (2002) 105.
[17] C.A. Johnson, R.A. Savidge, J. Pharm. Pharmacol. 10 (1958) 171T.
[18] C.F. Hiskey, N. Levin, J. Pharm. Sci. 50 (1961) 393.
[19] R. Karlicek, P. Solich, Anal. Chim. Acta 285 (1994) 9.
[20] F. Walters, L. Parker, S. Morgan, S. Deming, Sequential Simplex Optimization, electronic ed., Multisimplex AB, http://www.multisimplex.com, 1999.