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Spectrophotometric determination of adrenaline with an oxidative column in a FIA assembly*

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Abstract: A single channel FIA assembly is proposed for the spectrophotometric determination of adrenaline, the aqueous sample solution is directly injected into the carrier stream leading the sample through a manganese dioxide column at 80°C, and on to the spectrophotometer flow-cell. The calibration graph is linear up to 17 ppm of adrenaline. The influence of other substances has been studied and the method has been applied to the determination of adrenaline in a pharmaceutical formulation.

Keywords: *Oxidizing minicolumns; spectrophotometry; FIA; adrenaline.*

Introduction

Adrenaline (epinephrine) is a white to nearly white, odourless, microcrystalline powder or granules, gradually darkening on exposure to light and air; it was the first hormone to be obtained in crystalline form [1]. It is not active orally owing to destruction in the gastro-intestinal tract and to conjugation and oxidation which occur in the liver. Generally the drug is administered parenterally although preparations suitable for inhalation are available largely to provide a local effect in the lungs [2].

Adrenaline is oxidized by different agents such as iodine, potassium hexacyanoferrate(III), potassium permanganate and manganese dioxide. Oxidation occurs through the transient formation of adrenaline quinone with formation, under proper conditions [3], of adrenochrome. Study of the oxidation of the drug by molecular oxygen [4] has shown that the reaction involved is extremely complex. Results suggest that oxidation in aqueous solution can occur in the absence of heavy metal ions and probably involves free radical sequences.

The purity of the bulk drug is established using a non-aqueous system; adrenaline can be titrated in glacial acetic acid with perchloric acid using Crystal Violet as the indicator [1]. Spectrophotometric micro-titration procedures have been developed [5].

Colorimetric procedures which can be applied to the analysis of adrenaline are based on the presence of catechol or phenolic groups and hence have a variable degree of selectivity. Some other colour-producing reactions for adrenaline have been described [2]. Measurement of native or induced fluorescence provides a useful means of analysis [6].

Chromatographic methods have been proposed; thin-layer and paper chromatography have been used extensively. Since adrenaline is a polar, non-volatile compound, derivatization is a requirement for gas chromatography [7, 8].

One interesting trend in flow injection analysis (FIA) methodology is the use of solid-bed reactors. These have the advantage of simplification of manifolds and a reduced necessity for sample dilution and expensive reagents [9]. Immobilized enzymes have been widely exploited in pharmaceutical and biomedical analysis as have other solid or immobilized reagents such as potassium hexacyanoferrate(III) immobilized on an anionic exchange resin for the determination of paracetamol [10, 11], and copper carbonate for the atomic absorption spectrometric determination of amino acids [12].

This paper deals with the determination of adrenaline using manganese dioxide as an

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oxidative column in the FIA manifold. The sample is injected into the carrier stream passing through the solid reactor and the oxidation product is monitored by the spectrophotometric detector.

Experimental

Reagents and procedures

All chemicals used were of analytical-reagent grade. Aqueous solutions of adrenaline were obtained from Llorente S.A. and manganese dioxide from Matheson, Coleman and Bell.

Figure 1 schematically illustrates the overall configuration of the continuous-flow system. The assembly includes a Model 5041 sample injector (Reodhyne) and a Minipuls 2 peristaltic pump (Gilson). Spectrophotometric measurements were made with a Model 8452 A spectrophotometer (Perkin Elmer) provided with a 30-μl flow-cell (Hellma); PTFE tube coils were of 0.8 mm i.d. for the oxidative column and 0.5 mm i.d. in the flow injection assembly.

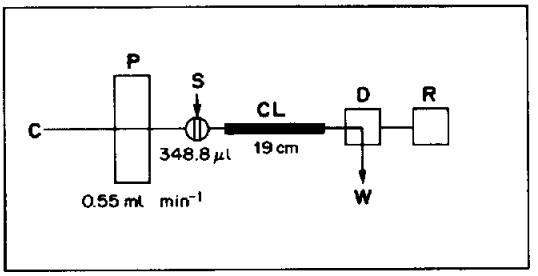

Figure 1
Flow injection manifold.

Oxidative columns were prepared by introducing the manganese dioxide particles into PTFE coils by suction. When the column was in the manifold, a water stream was circulated for 5–10 min before injecting the sample solutions.

Continuous-flow procedure. 348.8 μl of sample solution containing adrenaline was injected directly into a distilled and de-areated water carrier; after passing through the manganese dioxide column the oxidized sample was led to the spectrophotometer flow-cell and the absorbance read at 300.0 nm. The carrier solution, sample loop and column were immersed in a water-bath at 80°C.

Modified simplex method of optimization. The initial simplex method was chosen according to Yarbro and Deming [13] with the modification proposed by Morgan and Deming [14]. The modified simplex program for this work was based upon the method and flow-line of Nelder and Mead [15].

The range of FIA variables considered is shown in Table 1; the parameter to be optimized was peak-height. When a stable baseline was obtained on the monitor, a sample was injected and the subsequent absorbance read at 300.0 nm. This was repeated until the repeatability of peak-height measurements had a relative standard deviation (RSD) less than 1% (four or five replicates). Zero values for peak-height were assigned to wild results or those outside the accepted range. Once the program had been completed the highest transient signals were selected; 10 replicate runs were carried out to select a suitable set of parameters for optimal sensitivity and reproducibility.

Table 1
Simplex method of optimization

Parameter (units)	Tested range	Selected value
Solid reactor length (cm)	4–50	19
Flow rate	200–999	0.55 ml min^{-1}
Sample volume	0–150	348.8 μl

Constant parameters; adrenaline 10 ppm; temperature 80°C (solid reactor, sample loop and the carrier solution immersed in water-bath); carrier-distilled and deaerated water.

Results and Discussion

Preliminary investigations

Experiments were carried out by using a batch procedure to test the oxidation reaction in different aqueous media and the influence of temperature. Aqueous solutions (9.1×10^{-5} M) of adrenaline were placed in a beaker containing about 0.3 g of manganese dioxide and the pH was adjusted by addition of NaOH or HCl. Tests were carried out at room temperature and at 40, 60, 80, 90 and 100°C by placing the beaker in a water-bath. Spectra were recorded at timed intervals up to 10 min. Figure 2 shows the results optimised for pH; the reaction is strongly influenced by temperature.

Flow injection experiments

Experiments carried out using a FIA

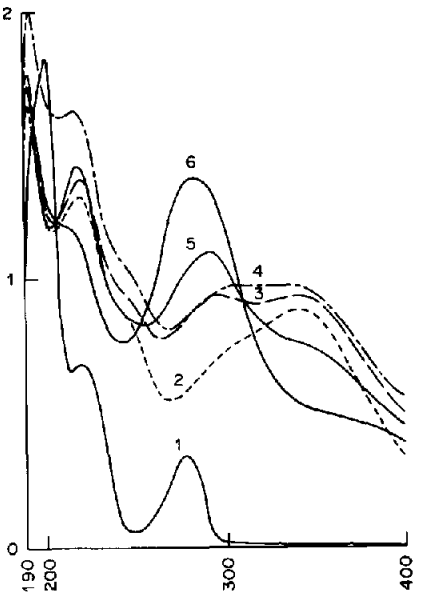

Figure 2
Absorption spectra of oxidized adrenaline (10 ppm). (1) Unoxidized drug; (2–6) oxidized by solid manganese dioxide at 20°, 40°, 60°, 80° and 100°C.

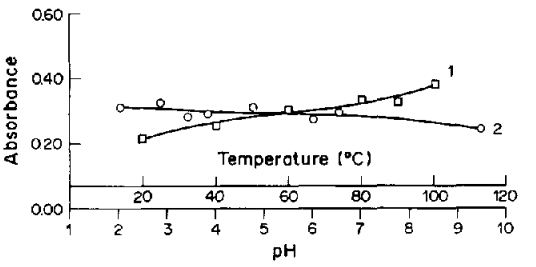

Figure 3
Influence of temperature (1) and pH (2) on the oxidation of adrenaline. 10 ppm adrenaline; column length 50 cm; flow rate 0.38 ml min^{-1} and sample volume 191 µl.

assembly confirmed some of the results reported in the above paragraph. The flow injection assembly included an oxidative column of 50 cm; the sample was directly injected into the carrier stream using a flow rate of 0.38 ml min^{-1}; a sample volume of 190 µl and coil length from column to detector flow-cell of 50 cm; the pH was tested by preparing different carrier solutions. The influence of temperature was studied by placing separately in the water-bath: the carrier solution container; the carrier solution and oxidative column; and the carrier stream, sample loop and oxidative column.

The pH of the carrier solution was tested, the range studied was pH 2.0–9.5. Higher transient signals were obtained at pH 2–6.5. pH 5 and 80°C were selected for further work; a carrier of distilled water previously deaerated was used in order to prevent bubbles at the temperature used. The influence of pH and temperature in a continuous-flow manifold is depicted in Fig. 3.

Once the chemical variables had been selected, a study on the optimization of FIA parameters was carried out by means of the modified simplex method. The first vertex of the initial simplex gives a peak-height of 0.2274 absorbance units; in four experiments an improvement from 0.2274 to 0.4898 was obtained. After 25 experiments it was decided that the system did not merit further work; points 20, 23 and 26 were pre-selected and then 10 replicates were performed for each point in order to select the optimum set of experimental variables. Point 23 (Table 2) was selected, the values being those reported in Table 1 for the optimized parameters.

Analytical application

The calibration graph showed a linear behaviour in the 0–17 ppm range. The regression equation for absorbance (A) against concentration (C) of adrenaline (ppm) was: $A = 0.0050\ C + 0.0018$. The detection limit, defined as twice the mean of baseline noise, was 0.05 ppm of adrenaline.

The reproducibility and sample throughput of the procedure was tested by injecting 10 ppm of adrenaline solution (11 replicates) under the reported experimental conditions. The RSD was 0.19%. The throughput was 45 samples/h.

The influence of other compounds that can be found in pharmaceutical formulations containing adrenaline was studied by preparing solutions of 7.0 ppm of adrenaline with different amounts of the interfering compound. The errors were calculated by comparing the peak-height with that obtained by injecting an aqueous solution of pure adrenaline. Table 3 shows the results; procaine, amethocaine (tetracaine) and picric acid seriously interfered with the assay.

Adrenaline was determined in a pharmaceutical formulation (Colicursi Epinefrina from Laboratorios Cusi, SA) and the results compared with those supplied by manufacturer. The declared concentration was 20 mg ml^{-1}; the relative error was +3.29%.

Table 2
Simplex method of optimization

Point *N*	Reaction-column length (cm)	Flow rate (arbitrary units)	Sample volume (cm)	Peak-height (absorbance units)	RSD (%)
1	4	200	0	0.2275	
2	47.4	388.3	35.3	0.2900	
3	14.8	953.3	35.3	0.3622	
4	14.8	388.3	141.4	0.4898	0.5
5	47.4	953.3	141.4	0.4243	
6	3.99	1141.6	176.7	0	
7	36.5	576.6	70.7	0.3980	
8	50.9	325.5	200.3	0	
9	23.9	796.4	76.6	0.4545	
10	20.9	848.7	168.9	0	
11	32.6	644.6	95.3	0.4516	
12	0.18	266.3	67.4	0	
13	35.6	781.5	122.9	0.4848	2.5
14	16.9	666.2	132	0.4779	0.4
15	21.0	427.6	187.6	0	
16	22.2	704.2	104.4	0.4807	0.4
17	32.1	583.2	113.8	0.4575	
18	20.7	645.4	127.5	0.4592	
19	25.2	584.9	132.2	0.4758	1.6
20	15.8	527.2	136.7	0.4744	0.5
21	19	546.2	122.9	0.4712	0.6
22	18.3	454.0	150.6	0	
23	18.8	523.2	129.8	0.4957	0.6
24	23.4	470.4	132.2	0.4935	0.6
25	12.8	336.4	136.8	0.4894	0.6
26	15.9	398.5	135.6	0.5025	0.3
27	23.9	539.7	123.7	0.4912	0.9
28	21.6	501.9	128.1	0.4840	0.8
29	23.6	505.1	127.9	0.4840	0.8
30	21.3	531.5	126.8	0.4839	0.9
31	19.9	469.1	129.7	0.4589	0.8

Table 3
Influence of other compounds

Compound	Concentration (ppm)	Relative error (%)
Atropine	200	1.3
Lignocaine	100	2.8
Picric acid	0.5	2.8
Procaine	0.2	4.2
Amethocaine	0.1	1.9
Phenol	500	1.6
Borax	200	0.8
Zinc sulphate	200	2.5
Sodium bicarbonate	10	4.6
Mercuric cyanide	100	0.9

Conclusions

A spectrophotometric–FIA procedure is proposed for the determination of adrenaline for use in the control analysis of pharmaceutical formulations. The method is based on the oxidation by a solid-bed reactor of manganese dioxide and it is applicable with acceptable precision. The continuous flow assembly is very simple and is easy to prepare.

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