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Short communication

Rapid zero-order and second-derivative UV spectrophotometric determination of cyanocobalamin in pharmaceutical formulations containing dextran and preservatives

M.J. López de Alda [a], P. Paseiro [a,*], A.G. Goicoechea [b]

[a] *Department of Analytical Chemistry, Nutrition and Bromatology, Area of Nutrition and Bromatology, Faculty of Pharmacy, University of Santiago de Compostela, 15706 Santiago de Compostela, Spain*

[b] *Laboratory of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of the Basque Country, 01007 Vitoria, Spain*

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1. Introduction

Owing to its favourable effects on erythrocyte maturation, cyanocobalamin (vitamin B_{12}) is widely used as a co-adjuvant in drug treatments for iron-deficiency anaemias [1]. Most of the veterinary drugs used for treating this anaemia contain combinations of iron–dextran and not less than 10 $\mu g\,ml^{-1}$ of cyanocobalamin [2]. Except for some orally administered preventative treatments, cyanocobalamin/iron–dextran formulations are generally administered intramuscularly and also contain antimicrobial preservatives such as phenol. The presence of this preservative means that the microbiological methods commonly used for the determination of cyanocobalamin [3,4] cannot be applied to these formulations. Furthermore, the high opacity imparted to these solutions by the dextran excipient prevents the determination of the cyanocobalamin component by traditional spectroscopic methods [4–6].

This paper reports a simple, rapid and accurate spectrophotometric method suitable for the determination of cyanocobalamin in pharmaceutical formulations containing dextran and preservatives. The key step in this method is prior purification of the sample on Sep-pak C_{18} cartridges.

2. Experimental

2.1. Instrumentation

Absorption spectra were measured in 1 cm quartz cuvettes using a Shimadzu UV-160 spectrophotometer.

* Corresponding author.

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2.2. Materials

All reagents were of analytical grade. The Sep-Pak C_{18} cartridges used in the sample purification step were purchased from Waters (ref. 051910). An aqueous cyanocobalamin stock solution (100 μg ml^{-1}) was prepared from vitamin B_{12} (USP, Merck ref. 524950). Cyanocobalamin standards (5, 8, 10, 12 and 15 μg ml^{-1}) were prepared by diluting 5, 8, 10, 12 or 15 ml of the stock solution with 8 ml of ethanol and diluting this solution to 100 ml with distilled water; these solutions were stored in the dark before use. A commercial cyanocobalamin formulation containing 10 μg ml^{-1} of cyanocobalamin with 10% (w/v) of iron–dextran as excipient and an otherwise equivalent formulation without the cyanocobalamin (referred to as the drug-free formulation) were both purchased from a pharmaceutical laboratory.

2.3. Procedure

Purification

A 25-ml aliquot of the cyanocobalamin formulation was diluted with 25 ml of distilled water, and using a 50-ml syringe, passed at a rate of 10 ml min^{-1} on to a Sep-Pak C_{18} cartridge previously activated with 2 ml of methanol followed by 3 ml of distilled water. Three 10-ml portions of distilled water were used to complete transfer of the sample on to the solid phase. All aqueous eluates were discarded. The cartridge was then reversed and eluted with 2 ml of ethanol and the ethanolic eluate was diluted to 50 ml with distilled water. This solution was passed on to a second cartridge and eluted exactly as before, thus yielding 2 ml of ethanolic eluate. The sample for analysis was obtained by diluting this ethanolic eluate to 25 ml with distilled water.

Spectrophotometry

Direct UV absorption spectra were recorded in the range 200–400 nm; their second derivatives were then generated mathematically by the data handling system of the spectrophotometer.

3. Results and discussion

In the absence of interfering compounds, the absorbance maximum of cyanocobalamin (361 nm) can be used for its spectrophotometric determination, for example, by the method described in the USP [4], which is used to monitor the concentration of this vitamin in some pharmaceutical formulations. However, drugs of the type studied here, in which the cyanocobalamin is combined with iron–dextran, require removal of the dextran component before determination by the USP method. The purification method optimized in this work used Sep-Pak C_{18} cartridges to eliminate simply and rapidly the interfering dextran component, which is not retained on the cartridge under the conditions described. Elution of the retained material with ethanol subsequently yields a transparent solution suitable for the precise and accurage spectrophotometric determination of the cyanocobalamin.

Fig. 1 shows the UV spectra of a cyanocobalamin standard solution (10 μg ml^{-1}) and the two formulations after purification using the described procedure. In these latter spectra the interference due to the iron–dextran component has been almost completely eliminated in the wavelength range 300–400 nm. Moreover, the small amount of residual interference due to background absorption is effecively overcome by taking the second derivatives of the spectra of the purified formulations (Fig. 2). Quantification of the cyanocobalamin was therefore achieved by measurement of the peak-to-trough height of the signal corresponding to the second derivative of the direct spectrum between 352 and 361 nm.

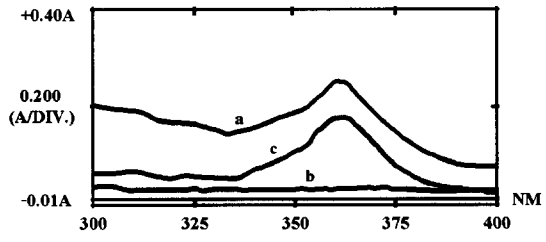

Fig. 1. UV absorption spectra of (a) the commercial cyanocobalamin formulation (10 μg ml^{-1}) and (b) the drug-free formulation, both after purification by the described procedure, and (c) a standard solution of cyanocobalamin (10 μg ml^{-1}).

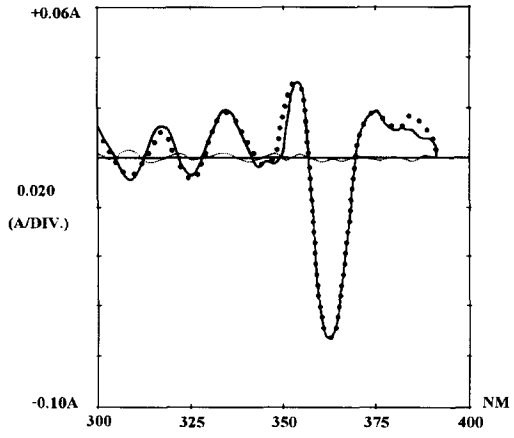

Fig. 2. Second derivatives of the UV absorption spectra of a standard solution of cyanocobalamin (10 μg ml^{-1}) (●), the drug-free formulation (· · ·) and the cyanocobalamin formulation (10 μg ml^{-1}) (—).

3.1. Linearity of the instrumental response and method precision and accuracy

The equation of the calibration line, constructed by regressing peak-to-trough heights (^2D) against cyanocobalamin concentration (c) for the five standards (5–15 μg ml^{-1}) was $^2D = 0.01072 (\pm 1.7 \times 10^{-4})c - 0.0018(\pm 0.0018)$; the correlation coefficient (r) was 0.9996.

The method precision, calculated as the RSD for the determination of cyanocobalamin in six replicate samples of the cyanocobalamin formulation, was 1.75%.

The method accuracy was determined by two approaches. First, the error attributable to background absorption was determined from the second-derivative spectrum of the drug-free formulation. This indicated an absolute error of 0.2 μg ml^{-1} (expresed a cyanocobalamin concentration), which for the cyanocobalamin formulation (10 μg ml^{-1}) is equivalent to a 2% relative error. Second, three 100-ml aliquots of the drug-free formulation were spiked with known amounts of cyanocobalamin and subjected to purification and then determination of the vitamin component. The recoveries (Table 1) also indicated a relative error of ca. 2%.

Table 1
Recoveries for the determination of cyanocobalamin in drug-free formulations spiked with cyanocobalamin

Sample No.	Amount added (mg)	Amount measured (mg)	Recovery (%)
1	0.800	0.752	94.0
2	1.000	0.979	97.9
3	1.200	1.220	101.7
			Mean: 97.9

4. Conclusions

A simple, rapid method is proposed for the determination of cyanocobalamin in pharmaceutical formulations containing a dextran excipient and a preservative, both of which are incompatible with pharmaceutical methods for the determination of cyanocobalamin. This method has satisfactory precision (RSD = 1.75) and accuracy (recovery = 98%) and is suitable for routine analysis such as quality control.

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