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Short communication

High-performance liquid chromatographic separation and determination of cobalt(II), cobalt(III) and iron(II) using bis(salicylaldehyde)tetramethylethylenediimine

M.Y. Khuhawar*, S.N. Lanjwani

Institute of Chemistry, University of Sindh, Jamshoro, Sindh, Pakistan

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Abstract

The reactions of bis(salicyclaldehyde)tetramethylethylenediimine (H_2SA_2Ten) with cobalt(II), cobalt(III), iron(II) and iron(III) were studied and conditions for their extraction as metal chelates from aqueous solution into chloroform were optimized. Cobalt(II), cobalt(III) and iron(II) were completely separated on a 3-μm Microsorb ODS column when eluted isocratically with methanol–water–acetonitrile (60:39:1, v/v/v), with spectrophotometric detection at 270 nm. Linear calibrations were obtained over the range 0–125 μg per 2 ml of extract. The detection limits were in the range 0.25–1.0 μg/ml. The oxidation of cobalt(II) to cobalt(III) with air and nitric acid was examined and the method was applied to the determination of cobalt and iron in pharmaceutical preparations. The results obtained were compared with those given by atomic absorption spectrometry.

1. Introduction

A number of complexing reagents have been reported for the high-performance liquid chromatographic (HPLC) determination of cobalt and iron, the main ones being 8-hydroxyquinoline [1], 6-hydroxy-5-nitrosonaphthalene-2-sulphonic acid [2], acetylacetone [3], diethyldithiocarbonate [4], picolinaldehyde-4-phenyl-3-thiosemicarbazone [5], 8-quinolinethiol [6] and various azo derivatives [7–11].

Zhao and Fu [12] separated iron(II), cobalt(II) and iron(III) chelates with 4-(5-chloro-2-pyridylazo)-1,3-diaminobenzene on a C_{18} bonded stationary phase with methanol–water as the mobile phase. Fernandez et al. [13] separated and determined iron(II) and iron(III) by reversed-phase HPLC using in situ complexation with *o*-phenanthroline.

The tetradentate Schiff bases are interesting complexing reagents owing to their reactions towards a limited number of metal ions [copper(II), nickel(II), palladium(II), platinum (II), cobalt, iron, vanadium(IV) and zinc(II)]. Their cobalt and iron complexes have attracted attention because of their possible use as biological models as oxygen carriers [14–16]. Averill and Broman [17] investigated the electroanalytical properties of cobalt chelates of bis(salicylaldehyde)tetramethylethylenediimine [2,3-dimethyl-2,3-N,N'-butanebis(salicylaldiimine); (H_2SA_2Ten]. H_2SA_2Ten has been reported as a

* Corresponding author.

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complexing reagent for the gas chromatographic (GC) and normal-phase HPLC determination of copper(II) and nickel(II) using precolumn derivatization and solvent extraction with toluene [17]. Recently, H_2SA_2Ten has been examined for the simultaneous extraction and separation of copper(II), nickel(II) and oxovanadium(IV) using normal-phase HPLC [19]. In this work, cobalt(II), cobalt(III) and iron(II) were separated and determined using reversed-phase HPLC. The method was applied to the determination of cobalt and iron in pharmaceutical preparations.

2. Experimental

H_2SA_2Ten was prepared by heating freshly distilled salicylaldehyde and 2,3-dimethyl-2,3-diaminobutane in a 2:1 molar ratio as reported [17,18]. The cobalt(II) complex was prepared by refluxing equimolar proportions (0.001 *M*) of cobalt(II) acetate and the reagent in methanol in a nitrogen atmosphere [20] (Fig. 1).

A Hitachi Model 220 spectrophotometer and a Hitachi Model 655 A liquid chromatograph, connected with a variable-wavelength UV monitor, Rheodyne Model 7125 injector and a Hitachi D 2500 Chromato-integrator were used.

A 3-μm Microsorb ODS column (150 × 4.6 mm I.D.) (Jones Chromatography) and a 5-μm Microsorb C_{18} column (Rainin Instruments, Woburn, MA, USA) were used.

2.1. Solvent extraction of cobalt(II) and iron(II)

Into a well stoppered test-tube were transferred 1–5 ml of solution containing 0–125 μg of cobalt(II) or iron(II), and nitrogen was bubbled through the solution. To the mixture was added sodium acetate–acetic acid buffer (pH 6) (2 ml) for iron(II) or sodium hydrogencarbonate buffer (pH 7.5) (2 ml) for cobalt(II), followed by solid ascorbic acid (4–5 mg) and reagent solution (1% w/v in ethanol) (2 ml). The contents were warmed on a water-bath for 10 min. Chloroform (2 ml) was added and the layers were mixed well. An aliquot of extract (1 ml) was transferred into a sample vial and the solvent was removed on a water-bath. The residue was dissolved in methanol (1 ml) and the solution (5 μl) was injected on to the Microsorb ODS column. The complexes were eluted with methanol–water–acetonitrile (60:39:1, v/v/v) at a flow-rate of 0.9 ml/min. Detection was achieved using a UV monitor fixed at 270 nm.

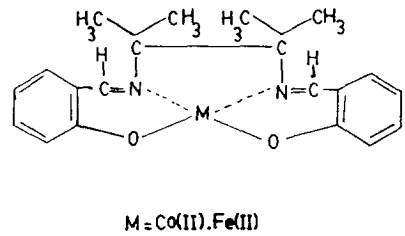

Fig. 1. Structure of metal chelates formed by the reagent.

2.2. Solvent extraction of cobalt(III) and iron(III) for HPLC determination

To an aliquot of solution (1–5 ml) containing 0–150 μg of cobalt or iron was added 37% hydrogen peroxide (0.5 ml) and the solution was evaporated to dryness. The residue was dissolved in ethanol (2 ml) and the procedure in Section 2.1 was followed except that addition of ascorbic acid was omitted.

2.3. Determination of cobalt in vitamin B_{12} syrup

Vitamin B_{12} syrup (Catacon; Glaxo, Karachi, Pakistan) (20 g) was placed in a crucible and potassium hydrogensulphite (0.5 g dissolved in 5 ml of water) was added. The mixture was heated on hot-plate for 15 min, 37% hydrochloric acid (10 ml) and 65% nitric acid (5 ml) were added, the mixture was heated nearly to dryness and the residue was heated on a flame. The white, powdery residue was dissolved in 5 ml of water. A 5-ml sample was taken and the procedure in Section 2.1 was followed.

2.4. Determination of iron in Fefol capsule

To a Fefol capsule (SK&F, Karachi, Pakistan) (0.4197 g) were added 65% nitric acid (5 ml) and 37% hydrochloric acid (10 ml) and the mixture was heated on a hot-plate. When the capsule had completely dissolved, most of the acid was evaporated. Hydrochloric acid (2 ml) was added and the mixture was heated nearly to dryness. The residue was dissolved in water and the volume was adjusted to 25 ml. A 0.1-ml sample was taken and the procedure in Section 2.1 was followed.

3. Results and discussion

Cobalt(II) and iron(II) complexes are easily extractable with chloroform. The effect of pH on the extraction of cobalt(II) and iron(II) with chloroform indicates that cobalt(II) shows maximum extraction in the pH range 7–8, but iron(II) extracts maximally at pH 6. At higher pH (7–8), iron(II) precipitates as iron oxide and does not form a complex. Cobalt(II) can be extracted only above pH 3, but iron(II) shows a colour reaction even at pH 2. For the simultaneous extraction of iron(II) and cobalt(II), pH 6 could be used. The complexes are highly stable and did not show any change in peak shape for up to 24 h.

The cobalt(II) and iron(II) complexes were easily eluted from the Microsorb ODS column when eluted with the methanol–water, giving symmetrical peaks. The excess of reagent added for derivatization eluted after cobalt(II) but before iron(II) and did not interfere. A peak was observed before cobalt(II) when the aqueous solution of cobalt(II) and buffer solution was not deaerated with nitrogen. This signal was considered to be due to cobalt(III) resulting from the air oxidation of cobalt(II) and subsequent complexation and extraction with chloroform. However, when the cobalt(II) solution was deaerated by passing nitrogen, the first peak disappeared. The cobalt(III) complex was therefore prepared by oxidation of cobalt(II) to cobalt(III) with hydrogen peroxide, followed by heating the solution nearly to dryness to remove the hydrogen peroxide. This treatment was considered necessary because in the presence of hydrogen peroxide cobalt(III) formed a coloured complex, which was difficult to extract with chloroform. However, when the cobalt(III) complex extracted with chloroform was injected it gave a single peak and eluted before cobalt(II). The oxidation of cobalt(II) to cobalt(III) was further examined by passing air through freshly prepared cobalt(II) for different times (15–60 min). An enhancement of the peak height of cobalt(III) was observed with a corresponding decrease in the signal for cobalt(II). However, even on passing air for 1 h through cobalt(II) solution, the conversion of cobalt(II) to cobalt(III) was far from quantitative. In contrast, the conversion was complete using nitric acid (1%).

An optimum separation between cobalt(II) and cobalt(III) was obtained when eluted isocratically with methanol–water–acetonitrile (60:39:1, v/v/v) at a flow-rate of 0.9 ml/min (Fig. 2). A similar attempt was made to resolve iron(II) and iron(III), but each time a slightly broader peak was obtained, which could not be resolved into iron(II) and iron(III).

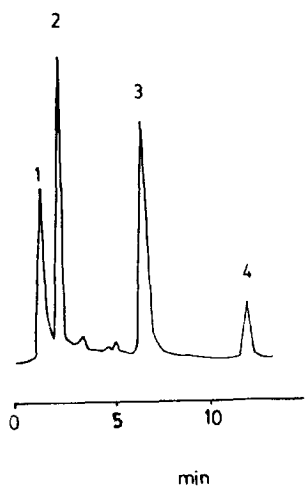

Fig. 2. HPLC separation of (1) cobalt(III), (2) cobalt(II), (3) reagent and (4) iron(II) chelates of H_2SA_2Ten. Column, 3-μm Microsorb ODS II (150 × 4.6 mm I.D.); eluent, methanol–water–acetonitrile (60:39:1, v/v/v), flow-rate, 0.9 ml/min; detection, UV at 270 nm.

Linear calibrations for cobalt(II), cobalt(III), iron(II) and iron(III), measured separately by plotting average peak height ($n = 3$) versus concentration, were found in the range 0–60 μg/ml of extract, using a 5-μl injection. The correlation coefficients for cobalt(II), cobalt(III), iron(II) and iron(III) were 1.0, 0.99, 1.00 and 1.01, respectively. The detection limits measured as three times the background noise were 0.5, 1, 0.5 and 1 μg/ml for cobalt(II), cobalt(III), iron(II) and iron(III), respectively, corresponding to 0.5, 1, 0.5 and 1 ng per injection, respectively.

Cobalt in vitamin B_{12} syrup and iron in a Fefol capsule were determined using the HPLC method. The cobalt concentration found was 0.28 μg/g and that of iron 42.33 mg in 0.42 g with relative standard deviations (R.S.D.) of 3.64% and 2.4%, respectively ($n = 3$). The results obtained were compared with those obtained by atomic absorption spectrometry, which were 0.32 μg/g of cobalt and 43.33 mg in 0.42 g of iron with R.S.D. 1.55% and 0.93%, respectively ($n = 3$).

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