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Cellulose-tethered tetradentate chelating agent for pyridoxine determination

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All standard solutions were prepared from chemically pure grade reagents in doubly distilled water. Pyridoxine hydrochloride [3,5-bis(hydroxymethyl)-2-methylpyridin-3-ol hydrochloride, Vitamine B_6, min 98%]; aniline (synthesis grade, 99%), phenol (synthesis grade, 99%, Merck) were used. Chromatographic paper containing 99% of hydrolyzed α-cellulose fibers (beats weight 80 g m^{-2}, thickness 0.18-0.20 mm, the content of Cu, Zn and Fe less than 0.5 ppm) was used as a carrier [S1].

The solid-phase reagent based on 2-hydroxy-4-nitroso-4-{2-[5-(2-diazo-5-nitrophenyl)-1,5-dioxapentyl]-4-nitrophenyl}-4-azabutylcellulose 4-toluenesulfonate (RIP-Diazo) in the form indicator strips was synthesized, prepared and azo coupled with pyridoxine to form of RIP-Azo-Pyridoxine as described [S2] (see Scheme 1 of the main text). Epoxy cellulose **1** was processed with 1,5-bis(2-amino-5-nitrophenyl)-1,5-dioxapentane to form of 2-hydroxy-3-{2-[5-(2-amino-5-nitrophenyl)-1,5-dioxapentyl]-4-nitrophenylamino}propylcellulose **2**, which was subjected to diazotization to form a reagent indicator paper RIP-Diazo **3**. Under the action of RIP-Diazo onto pyridoxine, a red-orange tetradentate reagent RIP-Azo-Pyridoxine **4** was formed. Test strips (TS) with a polymer holder 9.8 ± 0.05 mm and paper TS with a size of 40 x 9.8 ± 0.05 mm of yellow-white color (TS RIP-Diazo) were prepared from RIP-Diazo for quantitative sorption-spectrophotometric determination of pyridoxine. The diffuse reflectance spectra of the test strips [in the 400-700 nm wavelength range, as the Kubelka-Munk function $F = (1-R)^2/2R$, where R is the reflection coefficient] were recorded on Eye-One Pro mini-spectrophotometer (X-Rite Inc., USA). The determination was based on azo coupling reaction with the TS RIP-Diazo to form test strips with azo-pyridoxine (TS RIP-Azo) and the subsequent complexation reaction of the tetradentate TS RIP-Azo with Cu^{II} ion, selected after testing for chromogenic reactions of pyridoxine with 14 metal ions: Ag, Al, Au^{III}, Ba, Bi^{III}, Fe^{III}, Ca, Cd, Co^{II}, Cu^{II}, Mg, Mn^{II}, Ni, Pb and Zn. To do this, aqueous solution of pyridoxine (0.5 ml) was adjusted to optimal pH 3−8 with a buffer solution. The TS RIP-Diazo with the holder was immersed with a paper end into the manufactured pyridoxine sample for 1−5 min, the reaction solution was removed, the standard metal solution (50 μl) and an acetate buffer solution up to pH 5 were added. Color change of the TS was observed before and after the reaction with the metal. The diffuse reflectance spectrum of the dried test-strip RIP-Azo and the product of its reaction with the metal ion (TS RIP-M) were

recorded. To determine pyridoxine in a pharmaceutical formulation, 1 tablet (5 mg) or averaged sample weighing 5.00 mg was dissolved in water in a volumetric flask, diluted up to 100 ml, and 20 ml of sample was taken, placed in a Petri dish, the solution was adjusted up to pH of 5–7 and standard solution of Cu^{II} (50 μl) was added. The paper TS RIP-Diazo was lowered into the solution for 20 min, dried, and the diffuse reflectance spectrum of TS was registered and the function *F* was calculated. The concentration of the main substance in the sample was determined from the calibration curve for the range of pyridoxine concentrations of 0.2−10 mg dm^{-3}.

References

[S1] M. I. Berezhnaya, N. P. Ivanova, V. M. Ostrovskaya, P. P. Gura, Yu. N. Prikhod'ko and P.V. Poddubny, *SU Patent 1219698*, 1986.

[S2] V. M. Ostrovskaya, L. K. Shpigun and Ya. V. Shushenachev, *RU Patent 2680391*, 2019.