Colorimetry and Indirect X-ray Fluorescence Determination of Active Ingredients in Tetracycline Hydrochloride Drug and Injection Solution of B_{12} Vitamin Using of Polyurethane Foam Sorbents

Aleksandr A. Chaplenko[*,1], Oksana V. Monogarova[*] and Kirill V. Oskolok[*]

[*]Department of Chemistry, Lomonosov Moscow State University, Leninskie gory, 1-3, 199991, Moscow, Russia.

Abstract

The simple and available technique of colorimetry and indirect X-ray fluorescence determination of tetracycline hydrochloride (in the form of the colored complex with iron(III) ions) and cyanocobalamine (in the form of the colored thiocyanate complex with cobalt(II) ions) is offered. The analytes were separated from the accompanying components by sorption to polyurethane foam based on ethers. The conditions of sorption separation and measurement of the analytical signal of these substances are optimized. The obtained results of tetracycline drug and injection solution of the B_{12} vitamin are in satisfactory agreement with data declared by the manufacturer.

Keywords: Tetracycline hydrochloride, B_{12} vitamin, Polyurethane foam sorbent, Chemical colorimetry method, Indirect X-ray fluorescence analysis.

Introduction

One of the important tasks of pharmaceutical chemistry is the development of effective ways of determination of active ingredients in drugs. According to pharmacopoeias [1-4], both chemical and instrumental methods (for example, high performance liquid chromatography and infrared spectroscopy) are used traditionally for determination of active ingredients in pharmaceutical medicines. Low selectivity is one of the main disadvantages of chemical methods, but the high cost of instrumental methods and requirements to the qualification of the operator limited its available for field laboratories and drugstores. In this regard, the development of technically simple and selective approaches of determination of active ingredients in drugs is an actual task of modern pharmaceutical chemistry.

Visual colorimetry is among simple and available methods of analysis. The colorimetry techniques are widely used in analytical chemistry, but combination of these techniques with digital processing of the received images allows to expand method scopes and to improve metrological characteristics [5].

The aim of our work is to illustrate opportunities of combined chemical and tool approach based on sorption extraction of active ingredients from pharmaceutical drugs with subsequent digital colorimetry determination. The function of a sorbent isn`t limited only to a stage of separation and concentration. Sorbent phase is a certain universal environment with constant properties; it took part in the formation of an analytical signal and providing unity of conditions of its measurement. Therefore, determination of big contents of medicinal substances should be carried out by means of conversion of analyte in a sorbent phase. Polyurethane foam (PUF) sorbents based on ethers are used for sorption extraction of active ingredients from solutions. PUF sorbents are cheap and available; they have high chemical, mechanical and thermal stability. PUF sorbents have high distribution coefficients (from 3 to 5 orders) in the system «water solution – sorbent» [6] for many classes of organic and inorganic compounds. Simple, inexpensive and available measuring device – office flatbed scanner – is proposed for measurement of analytical signal (the intensity of coloring of a sample) instead of expensive equipment. The graphics editor can be used for processing of images received from the scanner [7, 8].

Products of the physical and chemical interaction of PUF with medicinal substances contain functional and analytical chelate groups. These groups are capable of connecting ions of transitional metals to stable complex compounds. This fact can be used for adequacy test of colorimetric techniques using indirect X-ray fluorescence determination of mentioned above organic substances on «heavy labels». «Heavy labels» are ions of metals forming stable complexes with the corresponding active ingredients [9].

Experimental

Reagents and materials. PUF sorbent brand 5-30 (SPA «Radical», Kiev, Ukraine) is used for sorption of active ingredients from drugs. The size of PUF tablets for sample preparation is 10 × 10 × 1 mm. The geometric shape of these tablets is a parallelepiped. The mass of PUF sorbent is ~ 0.2 g.

[1]Corresponding author E-mail: a.a.chaplenko@yandex.ru

Received:23 /5/2017
Accepted: 5/7/2017

The prepared PUF tablets were washed with 1 M HCl and acetone during 1 hour and then with distilled water up to pH 5-6 because PUF contains organic impurities technological metal impurities. After that PUF tablet was dried between sheets of filter paper. The degree of purification was monitored by X-ray fluorescence method. In our work state standard samples containing 1 mg/ml Co(II) ions («Ecoanalytica», Moscow, Russia), sodium fluoride (not less than 99 %), potassium thiocyanate (not less than 99 %), sodium hydroxide (not less than 99 %), ferric(III) chloride (not less than 99 %), hydrochloric acid (more 99.9 %), sulfuric acid (more 99.9 %), acetone (more 99.9 %) («Sigma-Aldrich», USA), cyanocobalamin solution for injection 0.5 mg/ml in ampoules on 1 ml (Borisov, Belarus), pharmaceutical substance – tetracycline hydrochloride powder (North China Pharmaceutical Goldstar Co., China) are used.

As a standard sample of tetracycline hydrochloride solution of tetracycline substance (1 mg/ml) was used. The quality of tetracycline substance is confirmed by methods described in Pharmacopoeia article [(2)]. The content of tetracycline hydrochloride is 99.4 ± 0.2 %. The stirring device (model 6500, «Ecros», Russia) is used in our work.

Sample preparation of medicinal substances and preparation of calibration solutions. Tetracycline hydrochloride solution. For the preparation of analyzed tetracycline hydrochloride solution pharmaceutical substance (m = 0.250 g) was thoroughly ground in an agate mortar to a homogeneous powder and was dissolved in 30 ml 0.01 M NaOH. The solution was shaken on a stirring device for 60 minutes and it was quantitatively placed into the volumetric flask (100.0 ml). The volume of solution was adjusted to 100.0 ml with the use of 0.01 M NaOH. Aliquot of analyzed solution (1.00 ml) was quantitatively placed into the volumetric flask (25.0 ml), add 1.00 ml of $FeCl_3$ solution (1 mg/ml). The volume of solution was adjusted to 25.0 ml / acidity to pH 2-3 with the use of deionized water and 1 M HCl. For the preparation of the calibration solutions standard sample of tetracycline hydrochloride substance was used. The calibration solutions were prepared similarly to analyze samples. Concentrations of tetracycline hydrochloride in calibration solutions are changed from 0.01 to 1.0 mg/ml.

Tetracycline hydrochloride was sorbed on PUF in the form of the colored complex with iron(III) ions. PUF tablet pressed with a glass rod to remove air bubbles for an increase of uniformity of analyte distribution by volume of sorbent. Solutions were shaken on a stirring device for 60 minutes. After that PUF tablet was removed from the solution, it was thoroughly washed with distilled water and was dried between sheets of filter paper.

Cyanocobalamin. sample preparation of cyanocobalamin solution for injection was included the stage of mineralization. The content of 5 ampoules was evaporated to dryness in a porcelain crucible, to the residue, concentrated H_2SO_4 was added and the heating was continued for 5 minutes. The residue obtained after calcinations was quantitatively transferred to a measuring fluoroplastic beaker and it was dissolved in 5 ml deionized water with 1M NaOH up to pH 10-11. 50.0 mg NaF and 50.0 mg KSCN were placed in a fluoroplastic beaker. The obtained solution was shaken on a stirring device to completely dissolving and it was quantitatively placed into the volumetric flask (25.0 ml). The volume of solution was adjusted to 25.0 ml / acidity to pH 2-3 with the use of deionized water and 1 M HCl. State standard samples containing 1 mg/ml Co(II) ions were used for preparation of calibration solutions. An aliquot of this solution (1.00 ml), 50.0 mg NaF and 50.0 mg KSCN were quantitatively placed into the volumetric flask (10.0 ml). The obtained solution was shaken on a stirring device to completely dissolving. The volume of solution was adjusted to 10.0 ml with the use of deionized water, acidified with 1 M HCl up to pH 2-3. The calibration samples were prepared from obtained solution by dilution. The content of Co(II) ions in calibration solutions is various from 0.0005 to 1.0 mg (it corresponds to a concentration of cyanocobalamin from 0.01 to 2.30 mg/ml).

The sorption of thiocyanate complexes of cobalt on PUF was carried out similarly one of tetracycline. The completeness of the recovery of cyanocobalamin was verified by the indirect X-ray fluorescence method with the repeated addition of PUF tablet to the analyzed solutions after sorption.

The measurement of analytical signal. The measurement of colorimetric analytical signal (lightness of the chosen color channel) was carried out with the use of office scanner LaserJet M1120 MFP (Hewlett-Packard, USA) and program for processing of raster images Gimp 2.8.16 (The GIMP Team, USA). The scanning parameters: the color mode is the color image; type is photo; resolution is 300 dpi. Processing of PUF tablets images and determination of lightness of the color B-channel (L_B) was made in the graphical editor.

The analytical signal was measured in a scale *RGB*-model as lightness of *B*-channel in conventional units from 0 to 255.Indirect XRF determination of medicinal substances on PUF sorbent was performed on a Spectroscan G Max XRF spectrometer (SPA "Spectron", St. Petersburg, Russia). The instrument was equipped with a sealed-off gas-filled proportional counter tube (filler gas 90% Xe + 10% CH_4 under atmospheric pressure, the thickness of the Be window is 150 μm), LiF(200) crystal analyzer ($2d$ = 402.8 pm), and low-power (4 W) sharp-focus (diameter 1.5 mm) X-ray tube of the transmitted type with a thin-film (2 μm) Mo-anode (the thickness of the Be window is 200 μm). The working voltage was 40 kV. The current is 100 μA. The working voltage was 45 kV. The incidence angle of the primary radiation on the sample surface was 80°, and the takeoff angle of the secondary radiation was 30°. To determine X-ray fluorescence spectra PUF tablets were placed in specially made cuvette with the low level of scattered primary radiation [10]. The peak value of the line intensities CoK_α, FeK_α in wavelength ranges 179.0 ± 5.0; 193.7 ± 5.0 pm respectively were measured for each sample. The exposure time is 60 s. The analytical signal is integrated intensities of the lines of these elements excluding the background signal normalized to the intensity of the scattered radiation MoK_α is incoherent X-raytube. For taking note of provision of a sample under the probe on uncertainty of results the analytical signal was measured by 4 times at the turn of a PUF tablet by 90°.

Results and Discussion

We varied pH of sorption from 1 to 12 and the time of sorption from 10 minutes to 2 hours (the concentration of ions was taken over and was 50 μg/ml) for the selection of optimal conditions of sorption. It was shown that the acidity is significant for the completeness of the sorption of the metals from the solution. The optimum pH value for cobalt and nickel ions is 2.5 – 3.5 (Fig. 1). The optimum time of sorption for both metals is ~ 60 minutes (Fig. 2).

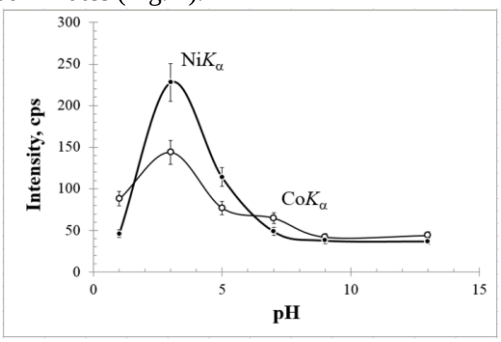

Figure (1): Selection of optimal pH value of $Zn^{2+}$and Co^{2+} sorption (n = 5, P = 0.95)

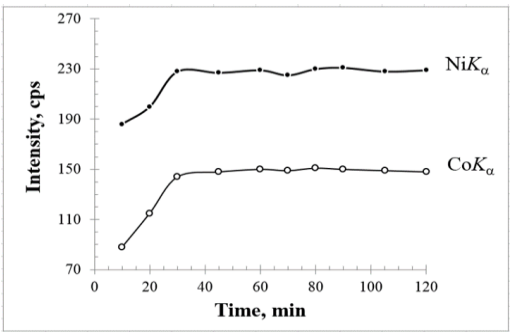

Figure (2): Selection of optimal time of Zn^{2+} and Co^{2+} sorption

Metrological characteristics of colorimetric and indirect X-ray fluorescence determination of active ingredients in drugs after sorption on PUF sorbent are presented in Table 1.

Table (1): The metrological characteristics of colorimetric and indirect X-ray fluorescence determination of tetracycline hydrochloride and cyanocobalamin using PUF sorbents (n = 5, P = 0.95)

Medicine substance	R^2	The range of determined contents, mg/ml	LOD, mg/ml	RSD
Colorimetry				
Cyanocobalamin	0.9933	0.3-1.5	0.09	0.03
Tetracycline	0.9881	0.03-0.2	0.01	0.01
X-ray fluorescence analysis				
Cyanocobalamin	0.9940	0.2-1.4	0.12	0.04
Tetracycline	0.9916	0.05-0.2	0.02	0.01

The new way of determination of B_{12} vitamin is proposed for illustration of possibilities of developed combined approach during analysis of drugs with metalloorganic active substances. The technique is based on chemical mineralization of cyanocobalamin, sorption extraction of Co(II) ions in the form of the colored thiocyanate complexes from

water solutions on PUF sorbent with subsequent measurement of analytical signal [11]. Thiocyanate complex of Co(II) ions stain the PUF sorbent in blue color, complex tetracycline with Fe(III) ions have lilac-gray color [12]. The accuracy of analysis results is confirmed by standard addition method (Table 2). The results of colorimetric and indirect X-ray fluorescence determination of tetracycline and cyanocobalamin in drugs are represented in Table 3. The obtained results are in satisfactory agreement with data declared by the manufacturer.

Table (2): The validation of accuracy of sorption colorimetric and sorption X-ay fluorescence determination of tetracycline hydrochloride and cyanocobalamin by standard addition method.

Introduced	Found ± confidence interval (n = 5, P = 0.95)	
Weight of cyanocobalamin, mg		
	Colorimetry	**XRF**
0.5	0.50 ± 0.05	0.45 ± 0.06
1.0	1.05 ± 0.07	0.94 ± 0.09
Weight of tetracycline hydrochloride, mg		
	Colorimetry	**XRF**
0.10	0.11 ± 0.01	0.09 ± 0.02
0.15	0.16 ± 0.02	0.14 ± 0.03

Table (3): Colorimetric and indirect X-ray fluorescence determination of drug substances in drugs using PUF sorbent (n = 5, P = 0.95)

Drug substance	m, mg/sample		
	According to pharmacopoeia methods	**Colorimetry**	**XRF**
Cyanocobalamin	2.50 ± 0.02	2.4 ± 0.1	2.6 ± 0.2
Tetracycline	248.5 ± 0.5	245 ± 15	250 ± 30

Conclusions

Thus, the developed combined chemical and instrumental approach can be used for determination of organic and metalloorganic active substances if one of transformation products is colored complex. Such pharmaceuticals include tetracycline hydrochloride and cyanocobalamin solution for injections. The proposed approach is available, technically simple and it doesn't require using of specialized and expensive devices. The developed technique can be used in low-budget laboratories and for preliminary screening of the drugs that don't meet the required standards during monitoring of quality of drugs.

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