CONTROL OF THE PHARMACEUTICAL INDUSTRIAL EQUIPMENT CLEANNESS: DETERMINATION OF CALCIUM GLUCONATE BY THE SPECTROPHOTOMETRIC METHOD WITH ERIOCHROME BLUE SE

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A simple spectrophotometric method for the determination of calcium gluconate in industrial equipment cleaning rinse waters using eriochrome blue SE was proposed. The method utilizes the formation of the coloured complex of the indicator with calcium ions and the colourimetric determination of the formed product. The calibration graph is linear in the range from 5 to 40 mg/l of calcium gluconate, the molar attenuation coefficient is 39000 m2/mol, the limit of detection is 2,8 mg/l, the limit of quantification is 8,5 mg/l, the method is selective with respect to the common excipients, shows a good accuracy (the relative uncertainty does not exceed 6%) and precision (the relative standard deviation does not exceed 6%), does not require lengthy sample preparation and sophisticated laboratory equipment and is suitable for the routine analysis of calcium gluconate at the industrial equipment surface and in the cleaning rinse waters.

INTRODUCTION

Calcium gluconate is a dietary supplement and a medication used to treat low blood calcium, magnesium sulfate [1] or verapamil poisoning [2], hydrofluoric acid burns [3], or cardiac arrest [1]. It is produced worldwide in large quantities [4]. In the state registry of medications of the Russian Federation, there are 40 registered preparations containing calcium gluconate, which are produced by 25 different manufacturers (Fig.1).

When several different preparations are manufactured at a single production line, the cleaning of the pharmaceutical industrial equipment and the determination of microgram quantities of produced preparations at its surface and in the cleaning rinse waters when the preparation is changed are the important steps of pharmaceutical production [5]. The regulated residual quantities of the formed products at the equipment surface after its cleaning usually do not surpass tens of micrograms. According to the European [6], US [7], British [8], Japanese [9], Russian [10], and International [11] Pharmacopoeias, calcium gluconate is assayed for calcium by EDTA titration, however, the method is not enough sensitive for the determination of microgram amounts of metal. Other common methods of calcium gluconate determination include flame emission spectrometry [12], polarimetry [13], and titrimetric determination of iodine [14] or spectrophotometric determination of formaldehyde [15, 16] after selective oxidation of calcium gluconate by periodate [14–16]. However, the flame emission method requires sophisticated equipment, the polarimetric method can not determine the microgram amounts of gluconate, and the oxidation of calcium gluconate is time-consuming. To ensure the production integrity, the method of analysis of the residual quantities of the preparation after the equipment cleaning should be as much rapid as possible, and therefore, the spectrophotometric determination is preferable. There are several spectrophotometric methods of determination of calcium cations. They differ in their simplicity and the usage of common reagents readily available in pharmaceutical laboratories. The method utilising eriochrome blue SE [17] is the most suitable one. Therefore, this study aims to adapt that method [17] for the spectrophotometric determination of calcium gluconate in industrial equipment cleaning rinse waters using eriochrome blue SE.

MATERIALS & METHODS

Reagents and equipment. Calcium gluconate (98%) was purchased from Rushim. Sodium hydroxide (99%) was purchased from Reahim. Eriochrome blue SE (98%), corn starch (97%), succinic acid (99%), and talc (97%) were purchased from Lenreaktiv. Tablets and intravenous injections containing calcium gluconate were purchased from the local market. The flat plates made of stainless steel 12X12H10T were used to model the cleaning of industrial equipment. The analytical balance Sartorius Cubis MSA 225P-ICE-DI was used for

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Fig.1. *Calcium gluconate and its pharmacy preparations*

weighting. Various micropipettes manufactured by Thermo Fisher Scientific were used for taking aliquots. The spectrophotometer Agilent Cary 60 was used for colorimetric measurements. The chemical glassware of 2nd grade was used. Water for preparation of solutions was twice distillated and then deionised with Sartorius Arium Pro VF Ultrapure Water system.

Preparation of the colour reagent. The 0,004% solution of eriochrome blue SE in 1 M NaOH was prepared and used as the colour reagent.

Preparation of the stock and working solutions of calcium gluconate. A stock solution of calcium gluconate with concentration of 50 mg/l was prepared. The working solutions with different concentrations ranging from 5 to 40 mg/l were prepared by appropriate dilutions. The working solutions were prepared daily.

Preparation of the solutions from tables and ampoules. The tablets of the calcium gluconate (content is 500 mg) or ampoules (content is100 mg/ml) were dissolved in water, and the working solutions with the concentration of calcium gluconate equal to 25 mg/l were prepared by appropriate dilutions.

Preparation of model rinse water solutions. Aliquots of 10,0 ml of the prepared working solutions, solutions from tablets, or solutions from intravenous injections were taken, placed onto flat plates made of stainless steel, and allowed to dry in the fume hood. The cotton swab was dunked in the test tubes with 10 ml of water, and the plates were swabbed with it several times during 2 minutes, the used swabs were immersed into the test tubes with water and mixed thoroughly over 5 minutes. The test tubes with the swabs were heated over 5 minutes on the boiling water bath, cooled, and the resulting solutions were transferred to the 10 ml volumetric flasks, and the volumes of the solutions were adjusted by water. The expected concentrations of calcium gluconate in the model rinse water solutions are equal to 25 mg/l.

General procedure for the determination of calcium gluconate. A total of 2,0 ml of the colour reagent was mixed with 4,0 ml of working, or sample solution of calcium gluconate or modeled rinse water in a test tube. The blank solution was prepared by mixing 2,0 ml of the colour reagent with 4,0 ml of water in another test tube. The content of the test tubes was mixed and left for 5 minutes. Then the absorbances of the working or sample solution of calcium gluconate, or rinse water at the wavelengths of 533 and 615 nm in the glass cuvette with the optical path length 1 cm were measured against the blank solution, and the difference between the absorbance at 533 nm and the absorbance at 615 nm ($A_{533} - A_{615}$) was calculated.

RESULTS

Selection of the wavelength. The spectra of the stock solution and the blank solution against water, and the difference spectrum of the stock solution against the blank solution were recorded in the wavelength interval from 450 to 750 nm with the wavelength step of 0,2 nm in the glass cuvette with the optical path length of 1 cm. The spectra are shown in Fig.2. The difference spectrum exhibits a maximum at the wavelength of 533 nm and a minimum at 615 nm. In the previous work [17], only the maximum wavelength was used for the quantification of calcium, however, in the present study, in order to increase the sensitivity, both the maximum and the minimum wavelengths were chosen for the further method development.

Selection of the reagent volume. In the series of the test tubes 4,0 ml of the stock solution were mixed with the various volumes of the colour reagent ranging from 0,2 to 4,0 ml. The corresponding blank solutions were prepared in another series of test tubes by mixing 4,0 ml of water with various volumes of the colour reagent. The solutions were left for 5 minutes. The absorbances of prepared solutions with the different sample volume at the wavelengths of 615 and 533 nm in the glass cuvette with the optical path length 1 cm

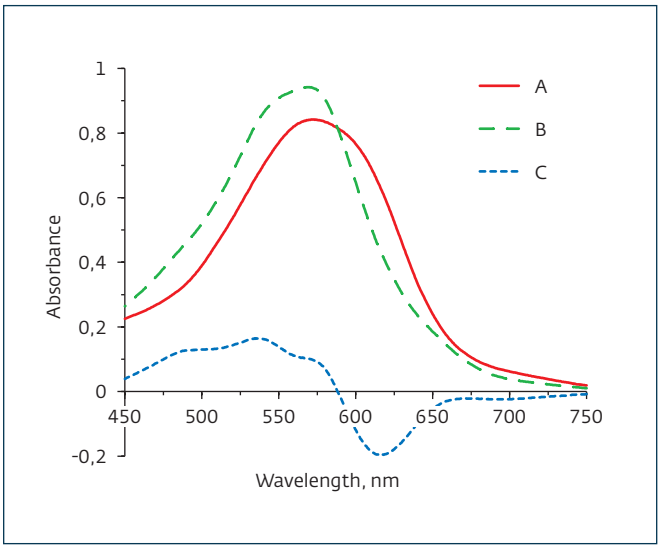

Fig.2. *The absorption spectra of (A) eriochrome blue SE in alkaline environment against water, (B) complex of calcium gluconate with eriochrome blue SE in alkaline environment against water, (C) complex of calcium gluconate with eriochrome blue SE against eriochrome blue SE in alkaline environment*

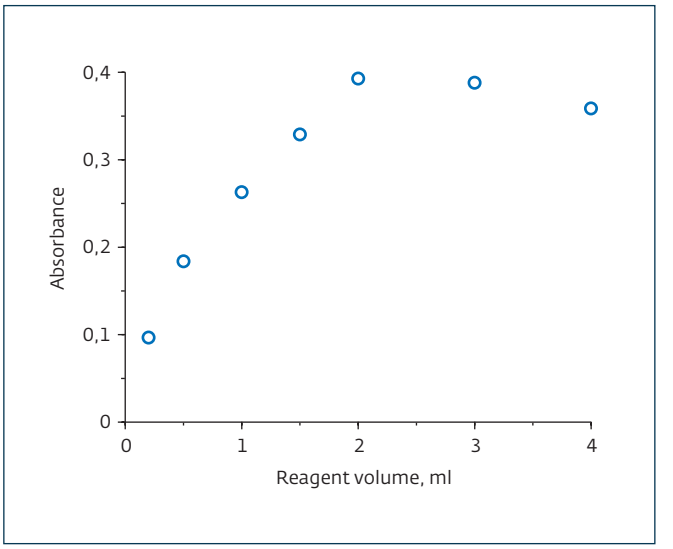

Fig.3. *The dependence of the absorbances of the coloured product on the volume of the added colour reagent*

were measured against the corresponding blank solutions. The results are shown in Fig.3. The maximum absorbances were observed at the reagent volume of 2 ml. This reagent volume was chosen for all further measurements.

Selection of the incubation time. In the series of the test tubes 4,0 ml of the stock solution were mixed with 2,0 ml of the colour reagent. The blank solution was prepared by mixing 4,0 ml of water with 2,0 ml of the colour reagent in another test tube. The solutions were left over various time intervals ranging from 0,5 to 30 minutes. The absorbances of prepared solutions with the different incubation time at the wavelengths of 615 and 533 nm in the glass cuvette with the optical path length 1 cm were measured against the blank solution. The results are shown in Fig.4. The maximum absorbances were observed after 3 minutes of incubation and then remained stable during 30 minutes. The incubation time of 5 minutes was chosen for all further measurements.

Construction of the calibration graph. The working solutions of calcium gluconate with different concentrations ranging from 5 to 50 mg/l and the blank solution were prepared. In the series of the test tubes 4,0 ml of the prepared working solutions were mixed with 2,0 ml of the colour reagent. The blank solution was prepared by mixing 4,0 ml of water with 2,0 ml of the colour reagent in another test tube. The solutions were left for 5 minutes. The absorbances of prepared solutions with the different concentration of calcium gluconate at the wavelengths of 615 and 533 nm in the glass cuvette with the optical path length 1 cm were measured against the blank solution. The results are shown in Table 1 and Fig.5. The dependency of the difference between the absorbances at 615 and 533 nm ($A_{533} - A_{615}$) on calcium gluconate concentration is linear in concentration range from 5 to 40 mg/l. This difference was used for the construction of calibration graph and for further method validation.

Analytical performance. The analytical performance of the method was determined in accordance with the State Pharmacopoeia of the Russian Federation [10] guidelines. The method was tested for linearity, limits of detection and quantification, selectivity, accuracy, and inter- and intra-day precision.

Linearity. According to Fig.5, the dependence of the difference between the absorbances of the coloured product at 615 and 533 nm on the concentration of calcium gluconate is linear in the range from 5 to 40 mg/l. The regression analysis was performed using the least-squares technique [18]. Additionally, the Ringbom's optimum range [19], the molar attenuation coefficient and the Sandell's sensitivity coefficient [20] were calculated. The parameters of the regression equation are listed in Table 2.

Limit of detection and limit of quantification. The limit of detection and the limit of quantification of the method were calculated according to [21]. The values are presented in Table 2.

Selectivity with respect to common excipients. According to the Russian State Register of Pharmaceutical Products, tablets of calcium gluconate contain corn starch and talc, and intravenous injections contain suc-

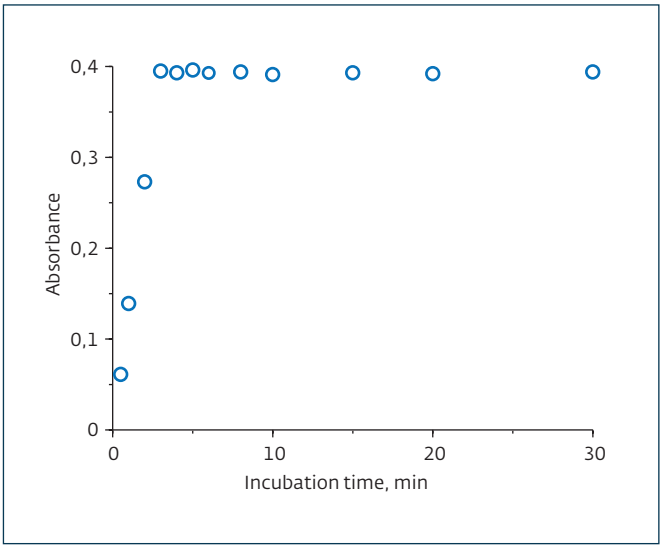

Fig.4. *The dependence of the absorbances of the coloured product on the incubation time*

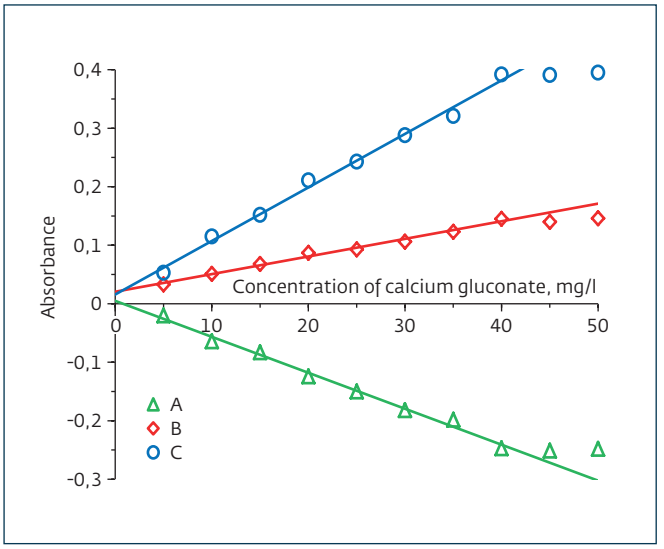

Fig.5. *The dependence of the absorbances of the coloured product on the concentration of calcium gluconate: (A) absorbance at 615 nm, (B) absorbance at 533 nm, (C) difference between the absorbances at 615 and 533 nm. The dashed lines represent the regression lines*

cinic acid as the common excipients. The possible interference of these excipients was studied. For that the 1 g/l water solution of succinic acid, and the 1 g/l suspension of talc in water were prepared. The 1% solution of corn starch was prepared in boiling water, was boiled for 30 min, and cooled down to room temperature. 4,0 ml of each solution were placed in the test tubes, 2,0 ml of the colour reagent was added to the each one, and the solutions were left for 60 minutes. No colour change was observed, this indicates that the tested excipients do not interfere. However, calcium stearate, if added to a tablet as the excipient, might interfere.

Accuracy and intra-day precision. Three series of experiments were conducted, in which ten working solutions, ten solutions from tables, and ten solutions from intravenous injections with the concentration of calcium gluconate equal to 25 mg/l were prepared. The solutions were

Table 1. *The dependence of the absorbance of the coloured product on the concentration of calcium gluconate*

Concentration of calcium gluconate, mg/l	Absorbance at 615 nm (A_{615})	Absorbance at 533 nm (A_{533})	$A_{533} - A_{615}$
5	–0,020	0,032	0,052
10	–0,064	0,050	0,114
15	–0,083	0,068	0,151
20	–0,124	0,086	0,210
25	–0,149	0,093	0,242
30	–0,182	0,105	0,287
35	–0,197	0,122	0,319
40	–0,246	0,144	0,390
45	–0,250	0,140	0,390
50	–0,248	0,146	0,394

Table 2. *The parameters of the linear regression of the dependence of the difference of the absorbances of the coloured product at 615 and 533 nm on the concentration of calcium gluconate, and the analytical parameters of the method*

Parameter	Value
Slope and its confidence interval (f = 6, p = 95%), l/mg	0,0091 ± 0,0003
Intercept and its confidence interval (f = 6, p = 95%)	0,016 ± 0,008
R^2 value	0,993
Linearity range, mg/l	5–40
Ringbom's optimum range, mg/l	20–40
Molar attenuation coefficient and its confidence interval (f = 6, p = 95%), m^2/mol	$(39 \pm 1) \cdot 10^3$
Sandell's sensitivity coefficient and its confidence interval (f = 6, p = 95%), $\mu g/cm^2$	0,11 ± 0,04
Limit of detection, mg/l	2,8
Limit of quantification, mg/l	8,5

treated as described in the general procedure, and then the absorbances of the coloured product were recorded, the concentrations of the solutions were calculated according to the regression equation, and the relative uncertainties and the relative standard deviations were determined. The results are collected in Table 3.

Inter-day precision. Three series of experiments were conducted, in which a working solution, a solution from tables and a solution from intravenous injections with the concentration of calcium gluconate equal to 25 mg/l were prepared each day during five consecutive days. The solutions were treated as described in the general procedure, and then the absorbances of the coloured product were recorded, the concentrations of the solutions were calculated according to the regression equation, and the relative standard deviations were determined. The results are collected in Table 3.

Accuracy and precision for the determination of model rinse water solutions. Three series of experiments were conducted, in which ten model rinse waters from working solutions, ten model rinse waters from the solutions from tables and ten model rinse waters from the solutions from intravenous injections with the concentration of calcium gluconate equal to 25 mg/l were prepared. The solutions were treated as described in the general procedure, and then the absorbances of the coloured product were recorded, the concentrations of the solutions were calculated according to the regression equation, and the relative uncertainties and the relative standard deviations were determined. The results are collected in Table 4.

DISCUSSION

The experiments show that the proposed spectrophotometric method is suitable for the determination of calcium gluconate in industrial equipment cleaning rinse waters. The method is rapid and simple; it does not require compli-

Table 3. *The accuracy and precision of the method*

Solution	Relative uncertainty, %	Relative standard deviation for intra-day precision, %	Relative standard deviation for inter-day precision, %
Working solution, 25 mg/l	2,2	2,7	2,8
Solution from tables, 25 mg/l	2,6	3,2	3,6
Solution from intravenous injections, 25 mg/l	2,4	3,0	3,4

Table 4. *The accuracy and precision for the determination of the model rinse water*

Solution	Relative uncertainty, %	Relative standard deviation, %
Rinse water from the working solution, 25 mg/l	4,2	4,8
Rinse water from the solution from tables, 25 mg/l	5,1	5,7
Rinse water from the solution from intravenous injections, 25 mg/l	4,7	5,3

cated sample preparation or sophisticated equipment. The method is selective with respect to the common excipients, sensitive (the molar attenuation coefficient equals 39000 m^2/mol, the limit of detection equals 2,8 mg/l, and the limit of quantification equals 8,5 mg/l), accurate (the relative uncertainty for the analysis of pharmaceutical formulations does not exceed 4%, the relative uncertainty for the analysis of modelling industrial rinse waters does not exceed 6%, which is acceptable for rinse water analysis), and precise (the relative standard deviation does not exceed 4% for intra-, 4% for inter-day precision, and 6% for analysis of modelling industrial rinse waters). The calibration graph is linear in the range from 5 to 40 mg/l of calcium gluconate with the good correlation coefficient. The method is recommended for the routine and quick analysis of calcium gluconate in industrial equipment cleaning rinse waters.

CONCLUSIONS

A simple spectrophotometric method for the determination of calcium gluconate in industrial equipment cleaning rinse waters using alkaline solution of eriochrome blue SE was proposed. The method is based on the formation of the coloured complex between calcium and the reagent, and the colourimetric determination of the formed product. The method shows a good analytical performance, does not require lengthy sample preparation and sophisticated laboratory equipment, and is suitable for routine analysis.

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