Drug Development and Industrial Pharmacy, 24(2), 109–114 (1998)

RESEARCH PAPER

Determination of Oligoelements in Parenteral Formulations by Planar Chromatography and Spectrophotometry

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ABSTRACT

The authors have studied the chromatographic behavior of parenteral preparations for pediatric use containing inorganic cations. After separation and identification by thin-layer chromatography, Mn^{2+}, Zn^{2+}, and Cu^{2+} were analyzed by a method based on reaction with an appropriate reagent and extraction with an organic solvent which yielded elution and preconcentration, resulting in an appropriate solution for colorimetric quantitation. Cr^{3+} cation was determined by atomic absorption spectrophotometry after appropriate chromatographic separation, using microcrystalline cellulose (adsorbent) and an acetone:water:hydrochloric acid mixture (80:5:8) as the mobile phase.

INTRODUCTION

Since long ago, man has been using mineral substances as medications, which, although never having been abandoned, are once again playing an important role in modern pharmacotherapy. Although when speaking about minerals it is common to mention oligoelements, trace elements, and principal elements, there is no sharp demarcation line justifying this differentiation. In general, when minerals occur or function in living tissue at concentrations expressed as μg/g or μg/liter, they are considered to be trace elements (1).

The pediatric use of these medications is necessary and should be evaluated using highly sensitive methods. We have developed a method for evaluation based on planar chromatography allied to spectrophotometry, which we consider to be a good alternative for the separation, identification, and quantitation of mineral substances even in complex systems such as pharmaceutical preparations, according to official specifications (2,3). Considering that reactions for the identification of active principles are of capital importance both for raw

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materials and for finished products (2,3,4), we can appreciate the necessity and importance of specific methods for the inorganic active principles studied, since a veritable proliferation of multimineral pharmaceutical preparations is occurring today.

Furthermore, we noticed that although inorganic cations have been studied extensively in different samples, there are few studies of these types of medications and their analysis by the technique (5) employed here.

MATERIALS AND METHODS

Reagents

All reagents used were analytical grade and deionized water was employed.

Equipment

The following instruments were used: Carl Zeiss-Jena spectrophotometer (Specord M 40) for the determination of Zn^{2+} λ = 530 nm, Cu^{2+} λ = 535 nm, and Mn^{2+} λ = 564 nm; and Intralab A A-1475 atomic absorption spectrophotometer for the determination of Cr^{3+} λ = 357.9 nm after chromatographic separation.

Sample

Analyzed samples included parenteral oligoelement solution (POES) for children older than 4 years (sample A) and parenteral oligoelement solution (POES) for children up to 4 years old (sample B). Both formulations (Table 1) were established by the University Hospital, Faculty of Medicine of Botucatu, UNESP, São Paulo, Brazil, within a series of pharmaceutical products for hospital treatment.

Sample A (beyond POES) was diluted so as to obtain an ionic concentration (mg · ml^{-1}) of sample B (up to POES) for the spectrophotometric determinations in the visible region. Cr^{3+} determination by atomic absorption spectrophotometry (AAS) was carried out on sample A with and without previous dilution.

Table 1

Sample Formulation

Component	A	B
$ZnSO_4 \times 7\ H_2O$	5	1×10^{-1}
$CuSO_4 \times 5\ H_2O$	1	2×10^{-2}
$MnSO_4 \times H_2O$	5×10^{-1}	6×10^{-3}
$CrCl_3 \times 6\ H_2O$	1×10^{-2}	1.7×10^{-4}

Standard Solutions

All standard solutions were prepared in deionized water at 0.1% concentration (w/v) in relation to the cations. Aliquots of the standard solution appropriately diluted for application on the plates in amounts equivalent to those of the cations of formulation B (0.9 μg Mn^{2+}, 5.0 μg Cu^{2+}, and 1.0 μg Zn^2 per spot) and those of chromium in formulation A, diluted to 4 μg · ml^{-1} (1.6 μg per spot, with a volume of 0.4 ml applied to the layer).

Adsorbent

Glass plates (20 × 20 cm) were covered with a 300-μm thick layer of microcrystalline cellulose (Merck and Riedel-de Haen) (25 g in 90 ml deionized water). The standard solutions and the samples were applied to the starting line at a distance of 2.5 cm from one another along previously demarcated lanes, with an area without application being used as the blank (B) or control lane. All assays were carried out on layers activated at 105–110°C for 10 min.

Development System

The system used was multiple one-dimensional ascending chromatographic development (mobile phases 1 and 2), because it has better resolution in the presence of contamination problems.

Methanol-concentrated hydrochloric acid (9:1, v/v) was used as prewash (approximately 13 cm). Acetone:water:concentrated hydrochloric acid (80:5:8, v/v) was used as the mobile phase proper (10 cm).

Detection Reagents

The following detection reagents were used: 0.08% alizarine in ethanol (m/v) followed by exposure to ammonia vapors (6) always applied to the right side of the plate, with the remaining part properly protected (Fig. 1, lanes 7 and 8); and ditizone chloroform solution (0.05%, m/v), mainly used in the qualitative phase of the study.

Standardization

Two calibration curves were constructed for each of the three ions (copper, zinc, and manganese), a direct

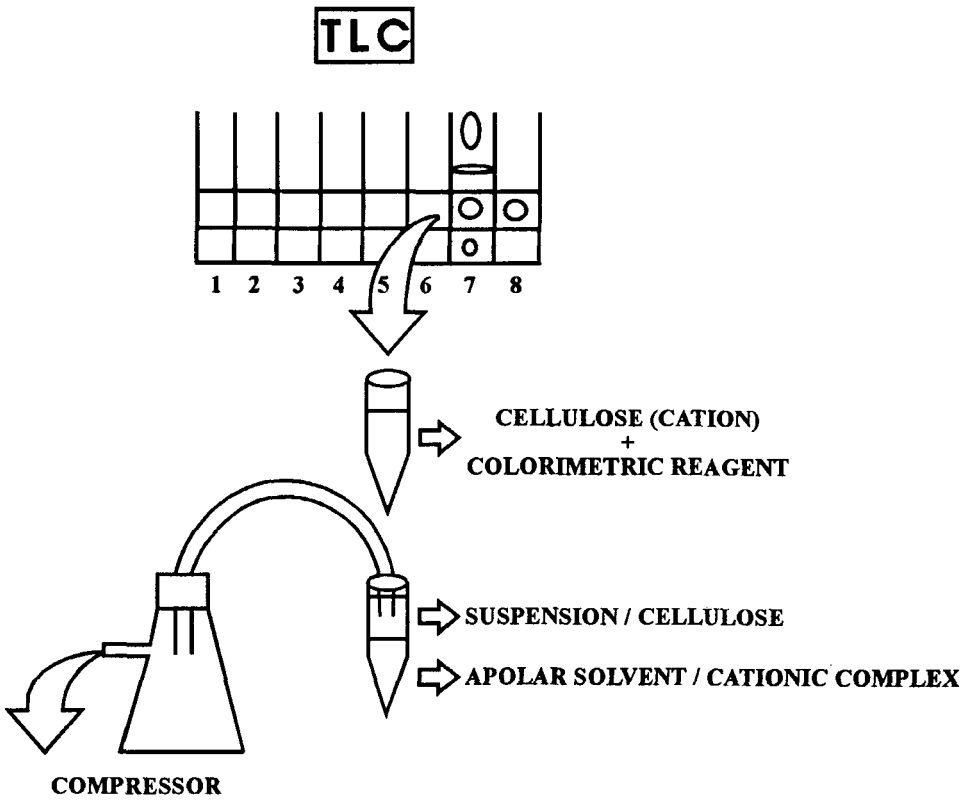

Figure 1. Planar chromatography–spectrophotometric system: 1–3: blank, 4–6: POES and/or standard solutions; 7–8: POES and individual standard solution, respectively, detected with the Alizarine.

curve and a chromatographed curve, using standard solutions before and after the chromatographic process (Table 2).

Chromium III was quantified from a direct calibration curve. Furthermore, we measured the absorbance of sample A directly, i.e., with 10 $\mu g \cdot ml^{-1}$, and therefore above the optimum range of the equipment which is 2–8 $\mu g \cdot ml^{-1}$ (Table 3).

Determination Conditions

The location, removal, and treatment of the cations isolated by thin-layer chromatography (TLC) was the basis for their quantification. Each area corresponding to the pure cations was removed directly to 15 ml centrifuge tubes. The next step was complexation of Cu^{2+}, Zn^{2+}, and Mn^{2+} and preparation of Cr^{3+} present in cellulose, and since cellulose presents a hydrophilic behavior in hydrochloric solutions, remaining in suspension in the aqueous phase after addition of the apolar solvent, it was removed by suction with the aid of a glass pipet attached to a vacuum pump (Fig. 1).

Quantitative Determination

For the construction of the calibration curves (direct and chromatographed) and for the quantification of the cations in samples A and B, the standard solutions and/or the cellulose containing the cation were transferred to centrifuge tubes and the following additions were made in sequence.

Manganese

Three milliliters of deionized water, 0.5 ml 7% hydroxylamine (m/v), 1.0 ml 0.07% [1-(2-pyridylazo)-2-naphthol] (PAN) in ethanol (m/v), and 10 min later, 3.0 ml carbon tetrachloride (7) were added; the mixture was then shaken for 2 min.

Copper

Three milliliters of 0.1 M hydrochloric acid and 2.0 ml ditizone solution (7) in carbon tetrachloride (0.0015%, m/v) were added; the mixture was then shaken for 3 min.

Table 2

Experimental Data Obtained from Calibration Curves of Cationic Complexes

	Mn^{2+}	Cu^{2+}	Zn^{2+}
Concentration range	0–20 μg · ml^{-1}	0–10 μg · ml^{-1}	0–12.5 μg · ml^{-1}
Initial volume	0.18 ml	0.20 ml	0.20 ml
Final volume (extraction)	3.0 ml	2.5 ml	4.0 ml
Number of solutions	10	10	10
Reading λ	564	535	530
Regression equations ($y = a + bx$)			
(a)	-0.0155 (-0.0248)[a]	-0.0002 (-0.0010)[a]	0.0006 (-0.0056)[a]
(b)	0.0642 (0.0636)[a]	0.0385 (0.031)[a]	0.0227 (0.0198)[a]
Regression coefficient (r)	0.9985 (0.9985)	0.9999 (0.9997)	0.9998 (0.9976)

[a]The values in parenthesis refer to the chromatographed calibration curves (for the complexes formed after thin-layer chromatography).

Zinc

Three milliliters of 0.1 M hydrochloric acid, 3.0 ml acetate buffer, pH 5 (7), and 4.0 ml 0.002% (m/v) ditizone in carbon tetrachloride were added; the mixture was then shaken for 4 min.

Chromium

Two milliliters of 0.01 M sulfuric acid was added, followed by 5 min of shaking and centrifugation at 2500 rpm for 5 min.

RESULTS AND DISCUSSION

Figure 2 shows an interesting fact about the detection reagents alizarine (a, violet spots) and ditizone (b, blue-greenish spots): the first produced better results for the four cations analyzed, whereas ditizone did not detect manganese or chromium, confirming the spectrophotometric reagent itself and consequently the quantitative determination of copper and zinc.

Before starting quantification we determined the appropriate concentration of the injectable substances in the layer, using a mixture of standard solutions (0.1% [m/v] in relation to the cations) at increasing concentrations, i.e., from 2 to 12 μl, therefore 2–12 μg for each application spot, as indicated in Fig. 3.

Table 3

Results Obtained from the Direct Calibration Curve in the Determination of Cr^{3+} by AAS

Concentration range	0–8 μg · ml^{-1}
Volume	0.15 ml
Number of solutions	10
Reading λ	357.9 nm
Regression equation ($y = a + bx$)	
(a)	0.0029
(b)	0.0189
Correlation coefficient (r)	0.9985

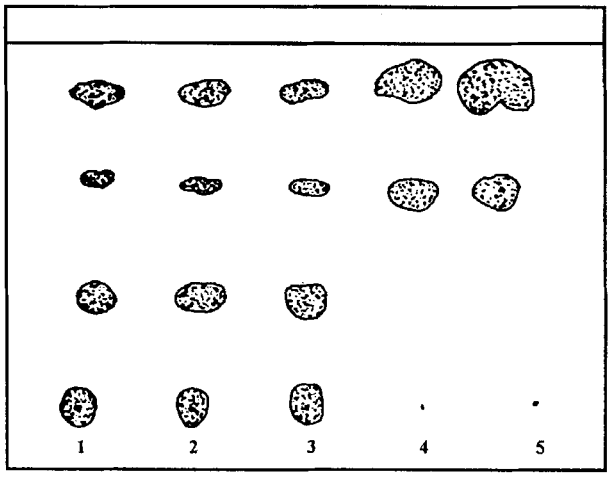

Figure 2. Representation of the chromatogram obtained with the parenteral oligoelement solution for children up to 4 years old (1–5: 10–20 μl) using Alizarine: 1, 2, and 3, and Ditizone: 4 and 5, as detection reagents.

Analysis of this figure shows that the most critical situation is in the range between copper and zinc (R_f) values × 100 = 80 and 90), since manganese and chromium, in addition to being present at much lower concentrations, are always fully separated regardless of the concentration used (R_f values × 100 = 55 and 0, respectively). Furthermore, the uniformity of the spots was found to be inversely proportional to the concentration.

In the case of chromium, the values obtained directly from the injectable substances without previous separation by TLC (II, Table 4) were always lower than those obtained after chromatographic separation (with scraping and elution of the spot areas, III, Table 4).

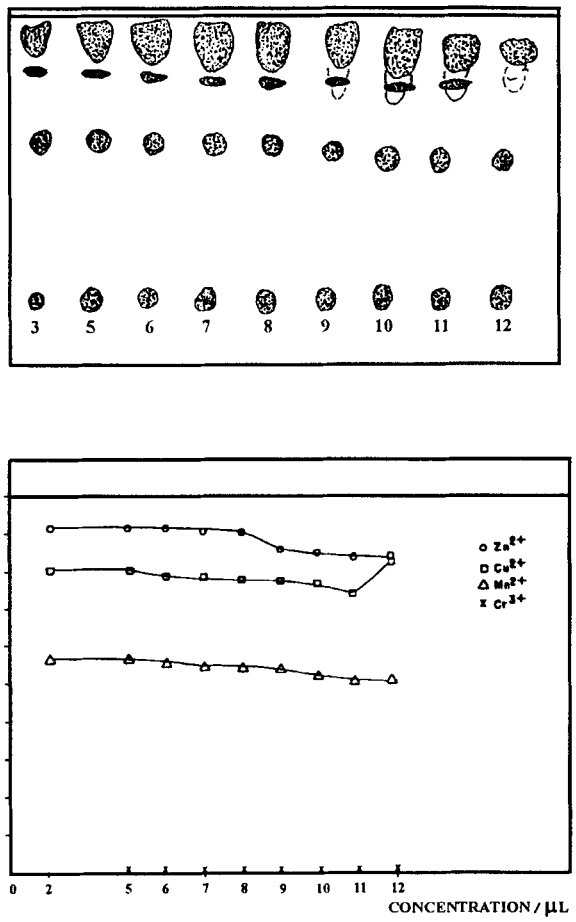

Figure 3. Top: Representation of the chromatogram obtained with the chromatographic system used with 2–12 µl volumes of 0.1% (w/v) standard solutions (SS) in increasing R_f order (Cr^{3+}, Mn^{2+}, Cu^{2+}, and Zn^{2+}). Bottom: graphic representation of the above figure. Relationship between the R_f values and concentration.

Table 4

Results Obtained During Construction of Calibration Curve (I), Determination of Cr^{3+} Directly in the Parenteral Solutions (II), and After TLC (III) by AAS

Standard solutions [µg · ml^{-1}]	Absorbance (λ = 357.9 nm)		
	I[a]	II	III
0	0.0001		
2	0.0410	0.0290[c] (a)	0.0390[c]
4	0.0840	0.0520[c] (b)	
6	0.1160		
8	0.1520		
		0.116[b]	
	$[Cr^{3+}]_{theoretical}$ = 10 µg · ml^{-1}		
	$[Cr^{3+}]_{experimental}$ = 5.8 µg · ml^{-1}		

[a]With standard solutions (SS), I = calibration curve.
[b]With parenteral solutions (sample A) without previous dilution.
[c]With parenteral solutions (sample A) previously diluted to 2 µg · ml^{-1} (a) and 4 µg · ml^{-1} (b).

The cation content of the parenteral solutions was calculated as a function of the direct calibration curve, of the chromatographed calibration curve, and of the formula $C_A = C_P \times A_A/A_P$ respectively corresponding to sample concentration, standard concentration, sample absorbance, and standard absorbance after subtraction of the absorbance of the blank (2).

In general, official monographs establish a range from 95 to 105% for finished products and, considering that our assays were elaborated as a function of the amounts of ions stated on the labels of the pharmaceutical preparations, it can be seen that except for Cr^{3+}, which was determined directly, the formulations studied here obeyed official standards (Table 5).

CONCLUSION

The proposed method is useful for the determination of oligoelements in parenteral preparations and no interference was observed on the part of the additives used in this type of formulation. The quantification of ions copper, zinc, and manganese after chromatographic separation was perfectly viable when elution by simultaneous extraction and concentration was used. The quantification of chromium supports this statement. Careful comparisons with the results obtained for directly applied injectable solutions (diluted or not) to the AAS within the linear range (2–8 µg · ml^{-1}), λ = 357.9 nm, have showed inferior results compared to chromium

Table 5

Cation Concentration Determination Under Analytical Conditions of POES, for Children Older than 4 Years (A) and up to 4 Years of Age (B) by Planar Chromatography and Spectrophotometry

Sample POES[a]	$[Mn^{2+}]$ $\mu g \cdot ml^{-1}$	$[Cr^{3+}]$ $\mu g \cdot ml^{-1}$	$[Cu^{2+}]$ $\mu g \cdot ml^{-1}$	$[Zn^{2+}]$ $\mu g \cdot ml^{-1}$
Labeled				
A	6.0[b] (500)[c]	2.0 (10)[c]	20.0[b] (1000)[c]	100[b] (5000)[c]
B	6.0	0.17	20.0	100.0
Experimental				
A	6.28 (104.7%)	1.90 (95%) 6.0 (60%)[d]	19.5 (97.5%)	98.5 (98.5%)
B	6.3 (105%)	-	19.48 (97.5%)	101.1 (101.1%)

[a]POES = Parenteral oligoelement solutions.
[b]Sample A diluted as a function of sample B.
[c]Sample A without previous dilution.
[d]Direct spectrophotometry (without previous chromatographic separation).

eluted from cellulose after separation of copper, zinc, and manganese. This was probably because of the elimination of interferents, which increased the reliability of the method.

In conclusion, the methodology presented here provides satisfactory and promising results in terms of application to the quality control of medications.

ACKNOWLEDGMENTS

The authors would like to thank Maria José Trovatti Frigero, Maria de Fátima Lopes, and Cilene do Carmo Federici Padilha for their technical assistence.

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