The determination of total calcium in urine: a comparison between the atomic absorption and the ortho-cresolphtalein complexone methods

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Análise do cálcio na urina: uma comparação entre os métodos de absorção atômica e ortocresolftaleína complexona

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key words

Urinary calcium

Atomic absorption

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abstract

Atomic absorption spectrometry has been recommended as the reference method for the analysis of total calcium in body fluids and the *ortho*-cresolphtalein complexone (*o*-CPC) method has been widely used as the field method. We evaluated the performance of the Mega-Bayer, a fully automatic selective analyser, in determining total calcium in urine utilizing the *o*-CPC method. We assayed native urines with low, normal and high calcium concentrations. The two methods agreed well, according to least-squares analysis and the F-test, with Mega-Bayer having the upper limit of linearity two times higher (10 mmol/L) than that of the atomic absorption. The present method achieved excellent analytical goals and sistematic errors bellow half of the allowed limit goals recommended by the Clinical Laboratory Improvements Amendments. Final Rule. Laboratory Requirements (CLIA). We concluded that *o*-CPC in the Mega-Bayer equipment can confidently perform the total calcium urinary analysis with the advantage of being a fully automatized biochemical procedure and of allowing a wider linear analytical range.

unitermos

Cálcio urinário

Absorção atômica

Ortocresolftaleína complexona

resumo

A espectrofotometria de absorção atômica é o método de referência para a análise do cálcio total em líquidos corporais, e o método da ortocresolftalína complexona (o-CPC) tem sido utilizado rotineiramente. Avaliamos a performance *do Mega-Bayer, um analisador seletivo automático, em determinar o cálcio total na urina utilizando o método da ortocresolftaleína complexona. Analisamos urinas com concentrações de cálcio baixas, médias e altas. Os dois métodos foram semelhantes de acordo com a análise de quadrados mínimos e o teste-F; o Mega-Bayer apresentou um limite de linearidade duas vezes mais alto (10mm/l) do que a absorção atômica. O presente método atingiu excelentes metas analíticas, além de erros sistemáticos menores que a metade do permitido pelo Clinical Laboratory Improvements Amendments. Final Rule. Laboratory Requirements (CLIA). Concluímos, portanto, que o-CPC no Mega-Bayer pode realizar a análise do cálcio total urinário com segurança, tendo a vantagem de ser um procedimento bioquímico totalmente automatizado, além de apresentar um intervalo analítico mais amplo.*

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Introduction

The determination of total calcium in urine provides important information during the evaluation and management of nephrolitiasis and the climateric phase.

Atomic absorption spectrometry has been recommended as the reference method for the analysis of total calcium in body fluids and the *ortho*-cresolphtalein complexone (*o*-CPC) method has been widely used as the field method (16). The atomic absorption spectrometry, however, has the disadvantage of being very time-consuming.

The reaction of calcium with *o*-CPC largely depends on buffer types, solvent and pH value. A serious problem is the reaction of *o*-CPC with magnesium; the absorption of the magnesium dye complex is aproximately one-third of that of the calcium at 570nm (3). Many studies were done in order to eliminate interferences, improve the linearity of the reaction, stabilize the reagent and adapt it to automated analysers (1, 3-5, 8, 10, 12-14).

Nowadays, the automated equipment have provided the possibility of making assays with, two wavelength readings (bichromatic analysis), the two point kinetic method, two independent reagents pipeted into the same reaction and/or reagent and sample blank readings. All of these procedures provided improvement in the calcium analysis and reduced analytical variation in the spectrophotometric method.

Our purpose was to evaluate the performance of the Mega-Bayer, a fully automatic selective analyser, in determining total calcium in urine utilizing the *ortho*-cresolphtalein complexone method.

Material and methods

Material

Calcium carbonate was used to prepare a 25 mmol/L aqueous stock solution for calibrating the atomic absorption spectrometer SpectrAA 250 Plus - Varian.

The Mega-Bayer determinations were performed with calibrators, reagents and controls obtained from the manufacturer.

Fourty-five fresh 24-h urine collections with either low, normal or high calcium concentration (a total of 135 samples) were obtained from patients under medical care in the Hospital das Clínicas, a 400-bed tertiary care hospital affiliated to the University of Campinas (Unicamp). After homogeneization and centrifugation, aliquots were stored at 4°C until the measurement on the following day.

Methods

The atomic absorption spectrometry was calibrated everyday with four aqueous $CaCO_3$ solutions – 0.625, 1.25, 2.5 and 5.0 mmol/L – prepared from the stock solution with a 23mM lanthanum chloride solution in 0.3N HCl.

All standards, samples and controls were diluted to 1:25 (v/v) with the lanthanum chloride solution before being assayed.

The Mega-Bayer was calibrated daily with the Multi-Calibrator Merck and urine samples were assayed without previous dilution.

The reaction program included independent buffer and *o*-CPC pipeting, 572 and 604nm readings, end point measurement mode with absorbance monitoration.

Results

Linearity

The linearity upper limit for our atomic absortion spectrometer is 5.0 mmol/L. The Mega-Bayer linearity was assessed by triplicate analyses of 12 aqueous $CaCO_3$ solutions and 12 mixtures of urine. It was possible to obtain good regression lines with Mega-Bayer for $CaCO_3$ solutions ($y = 1.107x - 0.0441$; $r = 0.9997$) and mixtures of urine ($y = 1.065x - 0.0486$; $r = 0.9992$) up to the concentration of 10 mmol/L. The obtained urine line is shown in **Figure 1**.

Precision

The within-batch precision was assessed by 20 consecutive determinations of different urines with low,

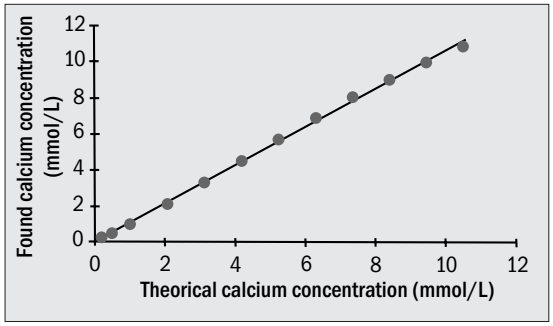

Figura 1 – *Regression line obtained with mixtures of urine. Each point represents the average of 3 measurements.*

Table1 Within-batch precision of urinary calcium determination

Urinary Concentration	Atomic absorption			*o*-cresolphtalein complexone		
(mmol/L)	x	SD	CV	x	SD	CV
Low	0.95	0.018	1.89	1.17	0.022	1.88
Normal	2.09	0.024	1.15	2.37	0.028	1.18
High	4.70	0.052	1.11	6.93	0.050	0.72

Table 2 Between-batch precision of urinary calcium determination

Concentration	Atomic absorption			*o*-cresolphtalein complexone		
	x	SD	CV	x	SD	CV
Qualitrol N	2.11	0.059	2.79	2.07	0.066	3.19
Qualitrol P	3.39	0.115	3.39	3.41	0.091	2.66

normal and high calcium concentration. The results are shown in **Table 1**. No precision differences were observed between the methods, for the three concentration levels, by the F-Test.

The between-batch precision was determined in normal and pathological control sera assayed in 20 different batches. The results are shown in **Table 2**. The F-Test did not show statistical differences of precision between the methods.

Comparison studies

The Mega-Bayer results were compared with the ones from SpectrAA 250 Plus - Varian using linear regression analysis. The data obtained from the 135 urine samples (range = 0.15 to 11.0 mmol/L) are y = 1.044 x - 0.022, r= 0.997 and the curve is shown in **Figure 2**.

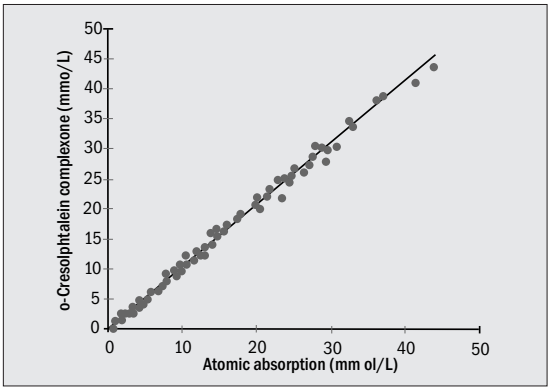

Figura 2 – *Linear regression obtained with 135 urine samples (range = 0.15 to 11.0 mmol/L).*

Although the average difference between the methods was small (-0.543), the t-test rejected the null hypothesis.

Discussion

The *o*-CPC method can be used for calcium determination in serum, urine and blood fluids (6, 7, 11). This study evaluated the use of this method in an automated system, for urinary measurements using the atomic absorption as the gold standard reference method.

The determination of total calcium in urine, utilizing the Mega-Bayer, agreed well with the atomic absorption reference method in the three urinary concentration levels tested, as shown by the least-squares analysis and the F-test. The probable explanation for the differences found in the t-test is the large sample number, since the obtained bias was small (-0.543). The similarity between the two methods permits the use of the same reference interval for both methods.

Atomic absorption and *o*-CPC are performing within the allowable error limits recomended by the Clinical Laboratory Improvements Amendments of 1988. Final Rule. Laboratory Requirements, USA (2) used as the goal in our laboratory; we observed sistematic errors bellow half of the allowed limit goals.

However, there was a difference between the two methods in their analytical ranges. The upper limit of

linearity of the *o*-CPC in Mega-Bayer was two times higher than the one of the atomic absorption. This brings technical advantages to the first method because of its independence of previous sample dilution.

The analytical goals for laboratory precision can be derived from different guidelines: clinical opinion, state of art and biological variation. Comparing these present results on analytical performance with the ones obtained by other authors (1, 15), we may conclude that the tested method achieved excellent analytical goals in all three approaches.

Therefore, this study permits the conclusion that *o*-CPC in the Mega-Bayer equipment can confidently perform the total calcium urinary analysis. It has the advantages of being a fully automatized biochemical procedure and of allowing a wider linear analytical range.

This study indicates that this is an excellent alternative method for urinary calcium measurements in automated systems. Multipoint calibration and careful control of the reaction temperature are recommended.

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