

Methods

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Comparison of Diluents for Serum Magnesium Estimation by Atomic Absorption Spectrophotometry

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Abstract. In order to determine the optimal diluent for the determination of Mg by atomic absorption spectrophotometry, the following diluents were tested: deionized water, 0.1 mol/l HCl, 2.5 g/l SrCl₂, 1.8 g/l LaCl₃, 17.7 g/l LaCl₃, half-saturated 8-hydroxyquinoline (oxine), 40 g/l trichloroacetic acid (TCA)/17.7 g/l LaCl₃, and 100 g/l TCA/0.1 mol/l HCl. The calibration curves, obtained on the same day, were passed through the origin in the form of linear regressions. The variances about the calibration curves did not differ significantly ($p > 0.05$), while the calibration slopes for the diluents differed significantly ($p < 0.001$). A standard serum solution was analyzed with the use of the eight diluents and the mean results did not differ significantly ($p > 0.05$). The % coefficient of variation varied from 0.8 to 2.0 and the percent recovery ranged from 95.5 to 102.5. The 99% confidence interval (CI) ranged from 0.02 to 0.09. Since conventional CIs do not take into account the random error of the calibration curve, the inverse CI were also calculated for each diluent. On both the conventional estimates and on the inverse CI, strontium chloride gave the best results, but only marginally in view of the interassay variability of approximately 1%.

Introduction

It is well known that atomic absorption spectrophotometry (AA) measurements of magnesium may be subject to interference by a variety of substances. Sodium at high concentration has been reported to interfere [10]. Elements which form acidic oxides, stable at high temperatures, e.g. aluminium, phosphorus, silicon, and titanium, interfere with AA

measurement. Other substances reported to interfere are strontium, hafnium, zirconium [3], calcium and barium [6], iron, potassium, lithium, chromium, tin, beryllium, rubidium, vanadate, sulfuric acid [12], and hydrochloric acid [7].

However, with the use of an air/acetylene flame, neither sodium, potassium, calcium, phosphate, nor iron appears to interfere [4, 16].

Many workers have reported that, in serum diluted with water, the protein present in the serum interferes as well.

It is of interest that two of the diluents in common usage, namely, strontium chloride (reported to remove all interference with the exception of that of iron, chromium, and titanium) and hydrochloric acid (known to remove protein interference) were themselves reported to give rise to interference [3, 7].

This inconsistency found in the literature and the fact that in serum, on use of lanthanum chloride, which is recommended in the manufacturer's instruction manual, we found difficulties in sample delivery, prompted us to review anew the diluents in current usage. The purpose of our study was to determine, if possible, which diluent gives the best precision on AA estimation of magnesium in human serum.

Materials and Methods

Diluents

The following diluents were compared: deionized water [14], 0.1 mol/l HCl [2], 2.5 g/l SrCl₂ [6], 1.8 g/l LaCl₃ (0.1% lanthanum) [5], 17.7 g/l LaCl₃ (1% lanthanum) [8], half-saturated 8-hydroxyquinoline (oxine) [9], 40 g/l trichloroacetic acid (TCA)/17.7 g/l LaCl₃ [N.W. Alcock, 1979, personal communication] and 100 g/l TCA/0.1 mol/l HCl [5].

In order to obtain water for serum dilution and for the preparation of all solutions, laboratory deionized water was passed through a Milli-Q Reagent-Grade Water System (Millipore, Bedford, Mass., USA). The HCl used was Merck (FRG). Solutions of SrCl₂ were prepared by dissolving 4.20 g of SrCl₂·6H₂O (Riedel-de Haën, Ark.) in water and diluting to 1 liter. A stock 88.3 g/l LaCl₃ (5% lanthanum) solution was prepared by dissolving 29.3 g of La₂O₃ (Fluka puriss., ≥ 99.9%) in 90 ml of 6 mol/l HCl and diluting to 500 ml. Diluent solutions were made by dilution of this stock. Saturated oxine solution was prepared by adding solid oxine (Merck, FRG) to 1 mol/l HCl with constant stir-

ring [9]. The half-saturated diluent was obtained *in situ* by adding to the serum sample equal volumes of water and saturated oxine solution. The TCA used was reagent grade (Riedel-de Haën, Ark.). The Mg contamination, according to the label analyses of four of the diluents, was as follows: 0.0001 ppm for HCl, 0.021 ppm for SrCl₂, and 0.23 ppm for 17.7 g/l LaCl₃ (1% lanthanum).

Standards

BDH Mg ribbon was cleaned by dipping briefly in 50 g/l HNO₃, rinsed with deionized water, and dried between two pieces of filter paper. A stock solution (1,000 mg/l) was prepared by dissolving the required amount of freshly washed and dried ribbon in the stoichiometric amount of HCl and diluting with 0.1 mol/l HCl. The Mg concentration was checked against a standard dolomite (Hoepfner Gebr.) and the difference found was 0.6%.

The calibration curves were obtained on the same day (using standards ranging from blank to 0.4 ppm Mg; fig. 1) for the eight diluents tested (see 'Statistical Methods').

Apparatus

All measurements were made on a Perkin-Elmer model 403 atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, Conn., USA) using an air/acetylene flame and 285.2 nm Mg resonance line. The absorbance readings of the diluents, relative to deionized water, ranged from 0.004 for 0.1 mol/l HCl to 0.076 for 40 g/l TCA/17.7 g/l LaCl₃, probably due to Mg contamination.

Assays

In order to compare the serum magnesium concentration (S-Mg) results obtained with the different diluents, a standard serum with constituents which were identical to the composition of human serum was used (Lab-Trol-E, Merz & Dade). The standard serum used had the following assay values for potentially interfering constituents: Na, 143 mmol/l; K, 4.65 mmol/l; Ca, 2.45 mmol/l; P, 1.06 mmol/l; serum-Fe, 14.5 μmol/l, and serum protein, 75.0 g/l. The manufacturer's assay value for S-Mg was 1.00 mmol/l.

Serum samples were diluted 1:100 and ten replicates were tested with each diluent. Recovery was measured by adding to the serum 0.1 and 0.2 mg/l of Mg. Six replicates of each addition were carried out.

Statistical Methods

The equation used for all calibration curves is of the form, $Y = bX$, namely, a straight line passing through the origin. This is so because in all AA spectrophotometers, the reading is arbitrarily set at zero while a blank solution is being aspirated. Further, to enable comparison of the slopes of the calibration curves, these were determined on the same day (fig. 1) for the eight diluents considered. To test the equality of the calibration curve error variances, Bartlett's test was used. The F test was used to test the equality of the slopes of the eight calibration curves.

Following the determinations of S-Mg in the standard serum samples, the conventional calculations were made of % coefficient of variation (CV), percent recovery, 99% confidence interval (CI) estimates of the mean concentration and of interassay variability. In addition to the above, an inverse confidence interval (inverse 99% CI) estimate was calculated for each diluent.

Use of the ordinary CI for the estimated concentration ignores the error in establishing the basic calibration curve. In effect, it assumes that the calibration curve is known without error. Consequently, the errors which affect the CI would be only those deriving from sample preparation and from instrument variability. Because there are, in fact, random errors involved in establishing the calibration curve, the proper basis for estimating the concentration is the inverse CI. Unlike the usual CI for Y, this is a CI for X, the concentration in an independent sample, estimated in an inverse manner from the calibration curve. The formula, which is complex [1], simplifies, when the calibration slope is highly significant (i.e.

when the experimental results fit closely to the regression line) to:

$$\text{Inverse CI} = \frac{\bar{Y}'}{b} \pm t_{\alpha/2, k-1} \cdot \frac{s}{b} \sqrt{\frac{1}{m} + \frac{\bar{Y}'^2}{b^2} \cdot \frac{1}{\sum_{i=1}^k X_i^2}}$$

where \bar{Y}' = mean absorbance value of the serum samples; b = slope of the calibration curve; $t_{\alpha/2, k-1}$ = Student's t for 100(1- α)% confidence and $k-1$ degrees of freedom; s = square root of the calibration curve error variance; m = number of serum samples measured; X_i = concentration of the given standard Mg solution used to determine the calibration curve; k = number of standard Mg solutions.

Results

The calibration curves for the eight diluents are seen in figure 1.

The variances about the calibration curve for the different diluents did not differ significantly ($\chi^2 = 4.90$, d.f. = 7, NS). The slopes of the calibration curves were significantly different [$F(7,24) = 217.6$, $p < 0.001$].

The mean S-Mg tested with the different diluents in the control serum of 1.0 mmol/l mean nominal concentration ranged from 0.986 to 1.034 mmol/l, as can be seen from table I. The means did not differ significantly

Table I. S-Mg with different diluents (mmol/l)

Diluent	n	Mean	SD	99% CI	Inverse 99% CI
Deionized H ₂ O	10	0.986	0.009	0.018	0.077
0.1 mol/l HCl	10	0.999	0.011	0.023	0.082
2.5 g/l SrCl ₂	10	0.990	0.008	0.016	0.019
1.8 g/l LaCl ₃	10	1.022	0.018	0.037	0.083
17.7 g/l LaCl ₃	9 ¹	1.034	0.021	0.047	0.066
Half-saturated 8-hydroxyquinoline	9 ¹	1.034	0.011	0.025	0.085
40 g/l TCA/17.7 g/l LaCl ₃	10	1.014	0.042	0.085	0.070
100 g/l TCA/0.1 mol/l HCl	10	1.018	0.022	0.045	0.059

¹ Outlying value rejected by Q test.

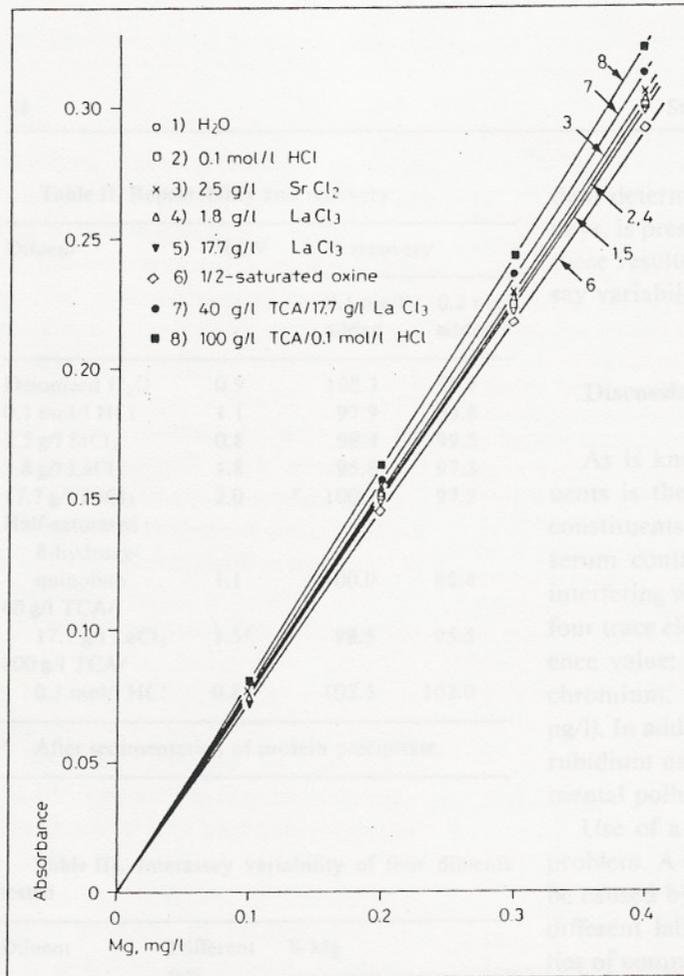


Fig. 1. Calibration curves of diluents tested.

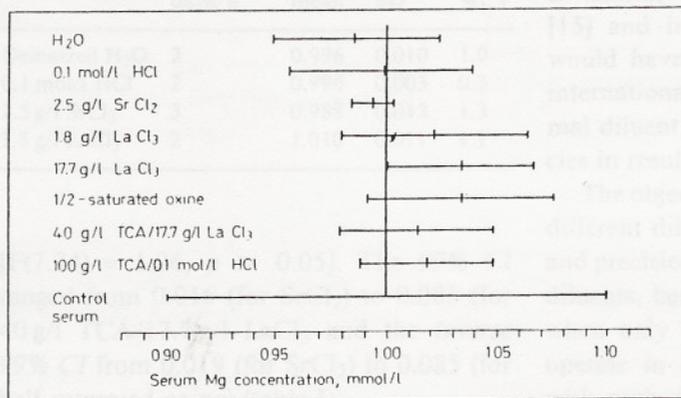


Fig. 2. Inverse confidence intervals for the diluents tested. (Inverse CI for control serum is based on confidence limits given by the manufacturers - Merz & Dade AG, Switzerland.)

Table II. Repeatability and recovery

Diluent	%CV	% recovery	
		0.1 mg/l added	0.2 mg/l added
Deionized H ₂ O	0.9	100.3	99.7
0.1 mol/l HCl	1.1	97.9	95.8
2.5 g/l SrCl ₂	0.8	98.4	99.5
1.8 g/l LaCl ₃	1.8	95.8	97.3
17.7 g/l LaCl ₃	2.0	100.2	97.2
Half-saturated 8-hydroxy- quinoline	1.1	100.0	98.4
40 g/l TCA/ 17.7 g/l LaCl ₃	1.5 ¹	98.5	95.5
100 g/l TCA/ 0.1 mol/l HCl	0.8 ¹	102.5	102.0

¹ After sedimentation of protein precipitate.

Table III. Interassay variability of four diluents tested

Diluent	Different test days, n	S-Mg		
		mean	SD	%CV
Deionized H ₂ O	2	0.996	0.010	1.0
0.1 mol/l HCl	2	0.996	0.003	0.3
2.5 g/l SrCl ₂	3	0.988	0.013	1.3
1.8 g/l LaCl ₃	2	1.010	0.011	1.1

[F(7,24) = 1.96, $p > 0.05$]. The 99% CI ranged from 0.016 (for SrCl₂) to 0.085 (for 40 g/l TCA/17.7 g/l LaCl₃ and the inverse 99% CI from 0.019 (for SrCl₂) to 0.085 (for half-saturated oxine) (table I).

The precision, expressed as %CV, ranged from 0.8 to 2.0 and the percent recovery from 95.5 to 102.5 (table II).

An estimate of the interassay variability, made for those diluents for which indepen-

dent determinations were made on different days, is presented in table III. On the basis of these results, a reasonable value for interassay variability would be 1%.

Discussion

As is known, the rationale for using diluents is the interference from other serum constituents in AA Mg estimation. Human serum contains six constituents potentially interfering with AA Mg estimation, as well as four trace elements (strontium, normal reference value: 50 µg/l; aluminium, 30–40 µg/l; chromium, 20–30 µg/l, and vanadium, 10 µg/l). In addition, it may contain lithium and rubidium used as medications, and environmental pollutants, e.g. beryllium.

Use of a diluent, however, may create a problem. A difference in measurements may be caused by the use of different diluents by different laboratories, thus causing difficulties of comparison. Since S-Mg is very stable in health, with very small interindividual [15] and intraindividual [13] variation, it would have been desirable to arrive at an internationally acceptable choice of one optimal diluent, to avoid extraneous discrepancies in results.

The object of the study was to evaluate the different diluents. To compare the accuracy and precision of the calibration curves for the diluents, best statistical results are obtained when only a minimum number of factors operate in an uncontrolled way. By using with each diluent the same standard solutions and the same standard serum, the outcome of the results is due to diluents [11].

In our study, on both conventional estimates and on the inverse CI, SrCl₂ gives the best results, but only marginally so in view of

the interassay variability of approximately 1%. Therefore, on the grounds of statistical results alone, it is difficult to single out one diluent as outstandingly best.

From a substantive point of view, however, we find that the use of LaCl_3 causes problems with sample delivery particularly at higher concentrations (upon prolonged aspiration of these solutions, it was found that the slot of the burner became clogged and the aspiration rate changed, with concomitant decrease in the absorbance reading). The use of TCA does not recommend itself, since it involves a more cumbersome procedure. As regards deionized H_2O , it is doubtful whether it could be used successfully to measure abnormal sera.

If the choice of a diluent is then to be made from the remaining three, the position of SrCl_2 becomes stronger on statistical grounds as its inverse CI is considerably narrower than those of HCl and half-saturated oxine (table I).

Thus, on a combination of substantive and statistical grounds, we recommend the use of SrCl_2 as a standard diluent. If agreement cannot be reached on this, we recommend, in the interest of clearer communication of research results, that every laboratory report the diluent used, and the inverse CI estimate for the mean S-Mg.

Comparaison des liquides de dilution pour l'évaluation du magnésium sérique par spectrophotométrie d'absorption atomique

Afin de déterminer le liquide de dilution optimal pour l'évaluation du Mg par spectrophotométrie d'absorption atomique, nous avons essayé les liquides de dilution suivants: eau déionisée; HCl, 0,1 mol/l; SrCl_2 , 2,5 g/l; LaCl_3 , 1,8 g et 17,7 g/l; 8-hydroxyquinoléine

semi-saturée (oxine), acide trichloracétique (TCA), 40 g/l/ LaCl_3 , 17,7 g/l et TCA, 100 g/l/HCl, 0,1 mol/l. Les courbes de calibrage, obtenues le même jour, ont été passées par l'origine sous forme de régressions linéaires. Les variances concernant les courbes de calibrage n'ont pas différé significativement ($p > 0,05$), alors que les pentes de calibrage pour les liquides de dilution ont été significativement différentes ($p < 0,001$). Une solution de sérum-étalon a été analysée en utilisant les 8 liquides de dilution et les résultats moyens n'ont pas différé significativement ($p > 0,05$). Le %CV a varié de 0,8 à 2,0 et la récupération, en pourcentages, a varié de 95,5 à 102,5. L'intervalle de certitude de 99% (99% CI) a varié de 0,02 à 0,09. Les CT classiques ne prenant pas en considération l'erreur fortuite de la courbe de calibrage, les CI inverses ont été calculés aussi pour chacun des liquides de dilution. Le chlorure de strontium a donné les meilleurs résultats, tant avec les estimations classiques qu'avec le CI inverse, mais seulement de façon marginale, compte tenu de la variabilité d'environ 1% entre les essais.

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