Determination of Calcium and Magnesium in Blood Serum by Automated Flame Microsampling

Application Note

Atomic Absorption

Author

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Introduction

The number of trace metallic elements of biological importance is increasing and a wide range of elements need to be analyzed in blood, urine and tissues. There is often a natural limitation of sample volume particularly in the analysis of blood and tissue digest samples. For example, serum calcium measurements may routinely be required on sample volumes of 0.05-0.10 mL. Therefore, a means of minimizing sample consumption while retaining high sensitivity for the measurement is of considerable importance. For elements such as calcium and magnesium, flame microsampling provides the necessary sensitivity and very small sample volumes can be analyzed with minimal sample preparation. A further advantage of flame microsampling is that it minimizes the problems associated with the aspiration of high protein content solutions encountered in serum analysis.

Agilent Technologies

Determination of Calcium and Magnesium in Blood Serum

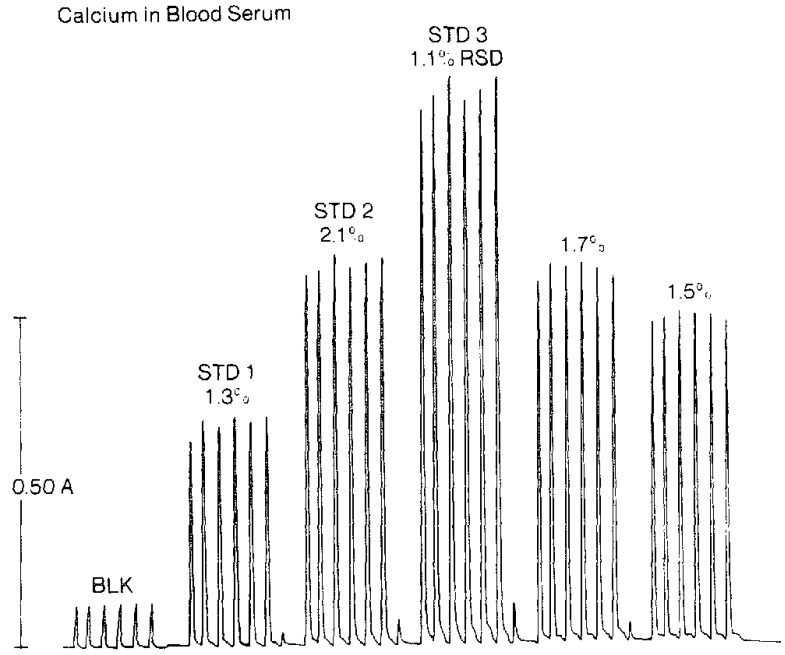
Unassayed normal serum samples were analyzed for calcium and magnesium. A serum volume of 100 µL was diluted to 5 mL, and potassium was added to give a final concentration of 5000 µg/mL in order to suppress ionization. The sample was acidified with concentrated HCl. The nitrous oxide-acetylene flame was used for both analyses to overcome chemical interferences.

The dipping method of flame microsampling utilizing the Agilent PSC 55 Programmable Sample Changer was used. The PSC 55 was programmed with the following parameters:

Number of standards	3
Number of samples	2
Rinse rate	1
(A distilled water rinse after every sample)	
Rinse time	10 (sec)
Dip time	0.2 (sec)
Multiples	5
Reslope rate	0
(Reslope function was not used)	
Sample flush	1
(Sample flush preceding every standard and sample)	

An Agilent AA-875 Atomic Absorption Spectrophotometer equipped with an adjustable uptake nebulizer was used. The uptake rate was adjusted to 7 mL/minute. Integration time was 8 sec. Measurements were made in the peak height mode.

The calcium determination was carried out at the 422.7 nm line. Background correction was not necessary. Calcium standards of 1.0, 2.0 and 3.0 µg/mL were prepared. Standards also contained 5000 µg/mL potassium and were acidified with HCl. Calibration was established taking into account the dilution factor to give results directly in milligrams per decilitre (mg/dL). Figure 1 shows calibration of 3 standards and the calculated mean values for the two samples. Measurements of 9.60 mg/dL and 8.16 mg/dL Ca are within the normal serum calcium concentration range of 7.5 to 13.0 mg/dL. The precisions for 5 measurements of the solutions ranged from 1.1 to 2.1 %RSD. The first peak in each set of six is the sample flush; it is disregarded in the calibration and sample measurements. This sample flush and 10 sec distilled water rinse between standards and samples eliminates the effects of carry-over.



ZERO	C	0.000
STD 1	C	5.00
STD 2	C	10.00
STD 3	C	15.00
01	C	9.86
02	C	9.62
03	C	9.68
04	C	9.45
05	C	9.41
MEAN	C	9.60
01	C	8.14
02	C	8.06
03	C	8.02
04	C	8.34
05	C	8.24
MEAN	C	8.16

Figure 1. Calcium in blood serum.

The magnesium determination was carried out at the 285.2 nm line without background correction. The PSC 55 parameters were the same as those used for the calcium determinations, however only one sample was analyzed. Calibration was again established directly in mg/dL. The actual standard concentrations were 0.2, 0.4 and 0.6 µg/mL magnesium. As Figure 2 indicates, the precision was excellent: from 1.1–1.4 %RSD. The determined concentration of 1.90 mg/dL magnesium is within the normally expected serum magnesium range of 1.5–5.8 mg/dL.

Routine Determination of Serum Calcium and Magnesium

It has been shown that flame microsampling is a feasible method of measuring serum calcium and magnesium. However, long term accuracy and precision must be considered in the routine analyses of larger numbers of samples. An assayed control serum was used to determine the long term accuracy and precision. The standard concentrations and the sample dilution factor were the same as the previous determinations. One serum sample dilution was carried out and analyzed 30 times, so that inaccuracies could be attributed to the AA method of analysis rather than to dilution errors. The following PSC 55 parameters were used:

Number of standards	3
Number of samples	30
Rinse rate	1
Rinse time	10 (sec)
Dip time	0.2 (sec)
Multiples	2
Reslope rate	10
Sample flush	1

The spectrophotometer parameters were the same as those used for the previous determinations of calcium and magnesium except that the integration time was reduced to 6 sec. Figure 3 shows the recorder tracing for the magnesium calibration and the first 20 samples. The first peak of each standard and sample is the sample flush. The calibration and sample measurements were determined from the mean of two readings. After the first 10 samples, the blank and second standard were rechecked. This was done by selecting 10 as the reslope rate parameter on the PSC 55. The sample probe returns to the blank solution and then to a reservoir of the standard selected for reslope. The actual reslope is achieved by the HP-85 computer accessory.

In this analysis drift was not significant enough to require reslope. This programmed remeasurement of the blank and second standard can be used as a monitor of drift of both baseline and sensitivity. Depending on the amount of drift encountered and number of samples (up to 67 with the PSC 55), the operator can program a "drift monitoring" (or calibration reslope) after any selected number of samples.

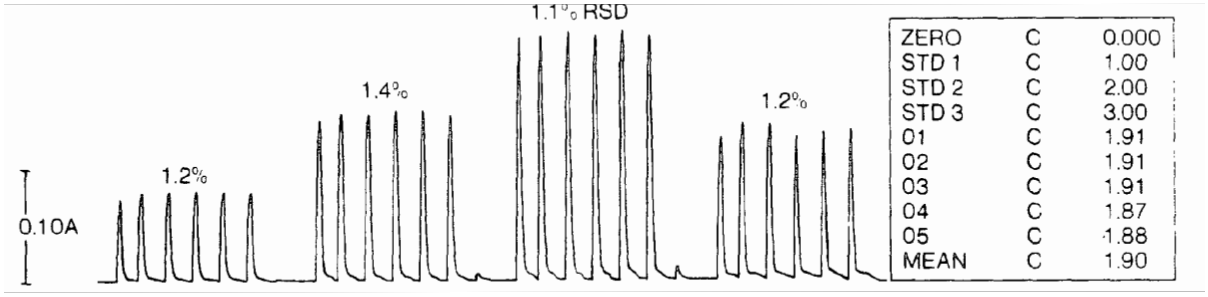


Figure 2. Magnesium in blood serum.

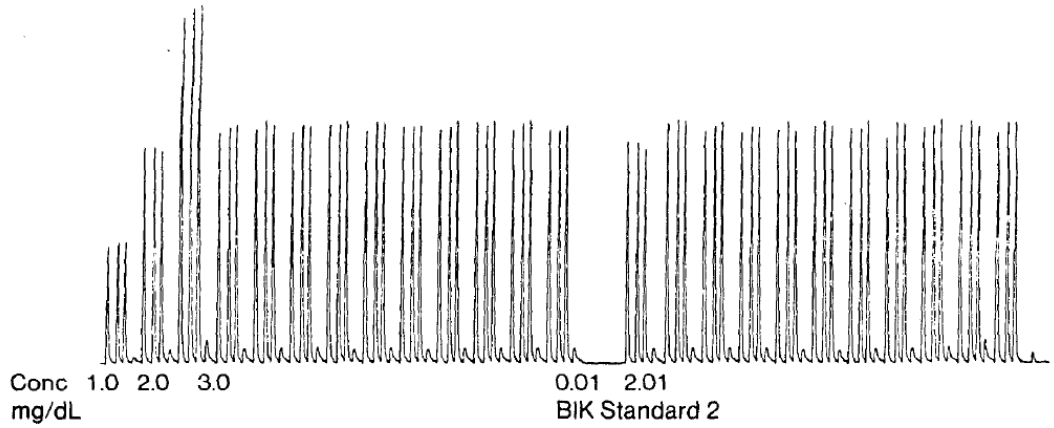


Figure 3. Serum magnesium. 0.2, 0.4, 0.6 µg/mL Mg standards (1.0, 2.0, and 3.0 mg/dL serum mg).

Table 1 summarizes the results of the magnesium determination. The normal control assayed value was 2.4 mg/dL with an expected range of ± 2 standard deviations (± 0.4 mg/dL). The mean value obtained was 2.15 mg/dL. The highest individual value was 2.21 mg/dL and the lowest value was 2.10 mg/dL. Standard calibration and the 30 determinations took 30 minutes. The analysis was repeated in a shorter period of time (15 min) with a distilled water rinse after every 10 samples rather than after every sample with only a slight decrease in precision.

Table 1 also shows the results of the calcium determination. The normal control assayed value was 10.0 mg/dL with an expected range of ± 0.4 mg/dL. The highest individual value was 10.1 mg/dL and the lowest value was 9.6 mg/dL. Standard calibration and the 30 determinations were completed in 30 minutes with a distilled water rinse after every sample.

Conclusion

Flame microsampling utilizing the Agilent PSC 55 Programmable Sample Changer is an excellent method for determining calcium and magnesium concentrations in small volumes of blood serum. The results were accurate and showed excellent precisions during the analysis of 30 samples. The same solutions could be used for the AA determination of the electrolytes sodium and potassium if cesium rather than potassium is used for ionization suppression. Flame microsampling minimizes sample preparation, permits the determination of calcium and magnesium on serum samples of 0.10 mL, and minimizes any clogging effects encountered in continual aspiration of high protein concentrations in serum.

Table 1.

Element	Normal control assayed value ±	No. of samples	Mean value	Highest value lowest value	Time
Magnesium				2.21	
(rinse after every sample)	2.4 + 0.4 mg/dL	30	2.15 mg/dL (1.4 %RSD)	2.10	30 min
(rinse after 10 samples)	2.4 + 0.4 mg/dL	30	2.15 mg/dL	2.10	15 min
Calcium				10.1	
(rinse after every sample)	10.0 + 0.4 mg/dL	30	9.8 mg/dL (1.5 %RSD)	9.6	30 min

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