



LITHIUM DETERMINATION IN WHOLE BLOOD BY FLAME ATOMIC EMISSION SPECTROMETRY

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A simple and rapid method for the determination of lithium in whole blood using Flame atomic emission spectrometry is described. No sample preparation was required apart from dilution with 0.02 N HNO₃. The reliability of the method was determined by analyzing Standard Reference Material (SRM) under identical experimental conditions and comparing the determined lithium concentration with the reported value. These were in good agreement with each other. The determined range of lithium in the whole blood of fifty-six healthy adult volunteers (28 males and 28 females) was 13.1 – 47.8 µg L⁻¹. The determined average concentration of lithium in whole blood was compared with the reported values of other countries. The data was statistically analyzed with respect to sex and different age groups.

Keywords: Lithium determination, Whole blood, Flame atomic emission spectrometry

1. Introduction

Lithium has many biological actions that make it relatively specific in behavior. Lithium is a low molecular weight monovalent cation, which is present in very low concentration in human and animal tissues, ranging from 2 - 200 µg kg⁻¹ [1]. Lithium possesses the smallest radius of all alkali metals in physiological solutions so it is radially hydrated and resembles calcium and magnesium. Lithium can replace sodium and maintain the resting and action potential of nerve cells. The normal concentration of lithium in blood is about 3.4 µmol L⁻¹ (23.8 µg L⁻¹) and therapeutic range is suggested to be between (2.1–9.1 mg L⁻¹), whereas 10.5 mg L⁻¹ represents the lower limit for intoxication. Serum/blood values exceeding 3.5-mmol L⁻¹ or 24.5 mg L⁻¹ should be regarded as potentially lethal [2]. An excess dose of lithium can produce irreversible damage to the nervous and renal system [3].

There are a few analytical techniques used for the determination of lithium in the blood such as potentiometry [4], chromatography [5,6], fluorimetry [7], neutron activation mass spectrometry [8] and atomic absorption spectrometry [9]. All these

techniques have their own merits and demerits but atomic absorption spectrometry is one of the preferred techniques due its rapidness and specificity.

The aim of the present study was to establish a rapid and accurate method for the determination of lithium in whole blood samples using flame atomic emission spectrometry (FAES). The emission mode was adopted because it is hundred time more sensitive than absorption mode of atomic absorption spectrometer [10]. The method was employed to analyze the lithium concentration in the whole blood samples of the inhabitants of Rawalpindi/Islamabad. Such study will help to establish baseline levels of lithium in whole blood of the population in the specified area.

2. Experimental

2.1. Instrumentation

All the measurements were made with Hitachi model Z-8000 polarized Zeeman atomic absorption spectrophotometer, which was coupled with a microprocessor-based data-handling facility. A water-cooled premix fish-tail type burner having a 10 x 0.05 cm² slot was used for the air-acetylene

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flame. Concentration mode of FAES was used for the measurements of lithium.

2.2. Reagents

Standard stock solution of 1000 mg L⁻¹ of lithium was prepared by dissolving appropriate amount of specpure metal oxide (Johnson Matthey Chemicals, Ltd.) in minimum amount of nitric acid and the volume was made upto 100 ml with water. For the preparation of calibration curve fresh working standards were made by appropriate dilution of stock solution in 0.02 N HNO₃ immediately before use. Glassware was cleaned by overnight soaking in HNO₃ (1:1) followed by repeated rinsing with water. Distilled and deionized water was used throughout this work.

2.3. Sampling

Blood samples of normal healthy persons were drawn by vein puncture under contamination controlled conditions and various blood samples were collected from the Federal Government Services Hospital, Islamabad, in 4 ml VACUETTES (Grainer laborotechnik) which contained EDTA as an anticoagulant. Samples were analyzed within twelve hours after collection.

2.4. Procedure

Calibration standard solutions were prepared by appropriate dilution of the stock solution in 0.02 N HNO₃. Blood samples were also diluted accordingly with 0.02 HNO₃. The emission signal was recorded by aspirating the solutions in the order of blank, standards, sample blank and samples into the air-acetylene flame employing the

optimized conditions given in Table 1. Minimum of three values were recorded for each solution and mean value of the signal was used for subsequent calculations. The signals were evaluated by subtracting the value of blank from the signal of the sample.

3. Results and Discussion

Lithium was determined in fifty-six samples of healthy adult volunteers including twenty-eight females and twenty-eight male of Rawalpindi/Islamabad area using flame emission mode of AAS. The whole blood samples were diluted with 0.02 N HNO₃ prior to the determination of lithium. This dilution is mandatory to avoid the blockage of burner orifice and for the deproteinization of blood sample. The instrumental conditions are given in Table-1. The criterion for the selection of the instrumental parameters was the conditions that produce maximum stable signal with low background.

Limit of detection (LOD) and sensitivity were determined for lithium in diluted-pooled blood sample by using instrumental conditions given in Table 1. The LOD was calculated as the concentration of analyte required to give a signal equal to twice the standard deviation of ten replicate of the blank measurements, whereas the sensitivity was computed as the characteristic mass of analyte, which corresponds to 0.0044 absorbance units. The determined values for LOD and sensitivity are 0.19 µg L⁻¹ and 0.6 µg L⁻¹ respectively.

The reliability of the method used was checked by analyzing the Standard Reference Material i. e. wheat flour (SRM-1567) from NBS, for its lithium contents using the instrumental conditions given in Table 1. The SRM was digested in a mixture of nitric acid and perchloric acid according to the procedure reported earlier [11]. The determined lithium concentration in the SRM was found to be 46.8 ± 0.8 ng g⁻¹, which is in good agreement with the reported value of 48.1 ± 1.0 ng g⁻¹ [8]. Additional support for the reliability of the method was obtained from the recovery study of the spiked lithium concentration in the whole blood sample having lithium concentration of 13.1 µg L⁻¹. The results are shown in Table 2, which indicate that the recoveries of the spiked lithium in sample of whole blood were better than 97%.

The proposed method was applied to the determination of lithium concentration in whole

Table 1. Instrumental conditions used for determination of lithium in whole blood.

Parameters	Values
Resonance line (nm)	670.8
Slit (nm)	0.4
Type of burner	Standard*
Burner height (mm)	7.5
Oxidant (air) pressure (kgcm ⁻²)	1.6
Fuel (C ₂ H ₂) pressure (kgcm ⁻²)	0.4
Measurement mode	Emission

*See experimental section.

Table 2. Recovery of lithium spiked in whole blood sample (n=3)

S. No.	Amount of lithium ($\mu\text{g L}^{-1}$)			% Recovery
	Added	Expected	Found	
1	10.0	23.1	22.8	97
			23.1	100
			22.9	98
2	20.0	33.1	33.2	100.5
			32.9	99.0
			33.3	101.0

blood samples of 56 healthy adult volunteers and the results are reproduced in Table 3-5 in the form of range, arithmetic mean and standard deviation. Lithium contents of the EDTA salt used as anticoagulant were also determined and were found to be less than 0.01 ng g^{-1} .

The perusal of the data in the Table 3 shows that the overall lithium concentration for both males and females ranged from $13.1 - 47.8 \mu\text{g L}^{-1}$ (mean = $27.8 \mu\text{g L}^{-1}$). The determined concentration of lithium in the whole blood samples for male volunteers of 21– 50 years age group ranged from $23.1 - 47.8 \mu\text{g L}^{-1}$ (mean = $33.0 \mu\text{g L}^{-1}$). Whereas for female volunteers of the same age group it ranged from $13.1-36.2 \mu\text{g L}^{-1}$ (mean = $22.7 \mu\text{g L}^{-1}$).

Table 3. Concentration of lithium ($\mu\text{g L}^{-1}$) in whole blood of normal healthy subjects.

Age	Sex	No	Range	Mean	\pm S D.
21-50	M+F	56	13.1 – 47.8	27.8	7.96
21-50	M	28	23.1 – 47.8	33.0	6.81
21-50	F	28	13.1 – 36.2	22.7	5.28

In order to study whether the apparent difference between mean blood lithium concentration of male and female is statistically significant, we applied student t-Test at 95% confidence levels to the data set. This shows that the t-statistical (6.588) is greater than t-critical (2.052) indicating significant difference between the studied population groups.

The data in Tables 4 and 5 show the range and mean values of lithium with respect to varying age groups among female and male subjects

Table 4. Distribution of lithium ($\mu\text{g L}^{-1}$) among various age groups of female subjects.

Age	No	Range	Mean	\pm S D.
21-30	10	13.1 – 27.7	19.7	4.90
31-40	8	17.4 – 36.2	23.1	3.21
41-50	10	16.3 – 36.2	25.1	5.81

Table 5. Distribution of lithium ($\mu\text{g L}^{-1}$) among various age groups of male subjects.

Age	No	Range	Mean	\pm S D.
21-30	9	23.1 – 47.8	32.6	4.60
31-40	9	25.0 – 47.4	38.1	7.76
41-50	10	23.1 – 35.5	28.8	4.57

respectively. Lithium concentration data in different age groups was also analyzed to test for any significant change with increasing age, using ANOVA test at 95% confidence level. The outcome of this statistical analysis shows that the value of F (2.779) is less than F critical (3.385), thus indicating no significant variation of blood lithium concentration among the female subjects with respect to different age groups. The data for male subjects reveal that the value of F (6.153) is higher than F critical (3.385) indicating a trend of blood lithium concentration among the male subjects with respect to various age groups. Since in the present work, the sample size in each age group is very small (≤ 10), therefore, in order to verify this relationship an extensive study with large number of subjects is required.

In the present study the determined over all average concentration of lithium in whole blood was $27.8 \mu\text{g L}^{-1}$ which is comparable to the normal concentration of lithium ($23.8 \mu\text{g L}^{-1}$) in blood [2] and to the value of lithium in whole blood reported by the Task group on Reference Man i. e., $30.3 \mu\text{g L}^{-1}$ [12]. These values are higher than the reported values from Canada $0.6 \mu\text{g L}^{-1}$ [7], USA $8.0 \mu\text{g L}^{-1}$ [13], UK $1.5 \mu\text{g L}^{-1}$ [14] and Spain $1.9 \mu\text{g L}^{-1}$ [15]. Therefore, in order to establish the base line levels of the area, analysis of more samples are being carried out.

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