STATISTICAL EVALUATION AND VALIDITY OF TWO ATOMIC-ABSORPTION SPECTROMETIC TECHNIQUES FOR THE DETERMINATION OF LEAD IN TOOTHPASTE SAMPLES

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Abstract

Two methods for the determination of lead in toothpaste were tested and compared. In the developed method (ETAAS), the toothpaste samples were dry-ashed with %10 magnesium nitrate and the resulting solution was injected directly into the graphite furnace using a combined platform and matrix-modification techniques for the determination of Pb in these samples. In British standard method, the toothpaste was slurred with ethanol, evaporated to dryness, dry-ashed and the resulting solution was extracted with APDC/MIBK system. The organic layer was aspirated into the FAAS.

Several statistical tools were carried out for the developed method to evaluate its analytical performance (LDR, MDL, LOQ, and RSD) and ANOVA Results for the calibration graph have shown that the regression line was statistically valid. With a %95 CL there was no significant difference in mean between the two methods by applying a paired t-test. Two procedures (direct and standard additions) of the developed ETAAS method were compared and tested statistically with British standard method based on RBD using two-way ANOVA. The results were revealed that all these methods have no significant difference and statistically valid.

Introduction

Lead is a toxic element and, even when present at very low levels, is stated to cause abnormal behavior especially in young children who lived in industrial areas and lead-contaminated homes(1,2). In recent study(3), it was found that lead is responsible for the persistent psychosis and contributes to the altered mental status.

Lead in toothpaste comes from the raw materials or contamination during manufacture. Consequently, the Iraqi (4) and British (5) standards specify that the lead content of toothpaste must not exceed 5 mgkg-1. Thus lead is required to be monitored in toothpaste for quality control. These necessitates the establishment of an accurate, rapid, and reliable method which is free from matrix interference effect to ensure that the lead levels in this matrix is with a safe limits.

A few methods have been reported for the determination of lead in toothpaste. Of these flame atomic-absorption spectrometry (FAAS) after dry-ashing,acid dissolution and solvent extraction(5). FAAS procedure for analysis of foodstuffs for lead has been modified for analysis of toothpaste and used by some laboratories (6). In these methods, the chemical and spectral interference has been reported. Errors related to the limited stability of the lead complex in the organic layer, the solubility of the solvent in water, and incompleteness of the extraction were also described. In addition, standard solutions must be extracted in the same manner for calibration, so the method is time-consuming and toilsome.

Electrothermal atomization atomic absorption spectrometry (ETAAS) offers a more practical opportunity than FAAS for determining lead in different matrices. But, when the organic extracts were analyzed by ETA, the only limitations being imposed by the difficulties of handling organic solvent with micropipettes, and the formation of organometallic compounds which may lead to some loss of the analyte (7) during the course of heating cycles . However, these interferences were less when matrix modification reagent(MMR) was used (8). This can greatly improve the atomization and ashing steps, to produce analyte atoms free from matrix material. It can change the properties of the matrix and stabilize the analyte against loss during the ashing stage even for high ashing temperature. One of the most commonly MMR is ammonium dihydrogen phosphate, which has been employed in the determination of lead and cadmium (9-10). This method was applied successfully for the determination of lead in toothpaste (11) .

In present work, several test statistics, including simple regression, ANOVA and randomized block design (RDB) were considered to evaluate the experimental data, by using Minitab. Version 11 computer software (12).

Experimental

Apparatus

All AAS determinations were carried out on a Perkin-Elmer 370A atomic-absorption Spectrometer equipped with an HGA 500 electrothermal and flame atomizers. The ET atomizer was fitted with commercially available tubes coated with pyrolytic graphite (PIN 109324). Argon was employed as the atomizer purge gas. The graphite tube and platform were cooled during operation by means of the HGA cooling system (B0091440). The standards and sample solutions (10µl) were introduced with the aid of an AS-40 autosampler. The AA signals for lead were measured at 283.3 nm and displayed in the digital read-out of the PE370A spectrometer and / or the PE 56 strip-chart recorder.

Reagents

Analytical-grade reagents and demineralized water were used in the preparation and dilution of solutions. A stock solution containing 1000 mg/l lead was prepared from Merck Titrisol® ampoules.

The working lead standards were freshly prepared by serial dilution of the stock solution with 0.2% V/V nitric acid after addition of matrix modifier at a concentration similar to that in the sample and blank solutions. The glasswares were soaked in 15% V/V nitric acid and washed ten times with demineralized water prior to use. The matrix-modifier solutions were 1% ammonium dihydrogen phosphate and 10% magnesium nitrate, and purified by solvent extraction with dithiozone according to the procedure reported by Khammas (13).

Samples

The commercial toothpaste samples were offered from local markets, and Baghdad factory for toothpaste production. A special toothpaste sample prepared according to the formula in British standard (5) is considered as reference.

Preparation of sample

The toothpaste samples were dissolved and prepared for the determination of Pb by ETAAS work as stated to the procedure described by Khammas et al (11), while the procedure described in British 5136 standard was performed for determination of Pb by FAAS.

Results and Discussion

Optimum Working Conditions

Similar instrumental parameters and the optimum furnace program for ETAAS work, which reported elsewhere (11) were used in this study (Table 1). For FAAS work, The measurements were carried out according to the instruction manual supplied by manufacturer.

Calibration graph for Pb and statistical validity

A calibration graph for Pb has been obtained for 10-µl volume injections of several concentrations of standard lead solutions under optimum conditions (Table 1). The analytical results are presented in (Table 2). The peak-height working calibration graph (figure not shown) was linear up to 150 µgl-1, which corresponds to an absolute mass of 1500 pg. If a calibration graph is linear from 4 to 1500 pg., this means that the linear dynamic range (LDR) obtained by the developed method is two and half orders of magnitude higher. Using the peak area mode can extend this linear range.

The estimated correlation coefficient(r) is commonly used to determine how well a straight line fits the data points. We present here two methods for calculation, namely, coefficient of determination (r2) and an ANalysis Of VAriance (ANOVA) technique to reveal whether the variability in absorbance is well expressed by linear relationship with concentration. The statistical calculations have shown that r2 = 0.9830 (Table 2). Thus we conclude that 98.7% of variations in absorbance are attributed to linear association with concentration. Since this percentage is large, with a correlation of 0.9871 (Table 2) give very strong evidence for linear association between two variables. The statistical validity based on ANOVA results, which treated as a simple one-factor classification is given in Table 3. The mean square(MS)obtained for regression can be tested for significance against that obtained from error. The ratio (F1,4) is 298.49 for one and four degrees of freedom. Since the 0.05 probability ratio is 7.71 (from

F-table) and since 298.49 is much larger than this, we can reject a null hypothesis (H0) and concluded that as expected, the assumption of linear regression is statistically valid. Practically speaking, this means that the predictions based on the estimated regression line
Y=0.005(±0.06) + 0.0043 (± 77× 10-4) should be acceptable.

Consequently all the concentrations of lead in the analyzed samples were determined from this relationship.

The repeatability (RSD) was measured under optimum conditions with a 50 µg/l lead solution (ten replicate) and found to be 1.9% compared to a value of 1.8% for tube-wall atomization. The characteristic mass (m0), method detection limit (MDL) and limit of quantitation (LOQ) were calculated from regression line and found to be 10.0, 0.8, and 4.0 pg. respectively.

Comparison of the methods and validity

Four different toothpastes produced by two different manufactures, in addition to special toothpaste were analyzed by both methods under optimum working conditions using direct working calibrations. In addition, toothpaste samples were spiked with 0,20,40 and 60 µgl-1and analyzed by standard additions procedure using the developed ETASS only. The results are presented in (Table 4), which also show the results analyzed by British standard method (FAAS). In order to validate the results of this work for the estimation of Pb concentration in different samples compared to British standard method (Table 4), a paired sample t-test is carried out, assuming that the difference in Pb measurements approximately normally distributed. This difference D is calculated as D=(British result – Iraqi result) and the |t| value was obtained from the following test statistic:

$$|t| = \frac{\bar{X}_D \sqrt{n}}{S_D} = 2.44$$

where XD = The mean difference = 0.062 , and n =5
SD = The standard deviation of the differences = 0.056

Based on the critical |t| value of 2.78(p = 0.05, dof = 4), the calculated value of |t| is less than critical value indicating that there appears insufficient evidence to suggest the accuracy of the two methods that differ significantly.

A Randomized Block Design (RBD) was also pursued to examine the source of variation and their effect on the concentration of lead determined by the three different methods (FAAS, ETAAS-direct calibration and ETAAS-standard additions). An RBD is essentially an extension of the paired sample t-test concept and thereby the detected methods differences can be precisely decided. Table 5 shows an RBD model structure for the concentrations of Pb
(g l-1) in five toothpastes by three methods investigated.

With the view to decide whether the methods or samples used are statistically significance in this model, two-way ANOVA must be applied to test the effect of these two controlled factors (sample and method) plus the uncontrolled factor (experimental error) on the response (concentration). The ANOVA calculations are shown in Table 6.

One of the important objectives of this work is to pinpoint the detected method differences, consequently, the Student-Newman-Keuls (SNK) multiple comparison procedure at p=0.5 significance level fulfill this role. From Table 5, n=5 (sample), k=3 (method) and from ANOVA (table 6) doferror=8 and an MSerror=0.00156, the null hypothesis to be tested in this follow-up is H0 : no difference in Pb concentration between analytical methods compared. The mean Pb concentration and the table differences in mean, the test statistic for the methods comparison is presented in Table 7. Also the corresponding %5 critical values Wr are shown in Table 8.

For comparison of FAAS and ETAAS (dir.cal.), the corresponding test statistic from Table 7 is 0.062. These methods in position i=1(1st), j=2(2nd) in order list. The critical value to use for comparison in this test statistic correspond to r=2+1-1=2, (r=j+1-1) and from Table 8, W2=0.461. This means that the test statistic is less critical value, we accept H0 and indicate that there is no significant difference between the two methods in Pb measurements (P>0.05). Similarly, for FAAS and ETAAS (std.add.) (i=1, j=3) then (r=3+1-1=3) , the test statistic is 0.076 (Table 7) and the associated critical value is W3=0.571 (Table 8) indicating that no significant differences exist for Pb concentration between the two methods(P>0.05). In same manner, the two ETAAS methods proved to give results with no significant differences as the test statistic (0.014) is less than W2= 0.461 at %95 confidence limits.

Conclusion

Statistical analysis of results greatly improves the reliability of analytical data for the determination of lead in toothpastes . ETAAS appears as precise and accurate method and give reasonable results. However, platform and matrix modification further improves the performance of ETAAS work in accordance with most recent reports that eliminate matrix interference. Moreover, the analysis time is shortening if compared with British standard.

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Table (1): *Graphite furnace program for the determination of pb in tooth-paste samples by ETA-AAS and the platform/matrix-modification technique (10-μl samples).*

Instrumental Parameters:
Wavelength: 283. nm
Slit bandpass: 0.7 nm
Lamp Current: 4mp
Signal Mode: Peak height
Background Corrector: On
Chart Speed: 10 mm/s
Injected Volume: 10

Furnace heating program:

	Step				
	1	2	3	4	5
Temperature, °C	80	130	800	1400	2650
Ramp, sec	5	20	5	0	1
Hold, sec	15	20	30	6	3
Recorder, sec				-4	
Read				0	
Internal gas flow, *ml/min*	300	300		0	300

Table(2): *Representative statistical results for the analysis of lead in aqueous solution using the developed platform/MMR ETA-AAS.*

Linearity (µg/l)	4-150
#Linear Dynamic Range (LDR)	2.5
*Characteristic Mass(m0) pg	10
▼Method Detection limit (MDL) pg	0.8
▲Limite of Quantitation (LOQ) pg	4
RSD% at 50 µg/l (N=10)	1.9
Regression Line:	Y=.0005+0.0043X
■ 95% C.L of Slope	±7.7 ×10-4
□ 95% C.L of Intercept	±0.06
Estimated correlation coefficient (r)	0.9871
Coefficient of Determination (r2)	0.9830

#LDR = $\log \frac{LOL}{LOQ}$ LOL = level of linear (highest mass)

*m0 = $\frac{0.0044}{S}$ S = slope of the calibration graph

▼M DL = $\frac{3\sigma_B}{s}$

▲LOQ = 5 × MDL
■b ± tSb S_b = std. devn. of slope.
□a ± tSa S_a = std. devn. of intercept.

Table (3***): Evaluation the strength of the linear relationship, ANOVA: Simple linear Regression.***

Source of variation	Degree of freedom	Sum of square SS	Mean square MS	F1,4
Regression (model)	1	0.34243	0.34243	298.49
Error	4	0.00459	0.00115	
Total	5	0.34702		

Table (4): ***Lead Concn. (µg l-1) in Different Toothpaste Samples Determined in This Work and British Standard (FAAS)***

SaMPLE	FAAS	ETAAS, Dir. Cal.	ETAAS Std. Add
Reference	0.39	0.45	0.41
Amber	0.43	0.46	0.47
Signal	0.26	0.28	0.31
Trisa	0.42	0.59	0.62
Macleans	0.53	0.57	0.60

Table (5): ***Structure for Pb Concentration in Different Sample By Three Methods:***

TREATMENT*		FAAS	ETAAS dir.cal	ETAAS std.add.
BLOCK#	1	0.39	0.45	0.41
	2	0.43	0.46	0.47
	3	0.26	0.28	0.31
	4	0.42	0.59	0.62
	5	0.53	0.57	0.60

*Treatment=Method Detected
#Block=Samples Used
Table (6): Ana
Table (6) : ***Alysis Of Variance for Concn. Pb µg -1***

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Source Of Variation	Degree Of Freedom	Sum of Square SS	Mean of Square MS	F
Treatment	2	0.01636	0.00818	5.23
Block	4	0.15177	0.03794	24.32
Error	8	0.01251	0.00156	
Total	14	0,1804		

for example F_4^2 = MS tret. /MS error
MS= SS/dof

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                                  Individual 95% CI
Treatment         Mean    ---------+---------+----------+----------+----
    ___
  FAAS               0.406    (---------*---------*)
  ETAAS (dir.cal.)  0.468                    (---------*---------*)
  ETAAS (std.add.)  0.482                        (---------*---------)
                              ---------+---------+---------+----------+-----
-                                 0.300     0.440     0.480     0.520
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                                  Individual 95% CI
 Block              Mean      -------+---------+---------+---------+-----
  __
   1                0.417              (-----*----)
   2                0.453                  (----*-----)
   3                0.283     (----*-----)
   4                0.540                             (----*----)
   5                0.567                               (----*----)
                              -------+---------+---------+---------+------
                                  0.300     0.400     0.500     0.600
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Table (7): ***SNK test statistic for Pb ((µg l-1) in differentmethods***

	Mean	FAAS	ETAAS Dir.cal.	ETAAS std.add.
FAAs	0.406	---	0.062	0.076
ETAAS (dir.cal.)	0.468		----	0.014
ETAAS(std.add.)	0.482			

Table (8): ***Critical Values Wr for SNK Multiple Comparison***

r	2	3
$q_{0.05,r,8}$	3.26	4.04
W_r	0.461	0.571

$W_r = q_{\alpha,r,doferror} \sqrt{} Ms_{error} / n$

Where- $q_{\alpha,r,doferror}$ is the 100α% critical value taken from
Tables of Studentized Range Statistic
- MS_{error} is mean square errors (Table 6)
- n = number of measurements per treatment.